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**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**
In order to study the differential sensitivity of peripheral pain generators in the residual limb of two classes of amputees, we use our existing research-grade image-guided intense focused ultrasound (ig-iFU) system to stimulate those pain generators. Targets for iFU stimulation include neuromas, intact nerves, TMR (targeted muscle reinnervation) sites, and patient-identified 'sensitive areas'. We have successful stimulated intact and transected nerves in our amputee patients and intact volunteers. We have observed significant differences in how the different groups respond to iFU stimulation and how the threshold value of iFU intensity necessary to generate sensations relates to different factors such as overall pain score, neuropathic pain, and the presence of phantom feelings and pain. Specifically, our data suggest that TMR surgery produces transected nerves less sensitive to iFU stimulation than 'standard' transected nerves, with those in neuropathic pain showing the greatest difference. We remain short of our enrollment goal of 45 TMR patients, with dormant plans to deploy at Northwestern University available to address that shortfall.

**15. SUBJECT TERMS**
Image guided intense focused ultrasound (ig-iFU) Intense focused ultrasound (iFU), Targeted Muscle Reinnervation surgery (TMR), limb amputation, Ultrasound

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## 1. INTRODUCTION.

Chronic pain is problematic for many amputees. That pain can have many causes, such as buildup of scar tissue that surround the transected nerves that can then irritate nerve ending. In addition, traditional amputation surgery frequently produces a neuroma at the end of the nerve, itself a source of tenderness and pain. An alternative to standard amputation surgery, called “Targeted Muscle Reinnervation” or TMR surgery cuts the nerve and then implants it into nearby muscle. TMR surgery has anecdotal evidence of reduced pain for amputees relative to standard amputee patients, an important though unstudied finding. Here we seek to address this question directly, through two means. One is use of questionnaires to assess patient’s pain, which gives a general sense of the patient’s experience of their pain; the other uses image-guided focused ultrasound device (ig-iFU) to directly test the sensitivity of cut nerve endings in residual limbs of amputee patients. To support this, we will work with two, 45-participant cohorts of patients: TMR and standard amputation surgeries. We will, in addition, enroll 45 non-amputee participants as a control cohort. Our ig-iFU device uses ultrasound imaging to locate neuromas, nerves, and tissue, and individual, short pulses of high-intensity ultrasound to stimulate the nerve endings in the residual limbs. In this way we will directly determine which are more sensitive: those of standard amputee patients or of TMR patients. An important outcome of this study is determination of the relative merits of each surgical procedure with regard to their relative impact on patient pain.

## 2. KEYWORDS:

- Image-guided intense focused ultrasound (ig-iFU)
- Intense focused ultrasound (iFU)
- Targeted muscle Reinnervation surgery (TMR surgery)
- Limb amputation
- Ultrasound

## 3. OVERALL PROJECT SUMMARY.

**Research Objective #1:** Determine the iFU threshold value required for reliable sensation induction and characterize those sensations, for **intact** peripheral nerves within healthy volunteers as well as within individuals with unilateral amputations, with or without TMR.

**Task 1.** Amend existing human subjects' protocol at HMC to include the more extensive studies described here.

- Completed: IRB and military HRPO approval have been obtained for work at UW.
- Incomplete: Obtain IRB approval at Northwestern University to increase our TMR patient subpopulation.

**Task 2.** Identify and consent volunteers with unilateral standard and/or TMR amputations or intact volunteers.

- Completed: We have identified and consented our allotted intact volunteers and standard amputees.
- Incomplete: We have done so for all candidate TMR patients in the Pacific Northwest. At Northwestern University we anticipate adding sufficient patient numbers to complete this Task.

**Task 3.** Amend our existing ig-iFU device as necessary.

- Completed: We have completed design and construction of a final ig-iFU device.

**Task 4.** Image and thereby locate with our ig-iFU device an intact major peripheral nerve in the appropriate contralateral limb of standard and TMR amputees or of controls.

- Completed: We have imaged and located major peripheral nerves for all of our intact volunteers and standard amputees.
- Incomplete: We have done so for all candidate TMR patients in the Pacific Northwest. At Northwestern University we anticipate adding sufficient patient numbers to complete this Task.

**Task 5.** Determine the iFU threshold value for an intact peripheral nerve and record the type and duration of the associated sensations.

- Completed: We have determined the threshold value and recorded associated sensations for all of our intact volunteers and standard amputees.
- Incomplete: We have done so for all candidate TMR patients in the Pacific Northwest. At Northwestern University we anticipate adding sufficient patient numbers to complete this Task.

**Research Objective #2:** Determine the iFU threshold value of **transected** nerves in all amputee volunteers.

**Task 6.** Image, hence locate the transected nerve ending and patient-identified sensitive areas as appropriate, in the patient's residual limb with ig-iFU.

- Completed: We have imaged and located major transected peripheral nerves for our allotment of standard amputees.
- Incomplete: We have done so for all candidate TMR patients in the Pacific Northwest. At Northwestern University we anticipate adding sufficient patient numbers to complete this Task.

**Task 7.** Stimulate the transected nerve ending of major peripheral nerves in the patient's residual limb with ig-iFU.

- Completed: We have stimulated the transected nerve ending of the major peripheral nerves for our allotment of standard amputees.
- Incomplete: We have done so for all candidate TMR patients in the Pacific Northwest. At Northwestern University we anticipate adding sufficient patient numbers to complete this Task.

**Task 8.** Apply questionnaires to patients to assay their pain.

- Completed: We have applied questionnaires to our allotment of standard amputees.
- Incomplete: We have done so for all candidate TMR patients in the Pacific Northwest. At Northwestern University we anticipate adding sufficient patient numbers to complete this Task.

**Task 9:** Write up all results for publication and presentation.

- Completed: We have written up our results that compare the iFU threshold stimulation value for intact volunteers and standard amputees (Mourad et al, 2018; Bobola et al, 2019). We have also written a review paper that summarizes how ultrasound in many forms may contribute to the diagnosis and alleviation of pain, including ig-iFU (Bobola et al, 2018). Finally, we have documented the apparent reanimation of a TMR nerve with ig-iFU (Ezekeke et al, 2019)
- Incomplete: We have not completed our comparison of the iFU threshold stimulation value for intact volunteers versus standard amputees versus TMR amputees due to an insufficient amount of data from TMR amputees. We have provided in an appended interim report the analysis that we could perform. Importantly, it shows that our requested work at Northwestern University will likely give us sufficient number of TMR amputees to complete our analysis.

**Task 10.** Visit Northwestern.

- Not yet started.

**Research objective #3:** Develop specifications of a clinical device that embodies intense focused ultrasound.

**Task 11.** Identify first-order ultrasound protocols and associated devices necessary to TAP.

- Completed: We have summarized this design in our review paper and in our interim report.

## 4. KEY RESEARCH ACCOMPLISHMENTS.

1. Following up on our first paper (Mourad et al, 2016 - attached), we have published our second paper supported by our DoD funding, a review paper (Bobola et al, 2018 - attached) on uses of ultrasound for diagnosing and treating pain, including ig-iFU, whose parameters and form factor we specify in that publication.
2. We published a third paper (Bobola et al, 2019 – attached) that summarizes our work to date, which demonstrates that intact and transected nerves respond differently to stimulation by transcutaneous, intense focused ultrasound. Transected nerves are much more sensitive to ultrasound stimulation that intact nerves. Moreover, that stimulation can generate phantom limb sensations..
3. We submitted a fourth paper (Ezeokeke et al, 2019 – attached) that documents the apparent re-animation of the motor and sensory function of a formally non-functional transposed peripheral nerve thanks to our use of iFU to successfully stimulate that nerve. .
4. We have generated our interim analysis of all of our data (Mourad et al, 2019 - attached). It shows that, on average, transected nerves after standard amputation are most sensitive to iFU stimulation, intact nerves from intact volunteers are the least sensitive to iFU stimulation, and that the sensitivity of TMR nerves fall in between those two cohorts. We also identified subsets of these cohorts with asymmetry in ipsilateral versus contralateral iFU threshold stimulation value that may correlate with spatial structure in central sensitization. Moreover, the biggest differentiator between sensitive versus insensitive TMR nerves is the presence versus absence of neuropathic pain. Importantly, we did not observe that for standard amputees. Finally, our power analysis with our existing data supports our desire for a final NCE that allows us to add to our TMR amputee cohort and thereby finish our analysis.
5. In support of that desire to collect data from more TMR amputees, we have obtained a verbal agreement with Northwestern University to deploy our ig-iFU device there to capture the last TMR patient data we need to complete our work.
6. We have completed amendment of our more advanced ig-iFU system, based upon the SSI device, in anticipation of deployment to Northwestern University.
7. We met with a representative of FusMobile, Inc, who have a x-ray guided iFU device whose details will inform the specifications of an eventual clinical device that embodies ultrasound stimulation under ultrasound-image guidance. We have under construction a grant application to the Focused Ultrasound Foundation to pursue this idea.
8. We have under construction an NIH HEAL proposal that uses our image-guided iFU procedure to generate a biomarker for ‘deep tissue tenderness’, where that tenderness may arise through peripheral and/or central mechanisms. We hypothesize that tracking tissue tenderness in this way may help the tracking of the efficacy of pain management,

## 5. CONCLUSION

During this last year we completed study of intact volunteers and standard amputees while collecting data from all TMR amputees available to us. We identified important trends in sensitivity to external stimulation between standard and TMR amputees (one that favors TMR surgery), as recounted in our attached interim report. Recognizing that we would not have enough TMR volunteers in our data base to complete our study, we established a verbal commitment to perform our remaining studies at Northwestern University, the home of TMR surgery. Regrettably, hard to identify and frankly bizarre email-based communication problems between DoD and UW (true for a few other universities as well, we are told), we did not complete arrangements with DoD to perform those studies at Northwestern. We remain hopeful we will get to do so.

## 6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS

Bobola M, Chen L, Ezeokeke CK, Kuznetslova K, Lahti AC, Lo W, Schimeck N, Selby M, Mourad PD (2018) Towards use of intense focused ultrasound for pain diagnosis and treatment: a review. *Current Pain and Headache Reports: Neuromodulation*. Jul10;22(9):60-72.

Bobola MS, Ezeokeke CK, Kuznetslova K, Lahti AC, Loeser JD, Olmstead TA, Friedly JL, Mourad PD (2019) A pre-clinical study of the response threshold of intact and transected nerves to stimulation by transcutaneous, intense focused ultrasound. *Ultrasound in Medicine and Biology.* Aug;45(8):2094-2103. doi: 10.1016/j.ultrasmedbio.2019.04.014.

Ezeokeke CK, Bobola MS, Selby M, Ko JH, Friedly JL, Mourad PD (2020). Case Study of an Amputee Regaining Sensation and Muscle Function in a Residual Limb after Peripheral Nerve Stimulation by Intense Focused Ultrasound. *Under revision at Brain Stimulation.*

Mourad PD, Friedly JL, McClintic AM, Olmstead TA, Loeser JD (2018) Intense focused ultrasound preferentially stimulates transected nerves within residual limbs: pilot study. *Pain Medicine*, V19:541-549. ePrint Sep 7 2017; doi: 10.1093/pm/pnx188.

Mourad PD**,** Bobola M, Ezeokeke CK, Selby M, Lahti AC, Loeser JD, Olmstead TA, Ko J, Friedly JL (2019) Interim report for W81XWH-15-1-0291 “Localizing and Assessing Amputee Pain with Intense Focused Ultrasound”

Various presentations on campus, some informal, one presented at the UW Neurological Surgery Grand Rounds.

## 7. INVENTIONS, PATENTS AND LICENSES.

Nothing to report, although we are in discussion with FUSMobile about possible translation of our work.

## 8. REPORTABLE OUTCOMES.

- Image guided intense focused ultrasound (ig-iFU) can identify and help locate deep and focal pain generators, as demonstrated in residual limbs of amputee patients.
- Ig-FU can locate peripheral nerves thanks to their enhanced sensitivity relative to surrounding tissue, typically muscle, ligaments and bone.
- Spatial patterns in the sensitivity of peripheral nerves ipsilateral and contralateral to an amputation might convey information about spatial patterns in central sensitization, a major contributor to chronic pain in the amputee population.
- The presence versus absence of neuropathic pain in TMR patients strongly differentiates those with versus those without sensitivity of their TMR nerves to iFU stimulation. This is not true for standard amputee patients in our cohort.

## 9. OTHER ACHIEVEMENTS.

We have in hand a draft grant application to the NIH HEAL initiative, which sponsors research targeting the improved diagnosis and/or treatment of pain, to test the ability of ig-iFU to identify tender peripheral tissue, which may arise from either or both of peripheral or central sensitization and which we believe can usefully track the efficacy of pain treatment.

## 10. REFERENCES

See above for citations to our publications.

## 11. APPENDICIES

We have attached via appendices the four papers listed above along with our interim report in which we detail our our analysis of the final data we have in hand.

Current Pain and Headache Reports (2018) 22:60
https://doi.org/10.1007/s11916-018-0711-7

NEUROMODULATION (M GOFELD, SECTION EDITOR)

# A Review of Recent Advances in Ultrasound, Placed in the Context of Pain Diagnosis and Treatment

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**Abstract**
Ultrasound plays a significant role in the diagnosis and treatment of pain, with significant literature reaching back many years, especially with regard to diagnostic ultrasound and its use for guiding needle-based delivery of drugs. Advances in ultrasound over at least the last decade have opened up new areas of inquiry and potential clinical efficacy in the context of pain diagnosis and treatment. Here we offer an overview of the recent literature associated with ultrasound and pain in order to highlight some promising frontiers at the intersection of these two subjects. We focus first on peripheral application of ultrasound, for which there is a relatively rich, though still young, literature. We then move to central application of ultrasound, for which there is little literature but much promise.

**Keywords** Ultrasound · Peripheral nerve stimulation · Pain diagnosis · Pain · Pain treatment

## Ultrasound for Peripheral Nerve Stimulation

In the early 1970s, researchers used both focused and unfocused ultrasound to evoke tactile sensation in human subjects. Gavrilov and colleagues investigated the use of stimulatory ultrasound to induce tactile, thermal, and painful sensations in the human hand [1–4]. For example, in Gavrilov 1977a, the authors studied the sensations generated by ultrasound delivered to the skin or below the skin as a function of intensity and water temperature. The piezoceramic transducers delivered ultrasound at resonant frequencies of 0.48, 0.887, and 2.67 MHz, and maximum intensities of 1300, 8000, and 30,000 W/cm$^2$. Both the subject's hand and the ultrasound transducer were submerged in warm bath water at temperatures of 30, 35, or 40 °C. Each subject described the presence or absence of sensation during ultrasound application to either their skin or the deep tissues for a duration of 1 ms, followed by 10 and 100 ms. Stimulation stopped immediately after the test subjects reported pain associated with ultrasound application. At lowest intensity values of ultrasound—the "threshold intensity"—test subjects felt a tactile sensation, described by them as a "local touch," a "slightly sensed stroke," or a "slight push." Interestingly, the threshold intensity for induction of a tactile sensation increased as the frequency of ultrasound increased. Also, the threshold intensity increased with movement of the focal region within the skin layer from the fingers to forearm. At larger intensity values, the sensation felt by subjects involved modification of temperature sensing. Specifically, in a way that varied between test subjects, ultrasound applied directly to the skin induced sensations of warmth and cold, sensations that disappeared when ultrasound of the same intensity focused below the skin layer. As was the case for tactile sensations, threshold intensity values for ultrasound-induced temperature sensations increased as its delivery point moved from the finger to the forearm. Moreover, depending on water temperature, stimulation of a given sensitive spot generally created a cold sensation (at 30 °C) or warm sensation (at 40 °C). As a means of studying the biophysical processes that lay behind these observations, the authors calculated the particle velocity, sound pressure, displacement, and temperature of tissue induced by ultrasound and

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correlated those calculations with the observed threshold intensity values. All those physical parameters other than tissue displacement increased as a function of intensity. Only the displacement amplitude of tissue within the focal volume of ultrasound application at its threshold value was independent of frequency [1]. Therefore, biophysical process of a mechanical nature plays a significant role, at least, in the stimulation of peripheral tissue.

Others, motivated by Gavrilov and colleagues, have explored many of the concepts implicit or explicit in their work. For example, one of the main goals of the work led by Dalecki [5] was to test the hypothesis that the tactile sensation experienced through direct exposure of tissue to ultrasound is caused by that tissue's exposure to the acoustic radiation force, itself related to the absorption by tissue of ultrasound. Experiments used an acoustically reflecting target, Corprene, set on the tissue site. This cork/rubber compound contained large amounts of trapped air so that it acted as an acoustic reflector. That reflection resulted in displacement of the Corprene, hence displacement of the underlying tissue. Importantly, the Corprene prevented the direct exposure of tissue to ultrasound yet produced local displacement of tissue without the induction of cavitation and of heat within tissue. Thresholds for tactile perception during ultrasound exposure of a portion of forearm away from bone with the Corprene target were compared to those of direct exposure of the forearm. They used ultrasound with a carrier frequency of 2.2 MHz and considered four pulse durations: 5,10, 50, and 100 ms. Across their protocol, the observed ultrasound threshold intensity values for perception of ultrasound did not depend upon the absence or presence of Corprene. This result supports the investigators' hypothesis that ultrasound-induced tactile sensations arise due to the acoustic radiation force. In addition, Dickey et al. [6] also determined threshold values for perceived sensations, using pulses of ultrasound that lasted for 0.1 s, delivering actual or sham ultrasound at a carrier frequency of 2 MHz into the fingertip pads of healthy test subjects. They observed an increase in sensitivity to ultrasound delivery as a function of increased intensity values. Of note, this increase in sensitivity to ultrasound stimulation correlated inversely with the density of peripheral nerve terminals in the fingertip pads, itself determined through use of a two-point discrimination test, consistent with the hypothesis of Gavrilov (1984) [3].

The work reviewed above used intense focused ultrasound (iFU)—ultrasound with intensities above FDA limits for diagnostic ultrasound) which has dealt with stimulating healthy tissue, in vivo and in humans. Of note, Gavrilov (1984) [3] hypothesized that clinicians could 1 day use iFU application to distinguish between healthy and neuropathic tissue, based on the qualities of the sensations evoked by ultrasound stimulation, thereby providing a noninvasive method for focally locating neural abnormalities in patients. Motivated by that hypothesis, researchers have shown it possible to elicit diagnostically relevant responses using inflammatory rat models of pain [7–9], neuropathic rat models of pain [10, 11], and patients [12, 13]. In all these papers, the authors found a lower intensity of ultrasound was required to elicit a discernible sensation or withdrawal response after application of iFU to damaged tissue than when applied to control (healthy) tissue.

For example, McClintic et al. [8] set out to show that iFU stimulation could be used as a noninvasive, targeted test for identifying inflamed tissue. A 0.375-s pulse of 2-MHz ultrasound was applied in a randomized fashion to the hind paws of rats, one of which was inflamed; the other, not. They observed that after application of iFU, the inflamed paw withdrew at a lower threshold of ultrasound intensity 100% of the time, and that iFU threshold values were two times higher for normal paws than for inflamed paws, a statistically significant result. An acute safety study [9] found that 20 separate 0.1-s iFU applications at 1000 W/cm$^2$ spaced 10 s apart produced no observable cell damage in rats in the subcutaneous area of ultrasound application, while 30 0.1-s iFU applications at 2000 W/cm$^2$ did produce observable damage in four rats. Interestingly, Garcia et al. (2014) [7] used the same rat model of inflammation to observe a significant diurnal difference in iFU threshold values, with high specificity and sensitivity. Specifically, the thresholds for stimulation for both single pulse and multiple pulse protocols were significantly higher at night than during the day, consistent with the diurnal pattern of pain for rodents, and lending hope to the idea that iFU stimulation could track pain management through time.

Motivated by these in vivo results for inflammatory tissue, these same researchers have used image-guided iFU (ig-iFU) to study peripheral pain generators in humans with known inflammatory pain in their shoulder. Specifically, Gelhorn, Gillenwater, and Mourad (2015) [12] applied ig-iFU to candidate trouble spots in the shoulders of patients with rotary cuff tendinosis. They used a Sonosite ultrasound imaging device, coupled with a 2.0-MHz iFU transducer to deliver iFU in individual pulses of length 0.1 s with escalating intensity values until either the test subject gave definitive reports of sensation induction or the iFU device reached its maximum intensity value. These researchers identified the iFU intensity threshold values of sensation induction in rotary cuff tendons along with several other sites in each group of participants, with values significantly less than that observed for control subjects. It was determined that while neither the healthy volunteers nor osteoarthritis patients reported any sensation upon application of iFU, patients with rotary cuff tendinosis did report reliable sensation induction in their rotary cuff tendon and surrounding tissue at a spatial and temporal average intensity ($I_{SATA}$) value of 680 ± 281 W/cm$^2$.

Regarding neuropathic pain, Tych et al. (2013) [10] and McClintic et al. (2013) [11] sought to demonstrate that iFU could distinguish between diffuse neuropathic tissue and

healthy tissue. Tych et al. (2013) used an ultrasound pulse of 1.15-MHz frequency, applied for 0.2 s into the hind paws of rats, one distal to pSNL (partial sciatic nerve ligation), the other left alone. Then, the rats were observed for instantaneous paw withdrawal. If there was no withdrawal, the intensity of the ultrasound was increased in 30% increments starting at 50 W/cm$^2$ until two consecutive single paw withdrawals were observed, defining this intensity as the iFU threshold. Tych et al. (2013) observed in pSNL rats that the rat withdrew its ipsilateral 98% of the time in 59 trials without withdrawing its contralateral paw at the same iFU intensity value. An average intensity and dose of 176 ± 56 W/cm$^2$ and 37.4 ± 11 (W/cm$^2$)*s was required to see a response in pSNL. The intensity and dose of ultrasound required to elicit a response in the sham surgery rats was 217 ± 25 W/cm$^2$ and 43.4 ± 5 (W/cm$^2$)*s, respectively, while in the control rats, the ultrasound device could not elicit a response even at its maximum intensity and dose of 283 W/cm$^2$ and 56.6 (W/cm$^2$)*s, respectively. This study demonstrates that diffuse neuropathic tissue is more sensitive to iFU compared to healthy tissue.

McClintic et al. (2013) [11] sought to extend this work to focal and subcutaneous neuropathic tissue, specifically a neuroma. The authors applied a single, 2-MHz ultrasound pulse that lasted for 0.1 s to the neuroma while the rat was under light anesthesia. (They located the neuroma through anatomical markers and verified its sensitivity through use of von Frey hairs.) For control tissue, they stimulated an area of the rat's leg 1 cm away from the neuroma towards the body. Starting at low intensity values, McClintic increased the iFU intensity in 10–30% increments targeting the neuroma until they observed three reliable flicks of its ipsilateral paw. After this observation, the authors applied the same intensity of iFU to the control area to see if it would elicit a response. In 21 out of 25 tests, the iFU elicited a response after application to the neuroma but not after its application to the control area. The results of this study agree with the results found by Tych et al. (2013) that neuropathic tissue is more sensitive than healthy tissue to iFU stimulation. These results further support the hypothesis that iFU stimulation can help differentiate painful tissue (here, subcutaneous neuropathic tissue) from normal tissue.

Motivated by McClintic et al. (2013) [11], Mourad et al. (2017) [13] used this same concept of ig-iFU stimulation to assay in a preliminary way the peripheral pain generators within the residual limbs of amputee patients. The researchers had access to five 2-MHz image-guided intense focused ultrasound transducers, each with a different depth of focus (specifically, 0.4, 1.3, 2.45, 2.75, and 3.0 cm) to target the transected nerve within the residual limbs of both standard amputee patients and those who had undergone a targeted nerve innervation (TNI) procedure. They applied 0.1 s of iFU stimulation to both the severed nerve endings and the immediately proximal section of the same nerve. When time permitted, they also applied iFU to the corresponding contralateral and intact nerve. They increased the applied intensity from 16 W/cm$^2$ until a reliable intensity threshold for sensation induction was found, or else stopped at 1032 W/cm$^2$, the maximum value of intensity achieved by the device. One or two neuromas were identified using ultrasound imaging for each of the four TNI patients. For three out of four TNI patients, they found an iFU threshold stimulation value below the maximum value produced by the transducer. For all three of those successful stimulation cases, the proximal nerve had the same iFU intensity value as for the neuroma itself. For the standard amputation group, neuromas were identified in three of the seven patients. For two of these three patients, the intensity threshold was the same in the neuroma and proximal nerve, while for the third patient, iFU applied to the proximal nerve did not generate a sensation, while for the distal neuroma, it did. For the remaining four out of seven standard amputees, iFU applied to the transected nerve end also generated a discernable sensation. Of particular interest, most successful iFU stimulation tests produced phantom limb sensations; also, for only one of the 11 test subjects could the authors elicit a discernable sensation with iFU applied to the contralateral and intact nerve, and then not in a reproducible fashion. Finally, the iFU threshold values trended inversely but were not statistically significant for phantom limb pain and pain associated with the participants' residual limb in the standard amputee patients while both had a statistical inverse correlation in the TNI group.

Wright and colleagues [14] conducted a study that tested the ability of rapidly repeated application of focused ultrasound to induce temporal summation within skin, muscles, and joints. The stimulations occurred in single or sets of four pulses applied across a range of application frequency and duration with a center frequency of 1.66 MHz. They found a lower threshold for observable sensation induction when they rapidly applied multiple pulses than when they applied a single pulse, consistent with the idea that ultrasound could induce temporal summation. Expanding on this, McClintic and colleagues [8] used a rat model of chronic inflammation to compare the threshold for immediate paw withdrawal through a single burst of focused ultrasound and a series of rapidly applied bursts. They used a transducer with a center frequency of 2 MHz and a range of $I_{SATA}$ intensity values between 100 and 1622 W/cm$^2$ and found that five rapidly applied 75-ms iFU pulses spaced 75 ms apart produced withdrawal of the inflamed rat paw at lower iFU intensity values than a single 75-ms burst, consistent with the presence of temporal summation in this chronic pain model and the results of Wright and colleagues. However, the total acoustic dose and predicted heat increase were the same in the single burst and multiple burst protocols which produced paw withdrawal. This suggests that while temporal summation may allow for sensation induction at a lower iFU intensity in the multiple pulse

condition through rapid pushing of this sensitive tissue, a temperature increase alone or in addition may have generated paw withdrawal behavior in these animals (Table 1).

## Ultrasound Alone for Anesthesia and Analgesia

As noted in the introduction, there exists a rich history of the use of diagnostic ultrasound imaging to facilitate delivery of drugs that temporarily block the function of peripheral nerves in order to generate regional anesthesia. Interestingly, evidence exists pointing to the possibility that more intense ultrasound than that capable of producing a sensation, applied directly to a peripheral nerve, can transiently and safely reduce that nerve's function. Because ultrasound is noninvasive, utilizing focused ultrasound to reversibly block nerve conduction for analgesia and anesthesia therefore has considerable appeal.

Colucci et al. 2009 [15] applied ultrasound to the sciatic nerve of bullfrogs. They tried two different ultrasound frequencies (0.661 and 1.986 MHz) and two different pulse durations (1 and 10 ms) and two different application rates (10 and 20 times per second), for 30 s in duration. Ultrasound stimulation significantly reduced up to 60% of the nerves' action potential, which returned to baseline several minutes after ultrasound application. A thermacouple placed inside of the nerve recorded focal heat generated by the ultrasound, with that heat increase correlated with the observed action potential reduction. This work therefore not only demonstrated transient decrease in nerve function after application of focused ultrasound but also pointed towards at least one mechanism by which ultrasound created this effect.

Hong et al. 1991 [16] showed that focused ultrasound could reversibly block nerve conduction in humans. They applied, transcutaneously, 1-min physio-therapeutic ultrasound at a frequency of 2 MHz to the peroneal nerve of healthy test subjects at intensities of 0.5, 1.0, and 1.5 W/cm$^2$. This stimulation produced a significant reduction in compound muscle action potential (CMAP), with a 41.4% decrease at 1.0 W/cm$^2$and 44% decrease at 1.5 W/cm$^2$, but not at 0.5 W/cm$^2$. Normal CMAP production returned to baseline 5 min after ultrasound stimulation.

Going beyond *transient* reduction of nerve function, Foley and colleagues demonstrated that much higher intensity ultrasound applied to a major nerve could stop nerve function through induction of distal axon degeneration. Specifically, Foley et al. [17] monitored the motor function of the hind limbs of twelve rabbits following application of high-intensity focused ultrasound, intra-operatively, to the rabbit's sciatic nerves. (They assayed motor function intra-operatively as well, through placement of a stimulating electrode proximal to the point of high-intensity focused ultrasound (HIFU) application and observation of paw movement in response to electrical stimulation.) They used HIFU with a spatial and temporal average intensity of 1930 W/cm$^2$ delivered at 3.2 MHz, in 5-s intervals, until electrical stimulation could not induce a motor response. They observed a lack of motor response (assayed intra-operatively as above) for up to 14 days after HIFU application, consistent with associated histological examination, which showed distal axon degeneration (Table 2).

**Table 1** Summaries of representative articles in section "Ultrasound for Peripheral Nerve Stimulation"

| Article | Model | US parameters | Result/conclusion |
|---|---|---|---|
| Tych et al. (2013) [10] | In vivo partial sciatic nerve ligation (pSNL) in Sprague-Dawley rats | 1.15-MHz pulses<br>0.2 s<br>Responses at:<br>pSNL: 176 ± 56 W/cm$^2$ $I_{SATA}$<br>sham: 217 ± 25 W/cm$^2$ $I_{SATA}$<br>normal: greater than 283 W/cm$^2$ $I_{SATA}$ | Neuropathic tissue is more sensitive to stimulation by intense focused ultrasound (iFU) than control tissue. |
| McClintic et al. (2013) [11] | In vivo neuroma in paw of Sprague-Dawley rats | 2-MHz pulses<br>0.1 s<br>Response at:<br>Mechanical: 5.7 ± 2.2 g<br>iFU: 343 ± 77 W/cm$^2$ | Successful stimulation of the neuroma by intense focused ultrasound required co-localization of the neuroma and intense focused ultrasound |
| Wright et al. (2002) [14] | Distal interphalangeal joint of index finger of human | Experiment 1<br>4 pulses at 2 Hz<br>25 ms<br>50 ms<br>75 ms<br>100 ms<br>Experiment 2<br>0.5 Hz, 1 Hz, 2 Hz, 3 Hz, 4 Hz, 5 Hz<br>Constant pulse of 50 ms | Experiment 1:<br>"...a progressive decrease in pain thresholds was found with increased stimulus duration"<br>Experiment 2:<br>"Analysis of variance showed a significant interaction between tissue stimulated and pulse-train frequency." |

**Table 2** Summaries of representative articles in section "Ultrasound Alone for Anesthesia and Analgesia"

| Article | Model | US Parameters | Result/conclusion |
|---|---|---|---|
| Colucci et al. (2009) [15] | In vivo, bullfrog, sciatic nerve | 0.661 MHz<br>1.986 MHz<br>1 ms, 10 ms for 10 and 20 pulses per second, for 30 s | "Thermal mechanism of focused ultrasound can be used to block nerve conduction, either temporarily or permanently." |
| Hong et al. (1991) [16] | In vivo, human, peroneal nerve | At 0.5 W/cm$^2$, 1.0 W/cm$^2$, 1.5 W/cm$^2$ | "Ultrasonic therapy with therapeutic dosage may cause a reversible conduction block on patients with painful polyneuropathy. " |
| Foley et al. (2006) [17] | In vivo, rabbit, sciatic nerve | 1930 W/cm$^2$<br>3.2 MHz<br>5-s intervals | Conduction nerve block of all 12 sciatic nerves was achieved with average HIFU treatment time of 10.5 ± 4.9 s (mean ± SD). |

## High-Intensity Focused Ultrasound to Treat Peripheral Sources of Pain

Though ultrasound, as a means of imaging, can guide RF ablation [18], ultrasound of a different sort—HIFU—of sufficient intensity can permanently destroy tissue. Here we first review MRI-guided HIFU systems, known as MRgHIFU, that represent a highly precise means of delivering HIFU. We then move on to their peripheral and central applications to pain treatment.

### MRgHIFU Systems for Delivering High-Intensity Focused Ultrasound

The first applications of MRI-guided high-intensity focused ultrasound began in the 1990s, with feasibility studies done on tissues to assess the thermal effects of focused ultrasound for the use of minimally invasive surgery.

Important early work showed the usefulness of MRI guidance for HIFU. In 1992, Jolesz and Hynynen al. [19] treated an acoustic silicone phantom gel and a bovine muscle with high-intensity focused ultrasound using MR imaging guidance. The experiments were done with a 1.1- and a 1.5-MHz transducer. With the 1.1-MHz transducer, the real-time MR imaging was able to show physical changes in the phantom gel and the bovine muscle at the site of the focus, as well as provide a temperature reading throughout the procedure. The procedures were done at various power levels and real-time MR imaging allowed for observation of HIFU-induced temperature increases from 30 to 60 °C. The study found reversible effects of HIFU with temperatures below 60 °C, with non-reversible effects above that temperature.

In 1993, Hynynen et al [20] also studied the feasibility of HIFU application under MRI guidance, as well as the feasibility of detecting tissue necrosis induced by HIFU with MR imaging, all in real time. For their experiments, Hynynen et al. used six, co-focused 1.1-MHz ultrasound transducers, each made of MRI-compatible materials, and a GE Signa 1.5T MR imaging system for the experiments, with the transducers placed within the MR bore. The experiments sonicated greyhounds' thigh muscle while monitoring the tissue temperature rise and structural changes via MR imaging. The study found that sonications of 20 s or longer produced visible (by MR) lesions in tissue. Lesions were immediately detected and the magnitude of the change (of lesions) correlated with the duration of the sonication. Postmortem analysis of the tissue found that the size and shape of lesions correlated with the MR images. The study showed that MR systems can be used to monitor HIFU therapy as well as give real-time feedback on the dimension and location of targeted volumes.

#### Advantages of MRI Guidance Over Ultrasound Guidance

MR imaging is more advantageous than ultrasound imaging [21] for guidance of therapy using HIFU because MRI provides more information. MR imaging provides high-resolution anatomical imaging as well as thermal mapping with uninterrupted feedback during therapy [20]. Thermal mapping of the tissue is done directly from MR images, providing information about the thermal diffusion within the tissue, which ultimately defines the length of the HIFU therapy [20, 22]. Unlike other imaging methods, MR imaging provides excellent resolution of soft tissues, such as brain, joints, and spine. MRI guidance also allows for imaging from different planes (axial, sagittal, coronal, and oblique) without repositioning the subject. This is very important and beneficial during therapy for optimal targeting and monitoring of surrounding tissues. In instances of transcranial focused ultrasound surgery (FUS) therapy, MR imaging provides real-time feedback of location of focus of the transducer within the brain.

This is all in contrast to ultrasound imaging, with its lower resolution, significant operator dependency, and limited view of the tissue of interest. Moreover, diagnostic ultrasound does not currently have the ability to effectively monitor temperature [21].

### Systems Available from Manufacturers

Currently, there are four MRIgHIFU systems available: the Sonalleve, the TULSA-PRO, the ExAblate O.R., and the ExAblate Neuro. The first three target the periphery while the fourth specializes in the brain.

The Sonalleve system was developed by Phillips to treat uterine fibroids and palliative pain of bone metastases. Philips sold the system to Profound Medical in June, 2017. The Sonalleve system includes an MR system with HIFU transducer built for noninvasive ablation. The HIFU transducer system includes a water cooling system to keep the patient's skin temperature constant, and the system is embedded into the MR table. The MR system provides 3D images for planning and real-time feedback of tissue temperature during HIFU therapy [23].

Profound Medical has an additional MRIgFU system, TULSA-PRO, for the treatment of prostate cancer and ablation of prostate tumors. The system incorporates a robotic therapeutic ultrasound transducer that provides ablation of prostate tumors. The MR system provides real-time feedback of temperature of the target volume as well as the surrounding tissues. The system also provides tissue cooling through the rectum and urethra for the protection of tissues surrounding the volume target [24].

Another MRIgHIFU system that was developed for treatment of uterine fibrosis and palliative pain of bone metastases is the ExAblate O.R., created through a collaboration between GE and Insightec. This system includes an MR system and a table-embedded HIFU transducer for ablation therapy. Similar to other systems, the MR system provides 3D images for therapy planning and tissue temperature monitoring during therapy.

## Ablation of Peripheral Tissue by Ultrasound to Ameliorate Bone Pain Due to Cancer

Several researchers have explored tissue ablation with HIFU to treat pain, especially bone pain [25]. In all cases, they used MRI-guided high-intensity focused ultrasound. Osteoid osteoma, for example, is a type of bone cancer that produces significant pain often along the cortical long bones. Current conservative treatments include aspirin, also known as acetylsalicylic acid (ASA) or nonsteroidal anti-inflammatory drugs (NSAIDs). Surgical interventions include surgical en bloc resection or curettage. The success of surgery for reducing or removing the cancer pain (88–100%) comes with the price of increased complication rates (35%) relative to conservative treatment [26]. HIFU introduces a noninvasive alternative to surgical treatment of this pain, one that may spare adjacent anatomical structures [26].

Hurwitz et al. [27] tested the ability of HIFU ablation of painful, generally metastasized cancer to reduce that pain. Here, patients with metastatic growths emanating from cancers such as breast, kidney, lung, and prostate were evaluated prior to treatment using MRI to determine the size and locations of the area designated for HIFU treatment. Pain levels were also recorded using a numerical scale to quantify pain experienced by the patient [28]. After establishing those baseline values, patients underwent 2–4-h (including 83 ± 43 min for sonication) MRI-guided focused ultrasound treatment and were evaluated for pain in the treated tissue using the Numeric Rating Scale (NRS) and the Brief Pain Inventory (BPI-QoL). The results of their study conveyed a statistical significance ($p < 0.05$) in both measures when patients receiving the ablation treatment were compared to the sham cohort. Although there were 51 reported adverse events in the cohort ($n = 112$) that received focused ultrasound, more than half of them were resolved on the day they arose.

Napoli et al. (2017) [29] found similar results in a clinical trial: a decrease in pain after application of HIFU therapy to painful but benign bone tumors. They studied 29 patients who were diagnosed with osteoid osteoma and treated with MRgFUS therapy. Twenty-seven of the 29 patients reported an absence of pain without consuming any analgesics following the treatment. Their recorded pain score on the visual analog scale (VAS) significantly decreased from a 7.9 baseline to a score of 0.7 up to 24 months after treatment [28]. Encouragingly, they did not observe any complications associated with the treatment. These results support using HIFU as a precise and minimally invasive method to ablate, hence treat pain caused by bone cancer. Similarly, Li et al. (2010) [30] conducted a study on patients with malignant bone tumors. They observed HIFU therapy to successfully ablate the tumor such that the patients experienced a reduction in their pain. In this study, 25 patients with malignant bone tumors received HIFU treatment. Of the 25 patients, 24 of them experienced pain from their bone tumors before treatment. In order to measure the pain experienced, Li et al. (2010) used a verbal scale from 0 to 3 to rate pain: 0 indicating no pain, 1 indicating mild pain, 2 indicating moderate pain, and 3 indicating severe pain. The pain scores in the patients decreased from 1.84 ± 0.85 before HIFU therapy to 0.12 ± 0.33 after HIFU therapy. In the 24 patients experiencing pain, 21 of them (87.5%) were completely relieved of pain.

Yarmolenko PS et al. [31] and Sharma et al. [32] also explored the feasibility and safety of using MRI-guided HIFU to ablate osteoid osteoma. Nine patients under the age of 25, resistant to medical treatment, with lesions targetable with MR-HIFU were selected to participate in this study. Five days prior to treatment, both patient groups of MR-HIFU had evaluations of VAS recorded. For MR-HIFU patients, they were put under general anesthesia with a Sonalleve V2 HIFU system paired with an Achieva 1.5T MR scanner. Temperature scans of low-power sonications sufficient to induce a temporary rise in tissue temperature without causing ablation were

applied prior to actual HIFU ablation to guide the HIFU treatment. Procedure time lasted, on average, 128 min with varying acoustic power and sonication duration dependent upon variables such as overlying bone thickness and osteoid osteoma characteristic.

The patients were interviewed several times (at 1, 7, 14, and 28 days post-treatment) to determine their VAS pain score and pain medication use. Patient median VAS score decreased from an average value of 6 down to 0 ($P = 0.0002$). Moreover, after treatment, eight out of nine patients stopped using NSAID. Finally, the patients experienced statistically significant increase in their sleep quality after HIFU treatment.

Taken together, these results and others' [33–35] point to the ability of high-intensity focused ultrasound, under MRI guidance, to ablate boney lesions in a way that reduced their associated pain (Table 3).

## (Towards) Use of Focused Ultrasound Applied to the *Brain* to Treat Pain

The skull represents a considerable barrier to ultrasound propagation into the brain, the major problem for those seeking to use focused ultrasound to treat portions of the brain associated with the experience of pain. Ultrasound not only attenuates in amplitude through absorption by the bone but also reflects and scatters the incident ultrasound as well as converts it from pressure to shear waves. These factors, as well as the variable thickness of the skull throughout its circumference, including inter-patient variability, while in play for diagnostic ultrasound, pose a quite significant problem for intense ultrasound, which puts the skull at risk for significant heating [36]. Current approaches alter the phase of the incident ultrasound based upon either CT scan-based mathematical modeling of ultrasound propagation or measurements of brain-tissue displacement caused by low levels of the acoustic radiation force [37]. In this way, intentional and designed initial defocusing of the ultrasound external to the skull yields a focused beam of ultrasound within the brain.

As mentioned above, GE and Insight created the ExAblate O.R., with its table-embedded HIFU transducer for ablation therapy. To treat brain, they modified this system to create the ExAblate Neuro, an MRIgHIFU system built to deliver HIFU across the skull. The system includes a novel HIFU system that incorporates one of the ultrasound focusing paradigms just discussed. It also has a helmet to help cool the patient's head. Specifically, it includes a water cooling system that keeps the patient's skin and skull cool, while the transducer's 650 kHz, 1024-element phased array provides precise targeting and treatment of the selected volume [38].

Thanks to the ability of MR to detect small changes in temperature, users of the ExAblate Neuro can refine the position of the ultrasound focus via measurement of the location of a sub-ablation thermal spot induced by HIFU. Once the clinicians have confirmation of the desired location of the HIFU focus, they then increase the acoustic power in a stepwise fashion until the tissue reaches the therapeutic target temperature (52–59 °C). To ensure the safety of patients, sonication temperatures are monitored throughout the procedure with MR thermometry while the patients are fully responsive, awake, and questioned repeatedly to avoid adverse effects.

### Ablation of the *Brain* to Treat Pain

The ExAblate Neuro system has had its most extensive tests applied to the treatment of essential tremor due to Parkinson's disease (PD), with one application to pain treatment. Since MRgHIFU treatment of essential tremor (ET) and pain each require ablation of brain tissue, we include essential tremor here to provide to the readers a sense of the range of HIFU

**Table 3** Summaries of representative articles in sections "MRgHIFU Systems for Delivering High-Intensity Focused Ultrasound" and "Ablation of Peripheral Tissue by Ultrasound to Ameliorate Bone Pain Due To Cancer"

| Article | Model | US parameters | Result/conclusion |
|---|---|---|---|
| Liberman et al. 2009 [33] | In vivo, bone metastases | - Avg. time: 66 min (range 22–162 min)<br>- Avg. sonications: 17.3 (range 8–32) | - VAS score reduction<br>- Edema at target area<br>- No lasting damage, some calcification of target area |
| Hurwitz et al. 2016 [27] | In vivo, bone metastases | - Avg. sonication time: 83 ± 43 min<br>- Max 65–85 °C | - NRS score reduction, $p < 0.001$ |
| Napoli et al. 2017 [29] | In vivo, human Osteoid osteomas | - 4 ± 1.8 sonications | - VAS score reduction ($p = 0.001$)<br>- 27/29 patients had pain absence and no intake of NSAIDs |
| Li et a. 2010 [30] | In vivo, human Osteosarcoma, malignant fibrous histiocytoma | - 70 to 169 W/cm$^2$<br>- Scanning speed = 1–3 mm/s<br>- Avg. sessions = 2.29 h | - Pain reduction of $p < 0.05$<br>- PET-CT revealed no abnormal radioactivity concentration in tumor areas. |

parameters necessary to ablate brain tissue associated with pain.

Chang et al. [39] studied the efficacy of using unilateral magnetic resonance-guided ultrasound thalamotomy for essential tremor treatment. Following treatment, patients were assessed on tremor severity and functional impairment using a critical scale for tremor (CRST [40]). Follow-ups occurred at 1 week, 1 month, 3 months, and 6 months after treatment. Eight out of the 11 patients that could be considered for analysis showed significant improvement in parts A, B, and C of the CRST.

Magara et al. [41] demonstrated that the use of MRgFUS can provide similar improvements to these patients compared to radiofrequency pallidothalamic tractotomy. Thirteen patients (range 37 to 82 years) with therapy-resistant PD were approved for MRgFUS treatment, divided into two cohorts. Group 1 (patients 1–4) received a single application of peak temperature while group 2 (patients 5–13) received applications of peak temperature four to five times. After treatments, follow-ups were held at 2 days and 3 months. At the 3-month follow-up post-treatment, group 1 showed a mean UPDRS (unified Parkinson's disease rating scale [42]) reduction of 7.6% while group 2 showed a mean reduction of 60.9%.

Chang et al. [39] observed comparable results in a prospective study of eleven patients' essential tremor, 8/11 of which received sufficient HIFU to ablate tissue, and all experiencing immediate reduction in tremor-related symptoms out to 6 months—the end of the study period. Interestingly and in contrast to some other studies, the MRI manifestation of the HIFU lesions disappeared 3 months after HIFU application. Chazen et al. [43] observed similar clinical results for MRgFUS treatment of essential tremors as well as demonstrated the ability of diffusion tensor imaging to target ablation sites for MRgFUS. Post-operative imaging of their four patients showed a lesion at the desired location and physician evaluation demonstrated a significant improvement of symptoms.

For patients with chronic and therapy-resistant neuropathic pain, there are few options to reduce their pain. In response to this, a team in Switzerland [44] recruited nine patients (aged 45 to 75) for a selective central lateral thalamotomy. Before treatment with MRgHIFU began, they confirmed the location of the HIFU focus with low sonications with 10 to 20-s durations applied to temperatures of 39 to 42 °C, below the threshold for ablation, but are visible on the MR thermometry to confirm an accurate focus point. After achieving confirmation, the authors gradually increased the HIFU power in order to achieve peak temperature of 53–60 °C with continuous wave sonication at individual durations of 10 to 20 s. During the procedure, patients experienced a variety of effects such as temporary pain relief, vestibular feelings, and dysesthesias. The treatment created lesions of 3 to 5 mm in length, with pain relief ranged from 30 to 100% relative to their baseline scores. In addition, they did not observe any adverse effects. These preliminary findings indicate that MRgHIFU could be a safe and reliably precise noninvasive option for neurosurgery interventions [44].

Three years later, Jeanmonod et al. [45] achieved similar results with central lateral thalamotomy, which they performed on 12 patients with chronic therapy-resistant neuropathic pain. Prior to treatment, they applied several low-power sonications of 10 to 20 s, achieving MRI-detected temperatures of 39 to 42 °C, thereby confirming the location of the focus relative to the anatomical target. After this step, they began therapy, continuing until they observed a temperature range of 51–64 °C at the HIFU focus. The mean VAS score of the preoperative patients was 59.5/100 with "significant" relief in pain (mean = 55%) after the procedure. Post-operative follow-ups occurred with patients at the end of the procedure, 2 days, 3 months, and 1 year. In all instances of follow-up, the VAS score was significantly decreased relative to baseline, with a mean score of 35.3/100 on year post-operation. In addition, five out of eight patients from the study did not use drugs to combat pain at the 1-year mark, while all did before treatment (Table 4).

## Towards Modulating Brain Function with Ultrasound to Treat Pain

In this section, we discuss observations that show that ultrasound can activate or deactivate the brain in a temporary and non-destructive fashion. As surely this audience knows, pain is a personal experience that requires brain function. Therefore—and speaking speculatively at this point—deactivation of relevant brain regions by ultrasound, or activation of a portion of the brain that inhibits downstream brain function, could have direct application to reducing a person's experience of pain. In anticipation of future studies that explore these possibilities, we review here recent observations of ultrasound's temporary effect on brain function.

Ultrasound-facilitated modulation of brain function (UNMOD) has experienced a resurgence since the early work in the 1950s performed by the Fry brothers [46] who, for example, observed that ultrasound delivered to the visual cortex of anesthetized cats could temporary deactivate it for 30 min. Leading this resurgence, Tyler and colleagues [47] directly measured neuron activation by ultrasound through placement of an electrode within the hippocampus of a slice of mouse brain, itself within the field of largely unfocused ultrasound at delivered at 500 kHz. Next, through the use of transcranial pulsed ultrasound that encompassed the majority of mouse brain, they induced generally bilateral peripheral motor activity such as tail and paw flicks and whisker movements [48]. King et al. [49] and Ye, Brown, and Pauly [50] produced comparable behavioral results, including with a wider exploration of neuromodulatory frequency. Younan et

**Table 4** Summaries of representative articles in section "Ablation of the Brain to Treat Pain"

| Article | Model | US parameters | Result/conclusion |
|---|---|---|---|
| Magara et al. 2012 [41] | In vivo, human<br>Pallidothalamic tract | 710 kHz<br>Mean application time: 13 s (range 10–21)<br>Peak temp: 52–59 °C | Group 1: avg. lesion of 83 $mm^3$ with disappearance at 3 months<br>Group 2: avg. lesion of 172 $mm^3$ with maintained visualization at 3 months |
| Chazen et al. 2017 [43] | In vivo, human<br>Thalamotomy | 650 kHz<br>mean application time: 10–20 s<br>Peak temp: 55–62 °C | Reduction in contralateral intention tremor |
| Chang et al. 2014 [39] | In vivo, human<br>Thalamotomy | 650 kHz<br>Mean application time: 10–20s<br>Peak temp: 55–62 °C | Immediate and sustained improvements in tremors<br>Lesion of thalamic nucleus |
| Martin et al. 2009 [44] | In vivo, human<br>Central lateral thalamotomy (CLT) | 650 kHz<br>Mean application time: 10–20 s<br>Peak temp: 53–60 °C | 3–5-mm lesion in 48-h post-op MRI<br>Long-term (~3.75 years) pain relief between 50 and 100% in 53% of patients |
| Jeanmonod et al. 2012 [45] | In vivo, human<br>Central lateral thalamotomy (CLT) | 650 kHz<br>Mean application time: 10–20 s<br>Peak temp: 51–64 °C | Lesions of 3–4 mm (d)<br>Mean post-op VAS score reduction of 42.3 and 40.7% at 3 months and 1 year respectively |

al. [51] produced similar observations coupled with a detailed numerical study of the ultrasound patterns within rodent brain. Moore et al. [52] demonstrated that ultrasound-induced EEG signals have temporal structure consistent with known activity in pyramidal neurons, as shown by comparison with their observed optogenetic stimulation.

To further increase the anatomical specificity of UNMOD, Tufail and colleagues [48] used an ultrasound collimator to produce directly measured action potentials generated within one hemisphere of the intact mouse brain. Yoo et al. [53] used a pulsed, 690-kHz focused ultrasound protocol on anesthetized rabbits, showing via functional magnetic resonance imaging (fMRI), electromyography (EMG), and gross observation that ultrasound delivered to one side of the brain induced observable brain function on the contralateral side, with focal volumes smaller than a hemisphere of brain. For their second study [54], the authors used a 350-kHz focused ultrasound protocol to stimulate at least a cranial nerve associated with control of an eye of an anesthetized rat, with motion induced ipsilateral to the stimulation zone. Kim et al. [55] showed with PET imaging that, among several results, the focal volume of the brain activated by 500-kHz ultrasound measures much smaller (given by a contour defined by the 90% of the peak value of pressure in the focus) than the focal volume of ultrasound defined physically (given by a contour defined by 50% of the peak value of pressure). This same group demonstrated in a rat model significant reduction of visually evoked potentials after application of ultrasound with one set of parameters (focused ultrasound with a 350-kHz carrier frequency, pulse repetition frequency of 100 Hz, spatial-peak pulse-average acoustic intensity of 3 $W/cm^2$), with a slight enhancement of visually evoked potentials after application of ultrasound with slightly greater intensity and dose. They were motivated by earlier work [53] that demonstrated, in rabbits, UNMOD's ability to reduce brain activity generated by light exposure and monitored by functional magnetic resonance imaging.

Mehic et al. [56] further demonstrated increased anatomical specificity of ultrasound stimulation of rodent brain, including large variations in motor response caused by small, lateral displacements of the ultrasound target—on the order of a millimeter. Kamimura et al. [57] achieved similar results as well as observed transient eye movement induced by ultrasound. Moreover, through the use of vibroacoustography at high frequencies (2.25 MHz and 1.75 MHz), Mehic et al. [56] were able to introduce low-frequency ultrasound (500 kHz, like many studies quoted above) into a smaller volume of the brain than that amenable to typical low-frequency ultrasound transducers. Finally, Yu et al. (2016) [58] used sophisticated analysis of signals derived from dense EEG arrays to map the propagation of brain activity induced by transcranially delivered ultrasound, from its point of application to other portions of the brain.

Several groups have demonstrated successful UNMOD in larger animals—sheep and non-human primates (NHPs). For example, Lee et al. [59] applied transcranial focused ultrasound to sensorimotor and visual areas of the brains of sheep and measured EMG signals in the hind legs elicited by ultrasound application to the brain, as well as visually evoked potentials. Also, Tanter and colleagues [60] altered the saccade patterns of awake NHPs through use of transcranial delivery of focused ultrasound with a carrier frequency of 320 kHz simultaneously with measurements of neural activity [61].

**Table 5** Summaries of representative articles in section "Towards Modulating Brain Function with Ultrasound to Treat Pain"

| Article | Model | US parameters | Result/conclusion |
|---|---|---|---|
| Kamimura et al 2016 [57] | In vivo<br>Mice strain C57BL-6 | 1.9 MHz<br>Pulse rep freq of 1 kHz<br>50% duty cycle (950 pulses)<br>On 1 s, off 1 s, ten times | Muscle movement at 1.9 MHz<br>Pupil dilation at 1.2 MPa<br>Pupil dilation at > 1.8 MPa |
| Airan et al 2017 [66] | In vivo<br>Fischer 344 rats | 1 MHz<br>0.5-Hz bursts for 2 min | Decrease in EEG at 1.0 and 1.5 MPA |
| Tufail et al 2010 [48] | In vivo<br>Anesthetized mice | Pulses between 80 and 225 acoustic cycles per pulse of 0.16–0.57 ms<br>Pulse repetition frequencies between 1.2 and 3.0 kHzSpatial-peak temporal-average intensities ($I_{SPTA}$) of 21–163 mW/cm$^2$ | Motor cortex activation;<br>Tail twitches and EMG activity in the lumbo sacrocaudalis dorsalis lateralis muscle;<br>EMG response in the contralateral triceps brachii muscle |
| King et al 2013 [49] | In vivo<br>CBL-7 mice | 500 kHz<br>Bandwidth of 340 to 650 kHz<br>Ultrasound intensities from 0.01 to 79.02 W/cm$^2$ (0.03 to 1.11 MPa)<br>20 to 480 ms | Brain activation from ultrasound can occur for ultrasound frequencies between 250 and 500 kHz. |
| Mehic et al 2014 [56] | In vivo<br>C57BL/6 mice | 88 bursts of 500-kHz ultrasound<br>Length 200 μs<br>Pulse repetition frequency of 1.5 kHz in a 1-s interval $I_{SPTA}$ = 5.25 W/cm$^2$ | Production of tail movement, unilateral and bilateral movement of legs and whiskers correlated with small O (1 mm) lateral movement of iFU focus. |
| Tyler et al. 2008 [47] | Hippocampus slice cultures of mice brain | Low-frequency US (0.44–0.67 MHz)<br>Spatial-peak pulse-average intensity ($I_{SPPA}$) = 2.9 W/cm$^2$ | Brain activation at 500 kHz via EEG. |

Finally, three intrepid groups [62–64] have applied neuromodulatory ultrasound in a transcranial fashion to the somatosensory cortex of healthy test subjects. The Tyler group [62] observed significant attenuation of the amplitudes of somatosensory evoked potentials elicited concurrently by median nerve stimulation. They also observed increased performance on sensory discrimination tasks without affecting task attention or response bias [63] as well as modulation of brain dynamics [64]. Yoo and colleagues [63] also stimulated the somatosensory cortex, thereby including tactile sensations in the hands of volunteers.

Three teams of researchers have applied UNMOD with therapeutic intent as of this writing. Min et al. (2011) [65] first induced acute epileptic seizures in a rat model. They then applied ultrasound, transcranially, to these anesthetized animals, with the following parameters: a carrier frequency of 690 KHz, with 500-ms-long pulses applied 100 times per second with an acoustic intensity of 130 mW/cm$^2$. EEG monitoring demonstrated reduced seizure activity as compared to untreated controls. In another approach to epilepsy treatment, Airan et al. [66] demonstrated the use of focused ultrasound to release neuromodulatory drugs from engineered nanoparticles that stopped chemically induced seizures in a rat model. After injection of the nanoparticles IV, they applied iFU with a carrier frequency of 1 MHz, in short, repeating bursts at a frequency of 0.5 Hz, for 2 min, to the brains of these anesthetized mice. As in Min et al. (2011) [65], they observed statistically significant decreases in total EEG power in mice experiencing seizures relative to control mice. Finally, and quite evocatively, Monti et al. (2016) [67] reported, in their letter to the editor, delivery of focused ultrasound to the thalamus of a patient who was in a comatose state for 19 days after TBI. The patient showed remarkable recovery from this state over a period of 3 days, with immediately observable clinical improvement. Their ultrasound had a 650-kHz carrier frequency, an estimated in situ intensity of 0.72 W/cm$^2$, and pulse lengths of 0.5 ms delivered 100 times per second for 30 s, with a 30 s pause, repeated ten times. This has some similarity to the results of Yoo et al. (2011) [68] who applied UNMOD to the thalamus of anesthetized rats, thereby reducing the time required for them to evince voluntary movement as they recovered from ketamine/xylazine anesthesia (Table 5).

## Summary and Conclusion

Here we have reviewed a range of literature that highlights the potential for ultrasound to address painful conditions beyond its current clinical use for image-guiding injections.

We began by reviewing the literature relevant to the use of ultrasound to stimulate tactile touch. This work was pioneered by Gavrilov [1–4], starting in 1974 and

continuing for more than a decade. The work by Dalecki's group [5] provided evidence to support Gavrilov's hypothesis that the tactile sensation arises due to tissue's exposure to the acoustic radiation force, the transfer of momentum from the sound field to tissue medium. We next reviewed research related to the use of ultrasound to diagnose tissue abnormalities, again as hypothesized by Gavrilov. These include identifying inflamed or neuropathic tissue in rodent models [7–9] and in humans [6, 12, 13].

In addition to this novel use of ultrasound for diagnostic purposes, more intense ultrasound can induce local anesthesia or analgesia affects. The nerve-blocking ability of ultrasound is associated with its ability to produce heat, as shown in Colucci et al. 2009 [15], in vivo. In humans, Hong et al. 1991 demonstrated temporary, ultrasound-induced reduction of peripheral nerve conduction.

A further increase in ultrasound intensity can ablate peripheral tissue, demonstrated to reduce bone pain associated with metastatic cancer. Hurwitz et al. 2014 [27] used MRI-guided HIFU to ablate the cancerous growths in a manner that reduced these patients' pain. Similar results were observed by Napoli et al. (2017) [29], Sharma et al. 2017 [32], and Yarmolenko PS et al. 2018 [31] for osteoid osteoma.

Pain can also be treated using focused ultrasound applied transcranially to the brain via ablative procedures [44, 45], a little studied but promising approach. Finally, transcranial ultrasound with significantly reduced intensity relative to ablative ultrasound can non-destructively and transiently activate as well as suppress brain function, shown in a range of animals (rodents [47–50, 52, 55–58, 65, 66, 68–70], rabbits [53], sheep [59], non-human primates [60, 61]) and modulated brain function in people [62–64, 67]. Perhaps, 1 day, neuromodulatory ultrasound can ameliorate the patient's pain, at least temporarily.

In short, recent advances in ultrasound biophysics have opened up new opportunities to ameliorate the patient's pain, worthy of further study and trial.

## Compliance with Ethical Standards

**Conflict of Interest** Michael S Bobola, Lucas Chen, Chikodi Ezeokeke Katy Kuznetsova, Annamarie C. Lahti, Alik Myroniv, Nels W. Schimek, Weicheng Lou, Madison L. Selby, and Pierre D. Mourad declare no conflict of interest.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.



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OXFORD

# METHODOLOGY, MECHANISMS & TRANSLATIONAL RESEARCH SECTION

## *Original Research Article*

# Intense Focused Ultrasound Preferentially Stimulates Transected Nerves Within Residual Limbs: Pilot Study

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### Abstract

**Objective. Identifying pain generators in tissue deep in the skin can require uncomfortable, complicated, and invasive tests. We describe pilot studies testing the hypothesis that ultrasound image–guided, intense focused ultrasound (ig-iFU) can noninvasively and differentially stimulate the end of transected nerves in the residual limbs of amputee patients.**

**Design. We applied iFU to the transected nerve ending as individual pulses with a length of 0.1 seconds using a carrier frequency of 2.0 MHz. After targeting, we gradually increased the iFU intensity to reach consistent patient-reported stimulation of the transected nerve ending. We also stimulated the proximal nerve, tissue near the nerve ending, and the intact contralateral nerve. We described the resulting sensations and correlated the results of the study participant's pre-iFU study responses to phantom and residual limb pain questionnaires.**

**Results. iFU spatial and temporal average intensity values between 16 W/cm$^2$ and 433 W/cm$^2$ that were applied to the transected nerve ending and proximal nerve elicited sensations, including phantom limb sensations, while the same intensity applied to control tissue centimeters away from the nerve ending, or to the intact nerve on the contralateral limb, did not. Two out of 11 study participants reported only mild and transient pain created by iFU stimulation. Successful iFU intensity values correlated with neither phantom nor residual limb pain scores.**

**Conclusions. Transected nerves had greater sensitivity to iFU stimulation than ipsilateral and contralateral control tissue, including intact nerve. These results support the view that ig-iFU may one day help physicians identify deep, tender tissue in patients who report experiencing pain.**

**Key Words. Intense Focused Ultrasound; Neuroma; Transected Nerve; Locating Deep and Tender Tissue**

### Introduction

A patient's pain may arise from readily identifiable peripheral sources via nociceptive pathways and/or amplify or occur primarily due to central sensitization. These factors can make pain diagnosis and treatment difficult. As part of a diagnostic armamentarium for either acute or chronic pain, physicians often perform evocative tests such as palpation in addition to imaging in order to try to locate, and hence identify, deep potential pain generators. Manual palpation (or its complement, anesthetizing via injection) of deep, potentially tender tissue also involves the intervening, generally superficial tissue, adding complexity to pain diagnosis. In addition, commonly

used imaging studies may identify multiple candidate pain generators or find only normal-appearing tissue at the site of sensitivity, making diagnosis and treatment of pain more difficult. For example, 50% to 80% of people with amputations experience pain that arises through a combination of peripheral sources such as neuromas and peripheral and central sensitization. These significantly impact function and quality of life [1–6] and generally have ineffective diagnoses and treatments despite extensive imaging [7,8]. Neuromas after amputation occur quite commonly, appearing readily on ultrasound and magnetic resonance (MR) imaging. Determining whether a neuroma actually contributes to a patient's pain, however, requires stimulating the neuroma to see if stimulation reproduces the experienced pain; problematic for deep neuromas and currently impossible without stimulating intervening tissue that may also contribute to the patient's pain.

Therefore, a noninvasive means of stimulating small, deep, and potentially tender tissue could significantly improve the ability of a clinician to locate painful tissue, a step toward identifying or ruling out the existence of pathology at the site of tenderness.

The use of intense focused ultrasound (iFU) under image guidance may represent a viable method for identifying deep painful tissue. Our previous work shows that iFU can elicit differential responses to stimulation in diffusely inflamed and neuropathic rat paws [9–12]. In addition, several researchers have shown that sufficiently intense iFU can generate sensations in healthy study participants when applied to superficial tissue [13–16]. Moreover, we have applied it successfully to identify focal and subcutaneous sources of shoulder pain in humans [17]. Finally, we showed that iFU could stimulate a neuroma in a rat model while the rats were lightly anesthetized, eliciting a motor response to their stimulation, while the same intensity of ultrasound applied to control tissue failed to induce a motor response [11].

Together, these results motivate the present study, which seeks to test the hypothesis that image-guided iFU can noninvasively stimulate transected nerve endings in the residual limbs of amputee patients who had undergone either standard amputation surgery or targeted nerve implantation, such that the stimulation differentiates the transected nerve ending from control tissue.

## Materials and Methods

### *Patient Population*

The Institutional Review Board of the University of Washington approved our human study. We recruited study participants from two groups of patients: those who had undergone lower limb amputation surgery using either standard techniques, where the transected nerve ending lies proximal to the end of the residual limb in soft tissue, or a targeted nerve implantation (TNI) technique [18]. Briefly, TNI consists of implanting the transected nerve ending into a secondary motor nerve point in muscle after partial surgical denervation at the time of amputation or during revision surgery.

Study participants were recruited via flyers at the Harborview Medical Center Amputation Clinic. Inclusion criteria were six or more months since lower limb amputation (transtibial, knee disarticulation, or transfemoral) and age 18 to 75 years. Exclusion criteria were current pressure ulcers, rashes, or open skin over residual limb, history of skin grafting or burns on residual limb, history of diabetes mellitus, cognitive or communication impairments that would impede participation in the testing procedures, history of muscle or nerve disease, including peripheral vascular disease, and evidence of alcohol or illicit drug use. We conducted studies on four TNI patients and seven standard amputation patients.

### *Ultrasound Device*

Our iFU system consisted of a portable commercial diagnostic ultrasound imaging machine connected to an intense focused ultrasound stimulation transducer, hence called an image-guided iFU (ig-iFU) system. Figure 1 shows a schematic of the device and of its application. We described it in detail in Gellhorn et al. (2015) [17]—so we include here only a summary description.

#### Ultrasound Device—iFU Transducers

We created five 2.0 MHz iFU transducers, each with its focus at a different depth relative to the skin surface (0.4 cm, 1.3 cm, 2.45 cm, 2.75 cm, and 3.0 cm). For a given patient, we used one transducer to deliver individual single bursts of ultrasound lasting 0.1 seconds, driven by a power amplifier controlled by two function generators. Input voltage, translated into spatial and temporal average intensity, was calibrated in advance of experiments using a hydrophone in a water tank, and it was monitored during the experiment using an oscilloscope. Full details regarding calibration can be found in Gellhorn et al. (2015) [17].

#### Ultrasound Device—Imaging System

Ultrasound image guidance was provided by a portable Sonosite M-Turbo ultrasound machine with a 13–6 MHz linear transducer whose imaging plane contained the iFU focus, as verified by an ultrasound needle hydrophone. Depths of the iFU transducers were marked on the screen of the Sonosite, such that we could see on the active ultrasound images the target of iFU stimulation.

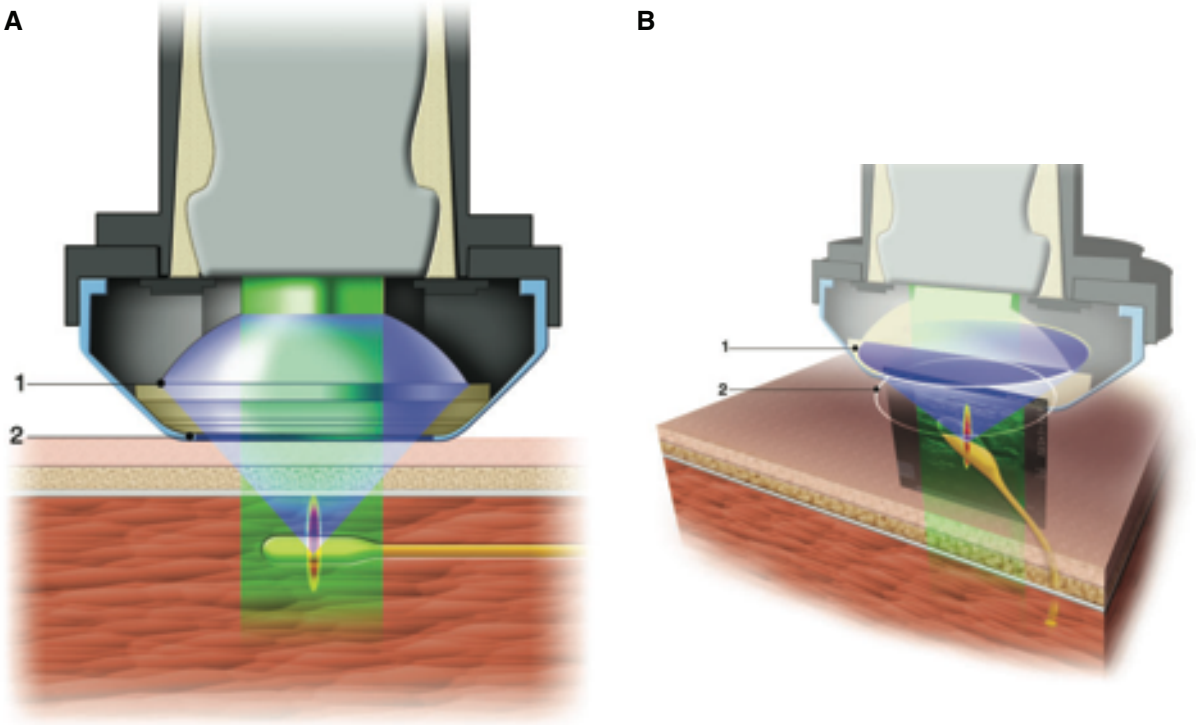


**Figure 1** **A)** Illustration of the ig-iFU device focused on a horizontal cross-section of a neuroma. **B)** Illustration of the ig-iFU device focused on a neuroma, using a three-dimensional view of the device with a sample B-mode image placed within the image.

#### Ultrasound Device—Integrated Ig-Ifu System

The imaging transducer was mounted within a custom housing that screwed onto the iFU transducers. This allowed us to image through a hole in the center of the iFU transducers, such that the imaging plane aligned directly with the iFU focus, verified in a water bath that included use of an ultrasound-sensitive needle hydrophone.

### *Study Procedures*

#### Pain Questionnaires

After successful consent, all study participants completed three pain questionnaires. The first questionnaire [19]—the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS)—yielded a composite pain score on a scale of 0 to 24, such that scores higher than 12 pointed to the likelihood of neuropathic pain during the week prior to the study. Each participant also reported the intensity of their phantom limb pain and of their pain associated with the residual limb itself as experienced over the last 24 hours (numeric pain rating scale from 0 = "no pain" to 10 = "pain as bad as you can imagine").

#### Initial Ultrasound Imaging and Manual Palpation

After a given study participant completed their questionnaires, the physician palpated areas in the distal portion of their residual limb to identify a region that contained tender tissue. Next, the sonographer imaged the study participant's residual limb using only the ultrasound imaging transducer, in order to identify anatomical structures of interest within the tender region, always the transected nerve ending, with or without an observable neuroma (Figure 2A). The location and depth of the tender sites were noted to facilitate subsequent iFU stimulation with the appropriate iFU transducer under ultrasound image guidance. With regard to the contralateral limb, the sonographer imaged the major nerve that corresponded to the target nerve in the ipsilateral limb, recording the location and depth as above. The physician did not, however, palpate the contralateral limb.

#### iFU Stimulation of Targets in the Residual Limb

After the initial exploratory imaging described above, we selected in a serial fashion the target tissue (the transected nerve ending with or without an identifiable neuroma), then assembled the ig-iFU system using the

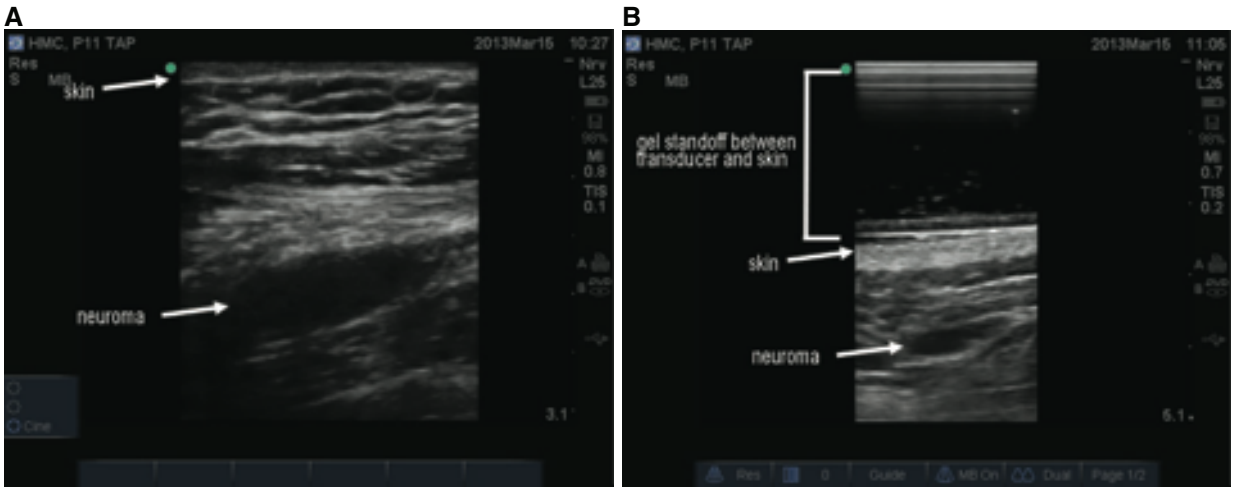


**Figure 2** **A)** Sample B-mode ultrasound image of a neuroma captured directly with the Sonosite ultrasound imaging device. **B)** Sample B-mode ultrasound image of a neuroma with the Sonosite device placed within the ig-iFU system.

iFU transducer with the appropriate depth of stimulation. Using the ig-iFU system, we then relocated the target tissue site via ultrasound imaging and aligned the focus of the iFU transducer with the target (Figure 2B). We then applied or sham-applied iFU in three to five individual 0.1-second bursts, at approximately 1 Hz, to the target tissue in a manner blinded to the study participant. We started with a low iFU intensity value (16 W/cm$^2$). After each actual or sham application, we asked the study participants if they felt any sensations associated with the application beyond that engendered by the touch of the device to the skin. If they did not feel any sensation with the iFU application, we increased the intensity and tried again. If they did indicate that they felt the stimulation, we repeated the iFU stimulation procedure using the same iFU intensity to verify the sensation. If they did not feel a sensation this second time, we increased the intensity of iFU stimulation and tried again. Once a study participant reported two consecutive sets of sensations (our definition of a "reliable" sensation) at the same intensity, we did not increase the intensity of iFU any further for that target. We define this intensity as the iFU threshold intensity value. For sites where we were unable to elicit a sensation as we raised the iFU intensity, we stopped our studies when we reached 1,032 W/cm$^2$, the maximum output of the device. We then moved the focus of the ig-iFU device to control tissue approximately 1 cm superficial to the transected nerve ending, applying actual iFU stimulation to it with the same intensity that generated a sensation when applied to the transected nerve ending or to the maximum intensity of the device, as appropriate. We then applied that same intensity of iFU to the proximal portion of nerve anatomically associated with the transected nerve ending, again asking about any sensations experienced by the patient due to iFU stimulation.

Finally, when not constrained by patient fatigue, we repeated the entire iFU threshold determination process for the corresponding nerve in the study participant's intact, contralateral limb.

## Results

Here we report our results as a summary of the individual cases for each study participant. In addition, Table 1 describes the results for each patient. Finally, in Figure 3, we present scatterplots relating the observed iFU intensity threshold value to each of the phantom limb pain and residual limb pain scores for each study participant.

### *TNI Patients*

In all four cases, we identified a neuroma and associated proximal nerve near the site of manual tenderness using diagnostic ultrasound imaging (Table 1). (In one case, we identified two neuromas and associated proximal nerves.) For three of the four study participants, iFU stimulation of their neuroma elicited a sensation (tingling to nonpainful shocks), but only once did it include sensations of the phantom limb. For those same three cases, the proximal nerve to the neuroma had the same iFU threshold–induced stimulation threshold as the neuroma. One study participant experienced mild and transient pain. For two of the four study participants, application of iFU to an intact nerve contralateral to the transected nerve ending did not elicit any sensations, including for the study participant whose neuroma was insensitive to iFU stimulation. We did not attempt to determine an iFU stimulation threshold value for the contralateral nerve in the other two study participants. One study participant had two neuromas, each with different iFU stimulation threshold values. In contrast, another study participant also had two neuromas, each insensitive to iFU stimulation.

**Table 1** Summary of Results

| | Total LANSS Pain Score (Scale 0–10) | Phantom Limb Pain (Scale 0–10) | Residual Limb Pain (Scale 0–10) | Phantom Limb Sensation due to iFU? | iFU Threshold Value for Nerve Ending (SATA W/cm$^2$) | iFU Value for Nerve Ending also Stimulated Proximal Nerve? | iFU Threshold Value for Nerve in Intact Limb (SATA W/cm$^2$) |
|---|---|---|---|---|---|---|---|
| Targeted Nerve Implantation Patients | | | | | | | |
| In the last: | Week | 24 hours | | | | | |
| P1 | 24 | 3 | 8 | No | 187 | Yes | NA |
| P2 | 5 | 0 | 0 | NA | Neither neuroma, to upper limit (1032) | No & no | None to upper limit (1032) |
| P3 | 18 | 10 | 8 | Yes | 16 & 66 | Yes & yes | None to upper limit (1032) |
| P4 | 23 | 6 | 3 | No | 16 | Yes | NA |
| Standard Amputation Patients with Neuromas | | | | | | | |
| In the last: | Week | 24 hours | | | | | |
| P5 | 14 | 4 | 3 | Yes | 433 | Yes | None to upper limit (1032) |
| P7 | 22 | 5 | 6 | Yes | 16 | Yes | NA |
| P10 | 13 | 0 | 0 | Yes | 65 | No | None to upper limit (1032) |
| Standard Amputation Patients without Neuromas | | | | | | | |
| In the last: | Week | 24 hours | | | | | |
| P6 | 15 | 2 | 5 | No | 433 | NA | NA |
| P8 | 14 | 0 | 2 | No | 64 | NA | None to upper limit (1032) |
| P9 | 0 | 0 | 0 | Yes | 258 | NA | None to upper limit (1032) |
| P11 | 18 | 4 | 4 | Yes | 16 | NA | 240 |

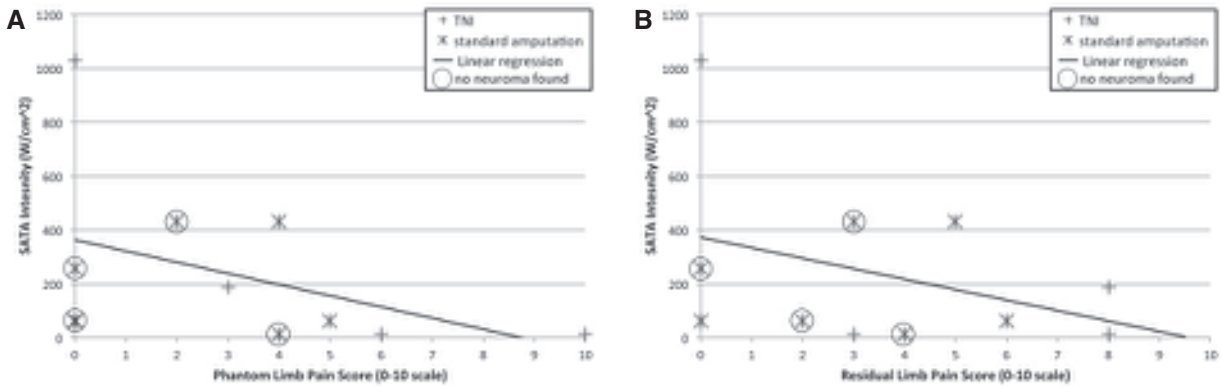


**Figure 3** **A)** iFU threshold stimulation intensity value vs pain scores, each score rated over the last 24 hours: Intensity (w/cm$^2$ SATA) vs phantom limb pain score (all data $R^2 = 0.18$). Neither inverse trend achieved statistical significance. **B)** iFU threshold stimulation intensity value versus pain scores, each score rated over the last 24 hours: Intensity (w/cm$^2$ SATA) versus residual limb pain score (all data $R^2 = 0.14$). Neither inverse trend achieved statistical significance.

*Standard Amputation Patients*

In three out of seven cases, we identified neuromas via ultrasound imaging at the site of palpable tenderness at the transected nerve ending (Table 1).

For two study participants, the neuroma and proximal nerve had the same iFU threshold stimulation value. Those values differed substantially between these patients, however. In addition, the neuroma for the third patient was sensitive to iFU stimulation while the proximal nerve was not at the same iFU threshold value. In all of these cases, iFU stimulation generated phantom limb sensations. For two patients, we tried but failed to observe successful iFU stimulation of the intact nerve in the contralateral limb.

In the other four cases, we found only a transected nerve ending with diagnostic ultrasound imaging (Table 1). For these study participants, we determined the iFU threshold intensity value for the nerve ending itself, specifically at a point that offered an unambiguous imaging target. All four of these study participants had transected nerve endings sensitive to iFU stimulation, with a wide range of iFU stimulation intensity values. In two of the four cases, iFU stimulation generated phantom limb sensations. In addition, we sought to identify the iFU threshold intensity value for the contralateral nerve in three of these four study participants. For two out of those three study participants, iFU stimulation of the intact nerve contralateral to the transected nerve ending did not elicit any sensations. For the remaining study participant, we elicited a sensation, but only once, without successful repetition.

*Pain Scores and Their Relation to iFU Stimulation Intensity Value*

The LANSS composite pain score measures the overall neuropathic pain level experienced by each study participant. All except two participants had LANSS scores above 12, indicating the likely presence of neuropathic pain in the majority (11/13) of our participants. iFU threshold stimulation intensity values for all patients trended inversely but without statistical significance for the phantom limb pain experienced by the participants over the last 24 hours ($R^2 = 0.18$, $P > 0.05$) (Figure 3A). Similarly, iFU threshold stimulation intensity values for all patients trended inversely but without statistical significance for the residual limb pain experienced by the participants over the last 24 hours ($R^2 = 0.14$, $P > 0.05$) (Figure 3A). iFU threshold stimulation intensity values for TNI study participants did have a statistically significant inverse slope as measured against each of the phantom limb and residual limb pain scores (respectively, $R^2 = 0.68$ and $R^2 = 0.55$ with $P < 0.05$; regression lines not shown). In contrast, the same analysis when applied to standard amputation study participants did not show a meaningful trend (respectively, $R^2 = 0.008$ and $R^2 = 0.003$ with $P > 0.05$; regression lines not shown).

## Discussion

In this study, we sought to determine if intense focused ultrasound (iFU) could stimulate nerve tissue deep to the skin (a transected nerve ending with or without an observable neuroma in a residual limb) and whether or not nerve tissue in residual limbs is more or less sensitive to iFU stimulation than control tissue. Our results demonstrated that sufficient iFU (the "iFU threshold intensity value") when applied to deep and focal nerve tissue generated discernable sensations in 10 out of 11 test subjects. This included generation of phantom limb sensations in six of those 10 test subjects, more often with a neuroma present (4/6) than not (2/6). Moreover, iFU stimulation of control tissue for a given patient (tissue within a centimeter of the neuroma or nerve ending that lay between the iFU source and its target, a major nerve in the contralateral limb) with the same iFU threshold

stimulation intensity value for that patient did not induce a discernable sensation. Finally, for intact, contralateral nerves, we could not identify an iFU threshold stimulation intensity value in six of seven cases (tested up to 1,032 W/cm$^2$) while we generated a sensation due to iFU stimulation only once (not twice in a row) in the remaining test subject.

As a secondary hypothesis, we anticipated that across patients the iFU threshold intensity values would scale inversely with a patient's residual limb and phantom limb pain scores. We observed only a weak and non–statistically significant inverse correlation for our entire cohort of study participants, leaving this hypothesis falsified thus far.

### *Potential Clinical Implication of iFU Stimulation*

Existing methods for characterizing painful tissue, such as manual palpation, thermodes, lasers, or Peltier devices, stimulate superficial tissue only, or superficial and deep tissue simultaneously. In contrast, iFU can stimulate focal and deep anatomical structures without stimulating the intervening tissue, a potentially useful difference in the clinic setting. When coupled with imaging, the clinician could use iFU to more readily locate and identify deep and tender tissue, a first step in the diagnosis and treatment of a patient's pain. More refined targeting via iFU could in turn motivate application of more refined diagnostic and/or imaging techniques in order to identify the presence and type of peripheral pathology at the site of tenderness. Such identification would then allow for more targeted peripheral interventions such as injections or surgery, if warranted. In the absence of identified peripheral pathology after this extra diagnostic attention, the physician may more readily move to treat potential central contributions to the patient's pain.

Our work offered here represents a first step toward testing the idea that iFU can act as a tool to identify specific peripheral tissues that may act as pain generators in amputee patients. Its use under image guidance may also track the efficacy of pain treatments. Pain management is especially problematic for patients with amputation as residual limb pain and phantom limb pain reduce the quality of life for most adults with amputation. We have shown previously [10] that iFU stimulation threshold values track thermal measures of diurnal variations in inflammatory pain in a rat model. This suggests it is possible that through the use of ig-iFU, a clinician may have the ability to track changes in a patient's pain during treatment. With this in mind, we hypothesize that an increase in iFU stimulation value over time for a given patient may indicate effective pain management, an especially useful finding as clinicians can apply iFU in a way blinded to the patient (and, we assert, blinded to the physician themselves, through design of an appropriate user interface). Interestingly, though not yet the focus of formal study, after targeted muscle reinnervation (TMR) surgery, quite similar to the TNI procedure, [20] patients appear to report less pain than standard amputee patients. Our pilot results suggest that it is possible to use ig-iFU to quantify the sensitivity of transected nerve endings that arise after standard amputation relative to the corresponding nerves at the implantation site of TMR patients, a focus of future study.

It is also possible that the utility of iFU may extend to conditions other than neuromas associated with amputation, such as chronic low back pain. It is well known that current imaging techniques identify abnormalities that often do not correlate with back pain [21] and the presence of abnormal findings on imaging can lead to ineffective or even counter-productive surgical treatments [22]. The use of iFU stimulation could allow clinicians to rule out the presence of specific peripheral pain-generating tissue and may therefore prevent unnecessary surgical interventions. Instead, iFU may help clinicians to more readily attend to central contributors to pain [23,24] rather than peripheral sources of pain. They may therefore more readily prescribe centrally acting medications, meditation, spinal cord stimulation, psychotherapy, or continued watchful waiting, among other choices [23].

## Future Research

Future studies might consider additional study of the mechanisms by which iFU stimulation may generate sensations. The choices we made of pulse duration and transducer frequency used in this study had their motivation in our existing work [12], where we applied a single pulse of iFU with a duration of 0.1 seconds to surgically create neuromas in rat legs. Other studies [13,14,16,25] have also investigated sensation induction by single pulses of iFU with a range of ultrasound frequencies (0.3–5.0 MHz) and duration (5–100 ms). By using a single short (0.1 second) pulse, we sought to activate mechanoreceptors, which the literature argues are activated upon ultrasound stimulation of that duration [14,25,26]. Future studies should consider application of individual and longer pulses, as applied to a neuropathic rat model by Tych et al. (2013) [9]. In this way, one could refine the study of the physical mechanisms by which ultrasound may generate sensations as a longer pulse may generate heat and activate thermoreceptors at the same time as mechanoreceptors. In addition, future studies might also apply multiple short pulses in rapid succession to study their potential to induce temporal summation and windup, as suggested by Wright et al. (2002) [15] and further explored by McClintic et al. (2013a) [11].

Finally, in the present, preliminary research, we observed only a weak and statistically insignificant inverse correlation between iFU stimulation intensity value and each of the residual limb pain score and phantom limb pain score for our entire cohort. Interestingly, though quite preliminarily given our patient numbers, we observed divergent results between the TNI and standard

amputee study participants. We need to perform additional studies with greater participant numbers to determine if our cohort-based results are intrinsic to iFU stimulation or simply a matter of low patient numbers.

### Limitations

One initial goal of this study was to explore the idea that targeted nerve implantation surgery could help reduce neuroma formation and therefore help to reduce amputee pain. Unfortunately, in our small cohort, all of the patients who underwent TNI surgery and participated in our study also had an observable neuroma, as did three of seven of the study participants who underwent standard amputation. We were not, therefore, able to make a meaningful comparison between tissue sensitivity associated with these different surgical procedures, a goal for future studies.

We were also not able to test the sensitivity to iFU stimulation of a major nerve in the intact contralateral limbs of all of our study participants due to patient fatigue. Future studies should consider determination of the stimulation threshold of intact nerves in healthy controls vs amputee patients.

In addition, our current studies allowed for blinding of the patient to iFU stimulation, but not the individual delivering the stimulation. We will seek to rectify this in future research through modification of our device to test the usefulness of delivering iFU stimulation in a double-blinded fashion.

Finally, as mentioned above, we observed only a weak and statistically insignificant inverse correlation between iFU stimulation intensity value and each of the residual limb pain score and phantom limb pain score for our entire cohort, although the (small) TNI subgroup showed an inverse trend. Perhaps more refined inclusion criteria, the addition of a QST study or, with more study participants, secondary stratification of the data against LANSS score or patient complaint, among other factors, would allow us to identify a subset of patients whose actual iFU threshold value provides additional diagnostic information.

### Conclusion

This study builds on our previous work that studied iFU stimulation of neuromas in a rat model [12]. Here, we have conducted a preliminary study of ig-iFU applied to the transected nerve endings of two cohorts of patients with lower extremity amputations—those who have undergone a standard amputation and those who have undergone targeted nerve implantation. We found that the transected nerves in the amputated limbs were more sensitive to iFU stimulation than both local control tissue and the corresponding major nerve in the intact limbs of the same participants. Additionally, we were able to image those targets while performing iFU application, showing successful use of iFU under image guidance.

We have therefore demonstrated the feasibility of non-invasively stimulating transected nerve endings using intense focused ultrasound under image guidance. Future work will explore the potential clinical usefulness of this new means of identifying deep, focal, and tender tissue.

### Acknowledgements

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● *Original Contribution*

# A PRE-CLINICAL STUDY OF THE RESPONSE THRESHOLD OF INTACT AND TRANSECTED NERVES TO STIMULATION BY TRANSCUTANEOUS INTENSE FOCUSED ULTRASOUND

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**Abstract—We used diagnostic ultrasound imaging to guide individual bursts (0.1 s) of 2 MHz intense focused ultrasound (iFU) to determine the sensitivity of intact and transected nerves. We found that all nerves had greater sensitivity to iFU stimulation than surrounding muscle. Intact nerves from healthy volunteers had less sensitivity to iFU stimulation (272 ± 35 W/cm$^2$ [median ± standard error]) than transected nerves (19 ± 37 W/cm$^2$). Intact, contralateral nerves of amputees dichotomized naturally into two groups—one very sensitive to iFU stimulation (6 ± 2 W/cm$^2$) and one relatively insensitive (539 ± 19 W/cm$^2$), compared with the intact nerves of healthy volunteers. Our study demonstrates the ability of iFU under ultrasound image guidance to stimulate deep, intact and transected peripheral nerves. It also highlights differences in the receptivity to ultrasound stimulation of the peripheral nerves of amputees versus healthy volunteers. (E-mail: doumitt@uw.edu)** © **2019 World Federation for Ultrasound in Medicine & Biology. All rights reserved.**

***Key Words:*** **Intense focused ultrasound, Image-guided intense focused ultrasound, Limb amputation, Nerve stimulation.**

## INTRODUCTION

In order to assay potential sources of a patient's pain, physicians often palpate potentially tender tissue as a supplement to diagnostic methods such as imaging, since imaging alone can only help identify tissue type, not link it causally to a patient's pain (Chou et al. 2012; Loeser and Cahana 2013).

In the case of a transected nerve after amputation, physicians cannot readily palpate it owing to its location, typically deep to the skin. Palpation, therefore, necessarily stimulates intervening tissue that, along with central sensitization (McMahon et al. 2013) and phantom limb pain (Flor 2002; Ketz 2008; Weinstein 1998), may also contribute to the pain experienced by an amputee (Ebrahimzadeh and Rajabi 2007; Ehde et al. 2000; Ephraim et al. 2005; Reiber et al. 2010).

Gavrilov et al. (1984, 1996 and reviewed in Bobola et al. 2018) showed that ultrasound, most likely *via* the acoustic radiation force, can induce discernable sensations when applied to a variety of tissues. Mourad et al. (2018), motivated by their earlier work in rats (McClintic et al. 2013), demonstrated the feasibility of using image-guided intense focused ultrasound (ig-iFU) to palpate the transected nerves and intact, contralateral nerves of participants with amputations. Here we extend this study to test the hypothesis that transected nerves have greater sensitivity to intense focused ultrasound (iFU) stimulation than (i) intact nerves contralateral to the amputation and (ii) intact nerves of healthy volunteers.

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## MATERIALS AND METHODS

### *Population*

The University of Washington Institutional Review Board and the military Human Research Protection Office approved this study. We recruited and consented participants aged 18 y or older from cohorts of healthy volunteers and patients who have had a major limb amputation for 6 or more mo. Exclusion criteria were as follows: presence of pressure ulcers, rashes or open skin

over the residual limb; a history of skin grafting or burns on the residual limb; a history of diabetes mellitus; and cognitive or communication impairments that might impede participation in the consenting and testing procedures.

*iFU system*

Our iFU system (Fig. 1) consisted of a commercial diagnostic ultrasound imaging machine connected to a custom-made iFU stimulation transducer. As in Gelhorn et al. (2015) and Mourad et al. (2018), the iFU transducer had an aperture diameter of 5 cm and a focal length of 4 cm. This transducer produced a focus measuring 5 mm in the axial direction and 0.7 mm in the transverse direction, as defined by the half-maximum pressure contour and calculated in MATLAB (MathWorks Inc., Natick, MA, USA) using linear, far-field acoustics. The patient interface used a soft gel standoff pad made from 10% clear ballistics gelatin (Amini et al. 2015), a non-toxic and moldable material without odor, stable across a range of temperatures and able to mimic the acoustic impedance of human tissue. This ig-iFU system, as applied to test patients with shoulder injuries (Gellhorn et al. 2015), was previously tested for feasibility as applied to amputees (Mourad et al. 2018). Refinements yielded a device capable of distributing iFU stimulation at a greater range of depths, guided by an imaging system with greater sophistication and image fidelity (a Philips EPIQ 5 [Philips Healthcare, Bothell, WA, USA] here versus a Sonosite M-Turbo [FUJIFILM Sonosite, Bothell, WA, USA]). As in Gellhorn et al. (2015) and Mourad et al. (2018), we calibrated the iFU transducers using a needle hydrophone (Onda Corp., Sunnyvale, CA, USA) in a de-gassed water tank. This produced intensity values, reported throughout this paper in terms of the median ± standard error of the spatial peak, temporal averaged value ($I_{spta}$).

*Ultrasound imaging and iFU stimulation of targets*

We first imaged the target area using diagnostic ultrasound imaging to identify the relevant nerves depending upon the amputation (always one of the median and sciatic nerves as appropriate, with tibial and peroneal nerves included when present, all subject to participant's pain and fatigue). For the healthy volunteers, we imaged at least the median and sciatic nerves, adding the tibial and peroneal nerves as above. We noted the location and depth of the nerves to facilitate subsequent iFU stimulation with the appropriate iFU transducer under ultrasound image guidance. (Of note, for transected nerves, we explicitly sought to locate the distal tip of the nerve.) Figure 2 shows an ultrasound image of an intact nerve without (Fig. 2a) and with (Fig. 2b) the gel standoff, while Figure 2c shows an ultrasound image of a transected nerve with a neuroma, imaged without the gel standoff.

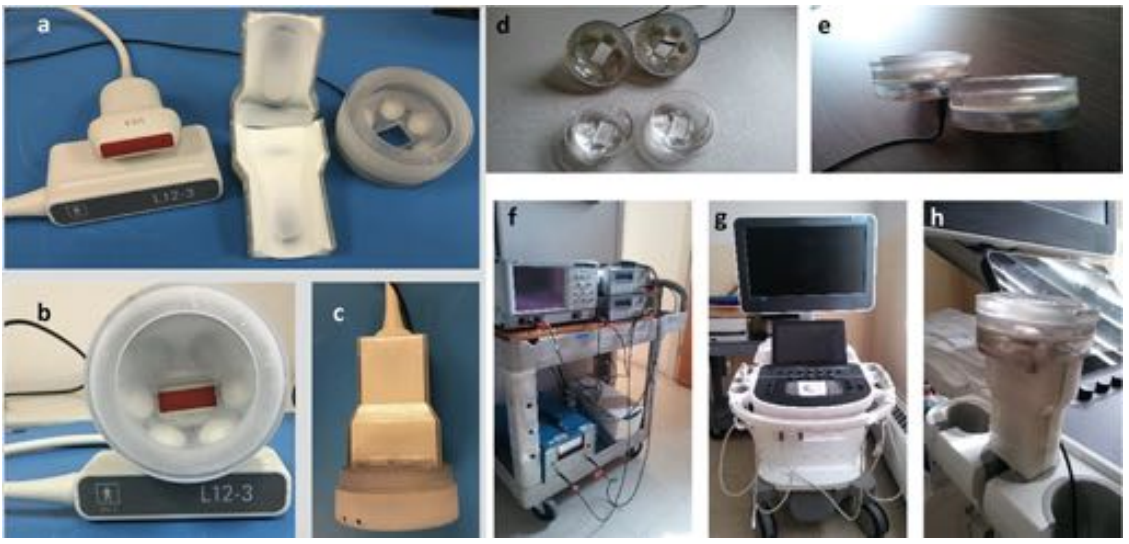


Fig. 1. Image-guided intense focused ultrasound (ig-iFU) stimulation system. (a) Phillips EPIQ L12-3 imaging probe (left) next to its housing (center) and an iFU transducer (right), whose central aperture allows transmission of diagnostic ultrasound in a manner co-located with the iFU focus**.** Assembled proximal aspect of the ig-iFU device in (b) axial view and (c) side view. (d) iFU transducer with gel standoff to control depth of stimulation. (e) Side view of iFU transducer with gel standoff placed on the transducer. (f) Electronic setup for the iFU device, including oscilloscope, function generators, amplifier and power regulator/surge protector. (g) Diagnostic ultrasound imaging machine with a completely assembled iFU transducer plus imaging probe. (h) A close up of the assembled probe for simultaneous ultrasound imaging and stimulation. iFU = intense focused ultrasound; ig-iFU = image-guided intense focused ultrasound.

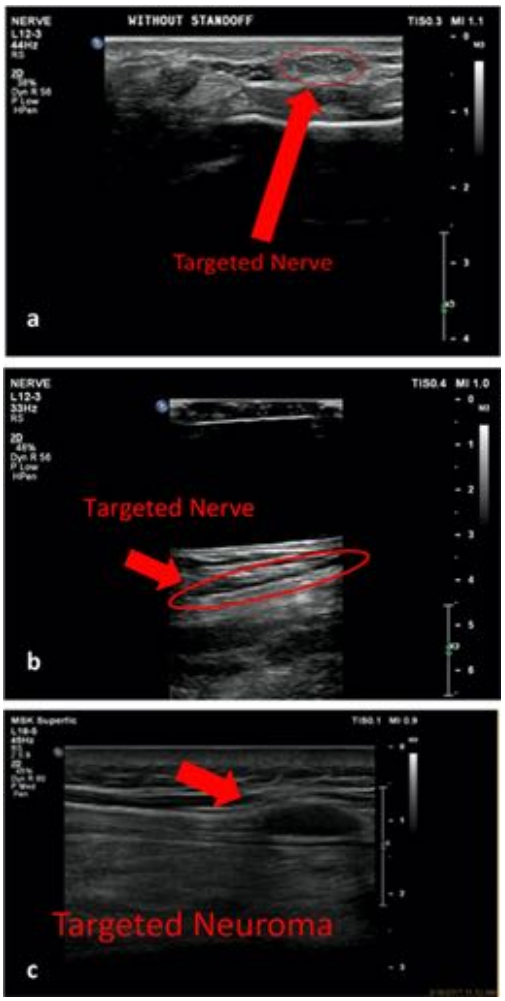


Fig. 2. Ultrasound images of nerves targeted by iFU. (a) Cross-sectional image featuring the nerve of interest using B-mode ultrasound imaging. (b) Longitudinal image of that same nerve imaged with ig-iFU system before application of iFU. (c) We have comparable images for transected nerve endings, here a peroneal nerve with a neuroma at its distal tip. iFU = intense focused ultrasound; ig-iFU = image-guided intense focused ultrasound.

After assembling our ig-iFU device, we then applied iFU in five individual 0.1-s bursts, at approximately 0.5−1 Hz, to the target nerve. If the transection occurred in a major nerve distal to the sciatic nerve, we first stimulated the sciatic nerve approximately 3−5 cm above the alar fold on the back of the knee. With sufficient time, we then applied iFU within 3 cm of the distal tip, always proximal to the neuroma, if present. We started by using a low iFU intensity value, approximately 16 W/cm$^2$. After each application of five iFU bursts, study participants reported the presence or absence of sensations either deep below the skin beneath the device or distal to the point of application and consistent with the enervation pattern of that nerve. We defined the iFU intensity threshold value (iFU_t) as the intensity at which the participant either felt a sensation as just described or the maximum intensity of the device was reached (820 W/cm$^2$). If we induced a sensation with iFU, we then stimulated the nearby muscle within the same limb at the same iFU intensity, recording whether or not iFU stimulation generated a sensation there, as a means of testing the ability of ig-iFU to locate nerves relative to surrounding muscle, consistent with Mourad et al. (2018). For amputee participants, we repeated the iFU threshold determination process for the corresponding nerve in the intact, contralateral limb. For the intact volunteers, we stimulated several major nerves, first the median nerve in the arm and the sciatic nerve in the leg then, when feasible, the peroneal and tibial nerves in the leg.

*Statistical analysis*

Our data did not conform to a normal distribution, so we chose nonparametric statistical methods throughout our analysis. Basic data analysis was performed using Excel (Office 365, Microsoft, Redmond, WA, USA), and nonparametric statistical analysis was performed using Stata 15.1 (StataCorp LLC, College Station, TX, USA). The Kruskal-Wallis equality-of-populations rank test was used to measure between-group differences. The Wilcoxon rank-sum test was used to determine if two sets of data differed from each other in a statistically significantly fashion. The Wilcoxon matched pairs signed-rank test was used to examine differences in paired data. The canonical discriminant analysis test was used to examine differences between three pairs of data.

## RESULTS

From January 2017 through March of 2018, we successfully consented and tested 46 healthy, intact volunteers and 22 participants who had amputations all from the greater Seattle area. Those amputations occurred through a balance of trauma (12 of 22) and other causes (10 of 22), such as cancer, diabetes or infection. Lower-limb amputations constituted the largest group of patients available to us (21 of 22). Of the 21 lower-limb amputations, 17 had a below-knee amputation, three had an above-knee amputation and one had a through-knee amputation. We found an iFU threshold value below 820 W/cm$^2$—the maximum intensity value of our device—for all 28 transected nerves and for 67 of 71 intact nerves across all participants (24 of 71 from amputees, 47 of 71 from intact controls). For the four patients for which we did not induce a sensation in an intact nerve, we represented that value as 820 W/cm$^2$ in our analysis.

The average depth of the nerves we stimulated measured 2.25 ± 0.5 cm. Scar tissue and edema often complicated the imaging of the distal tips of the peripheral nerves in the residual limbs of our amputee participants.

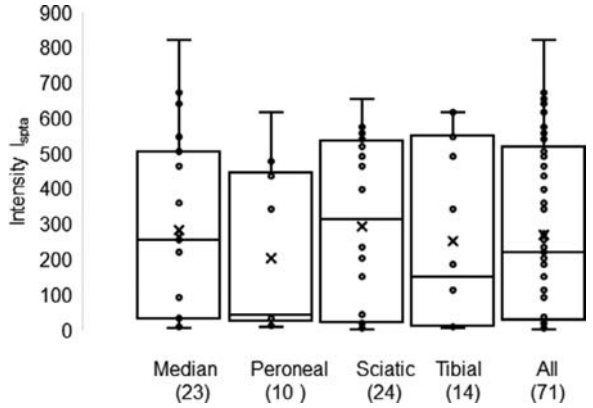


Fig. 3. Sensitivity of intact peripheral nerves to ultrasound stimulation pooled across volunteer cohorts. The intensity of ultrasound necessary to stimulate intact peripheral nerves of healthy participants and from the contralateral limb of amputees, when pooled together (N = 71), did not vary as a function of the type of nerve. ($X^2 = 4.996$, $p > 0.65$). $I_{spta}$ = spatial peak, temporal average intensity.

This resulted in the use by the sonographers of a considerable amount of time, which sometimes limited our study because of patient fatigue. We compensated for this by first choosing the sciatic nerve for stimulation after the survey of all their transected nerves because it was subject to the least amount of edema and scaring. We then moved on to other nerves ipsilateral then contralateral to the amputation as circumstances allowed.

*iFU stimulation of intact nerves*

We first examined the iFU_t required to induce a discernable sensation for all intact nerves, testing for differences between nerve type regardless of cohort source (Fig. 3 and Table 1). We did not observe any difference in the iFU_t value between the four different intact nerves, with an average iFU_t value across all nerve types and cohorts equaling 217 ± 30 W/cm$^2$. (We note, however, a trend for the peroneal and tibial nerves to have a lower iFU stimulation threshold values compared with the other nerves.) Stimulation of intact nerves produced transient sensations that test patients associated with anatomy distal to the stimulation site, sensations often described as “tingly” or “warm.”

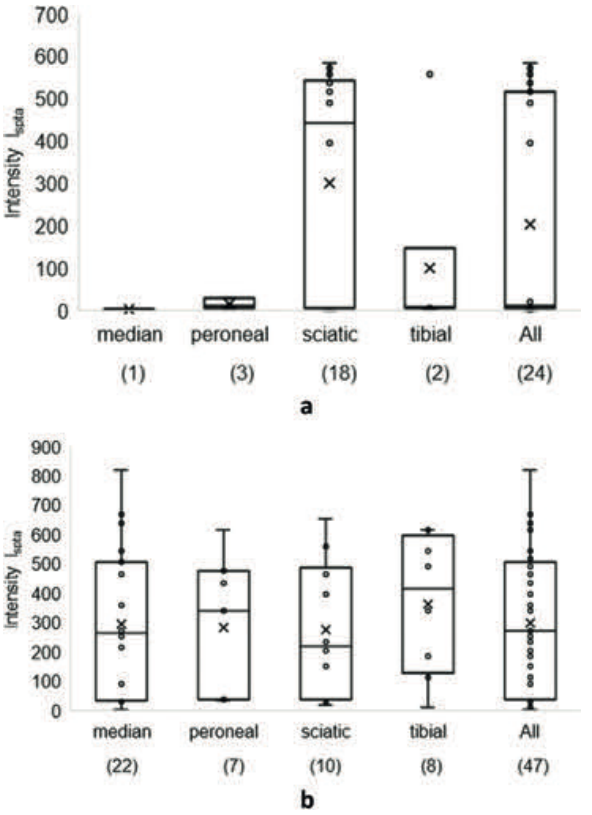


Fig. 4. Sensitivity of intact peripheral nerves to ultrasound stimulation segregated by volunteer cohort. (a) With regard to the intact nerves of amputee participants (N = 24), the intensity of ultrasound necessary to stimulate them did not vary as a function of the type of nerve, with a trend toward significance for the sciatic nerves. ($X^2 = 3.04$, $p < 0.4$). (b) With regard to the intact nerves of healthy test patients (N = 47), the intensity of ultrasound necessary to stimulate them did not vary as a function of the type of nerve. ($X^2 = 0.69$, $p > 0.87$). $I_{spta}$ = spatial peak, temporal average intensity.

We then compared the iFU_t value of the intact, contralateral nerves of participants with amputations (Fig. 4a and Table 2) with that of the intact nerves of the healthy volunteers (Fig. 4b and Table 2). We did not observe a difference in sensitivity of intact nerves within each of the cohorts as a function of nerve type, though we did observe a trend toward higher values of iFU_t for the intact sciatic nerves of amputee participants relative to the other nerves. We did find, however, a statistically

Table 1. Basic statistics for iFU intensity threshold value (iFU_t) for intact nerve types, pooled across all participants

| | All (W/cm$^2$) | Median (W/cm$^2$) | Peroneal (W/cm$^2$) | Sciatic (W/cm$^2$) | Tibial (W/cm$^2$) |
|---|---|---|---|---|---|
| Number (n) | 71 | 23 | 10 | 24 | 14 |
| Mean | 268 | 281 | 203 | 291 | 251 |
| Standard error | 30 | 54 | 75 | 51 | 70 |
| Median | 217 | 255 | 41 | 314 | 148 |
| Standard deviation | 249 | 258 | 237 | 248 | 262 |

iFU = intense focused ultrasound; iFU_t = iFU intensity threshold.

Table 2. Basic statistics for iFU intensity threshold value (iFU_t) for intact nerves of amputee and intact participants

| | All (W/cm$^2$) | Median (W/cm$^2$) | Peroneal (W/cm$^2$) | Sciatic (W/cm$^2$) | Tibial (W/cm$^2$) |
|---|---|---|---|---|---|
| **Amputee Participants** | | | | | |
| Number (n) | 24 | 1 | 3 | 18 | 2 |
| Mean | 248 | 4.5 | 15.81 | 327 | 11.7 |
| Standard error | 53 | NA | 7.4 | 59 | 0.5 |
| Median | 12 | 4.5 | 11.5 | 491 | 11.7 |
| Standard deviation | 258 | NA | 12.9 | 252 | 0.7 |
| **Intact Participants** | | | | | |
| Number (n) | 47 | 22 | 7 | 10 | 8 |
| Mean | 300 | 294 | 283 | 275 | 365 |
| Standard error | 35 | 55 | 92 | 73 | 84 |
| Median | 272 | 263 | 340 | 218 | 416 |
| Standard deviation | 240 | 257 | 243 | 231 | 237 |

iFU = intense focused ultrasound; iFU_t = iFU intensity threshold.

significant difference between the intensity of stimulation of intact nerves between our two cohorts (Fig. 5a and Table 3). Unlike for the intact volunteers, we observed, in addition, a bimodal distribution of iFU_t values for the intact, contralateral nerves of amputees. When separated out (Fig. 5b and Table 3), iFU_t values for the "sensitive," intact, contralateral nerves from the amputees had an average iFU_t value of 6 ± 2 W/cm$^2$, significantly lower than that of the healthy volunteers (272 ± 35 W/cm$^2$). Meanwhile, the iFU_t value for the remainder equaled 539 ±19 W/cm$^2$, significantly higher than that of the healthy volunteers as well as the sensitive, intact nerves from participants with amputation.

*iFU stimulation of transected nerves*

We stimulated 28 transected nerves with iFU (Fig. 6 and Table 4). In all patients, stimulation of these transected nerves produced comparable sensations to that generated by iFU when applied to the intact nerves of healthy test patients, including "tingly" and "warm" sensations associated with the phantom limb. We observed lower values of iFU_t, on average, for the transected nerves (18 ± 37 W/cm$^2$) relative to the intact nerves of healthy volunteers (272 ± 35 W/cm$^2$) (Fig. 7 and Table 5).

Figure 8 allows a view of the distribution of the results shown in Figure 7, through use of a Kaplan-Meier plot. Of note, the majority of the transected nerves of the amputee volunteers had iFU_t values lower than the majority of iFU_t values for the intact nerves of healthy volunteers.

Next, we compared the sensitivity to iFU stimulation of 26 out of 28 transected nerves from 22 test patients for which we also had data for the iFU threshold

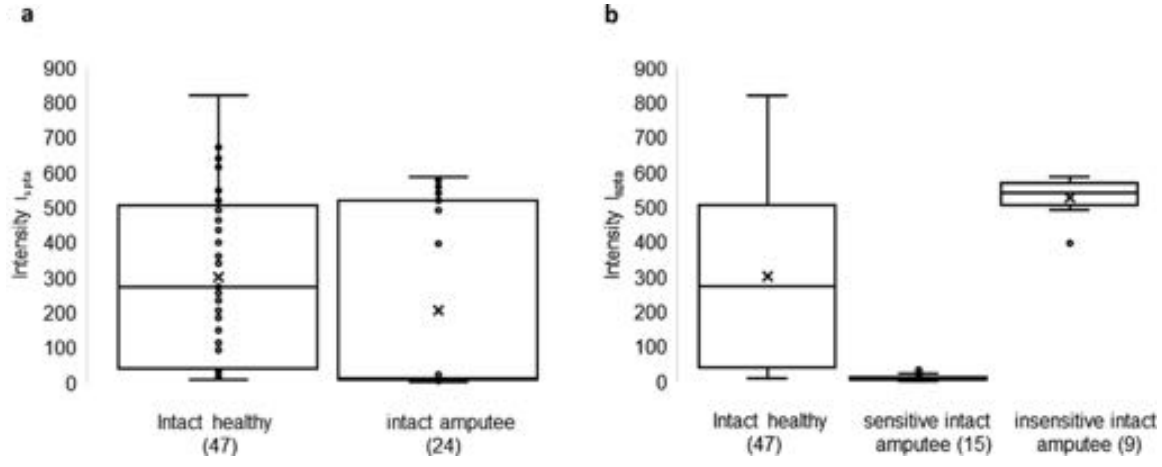


Fig. 5. Sensitivity of intact peripheral nerves to ultrasound stimulation between volunteer cohorts. (a) Intensity of ultrasound necessary to stimulate intact peripheral nerves within each of the two volunteer cohorts (intact, healthy controls; amputee volunteer). Analysis revealed that, on average, the intact nerves contralateral to an amputated limb had significantly lower iFU_t values compared with the intact nerves from healthy volunteers ($z = 2.668$, $p < 0.01$). (b) As in (a) with, however, data from the intact nerves of amputees divided into "sensitive" (N = 15) and "insensitive" (N = 9) subgroups. The iFU_t values differed significantly between all three intact nerves groups ($X^2 = 37.162$, $p < 0.0001$). When examined in a pairwise fashion, all three groups differed from each other in a statistically significant fashion ($z > 2.6$, $p < 0.01$). iFU_t = intense focused ultrasound intensity threshold; $I_{spta}$ = spatial peak, temporal average intensity.

Table 3. Basic statistics for iFU intensity threshold value (iFU_t) for intact major nerve types for each healthy and amputee participant and with a sub-population analysis for amputee participants

| | Intact nerve, healthy volunteer (W/cm$^2$) | Intact nerve, amputee volunteer (W/cm$^2$) | More sensitive, intact nerve, amputee volunteer (W/cm$^2$) | Less sensitive, intact nerve, amputee volunteer (W/cm$^2$) |
|---|---|---|---|---|
| Number (n) | 47 | 24 | 15 | 9 |
| Mean | 300 | 203 | 9 | 527 |
| Standard error | 35 | 53 | 2 | 19 |
| Median | 272 | 12 | 6 | 539 |
| Standard deviation | 240 | 258 | 8 | 57 |

iFU = intense focused ultrasound; iFU_t = iFU intensity threshold.

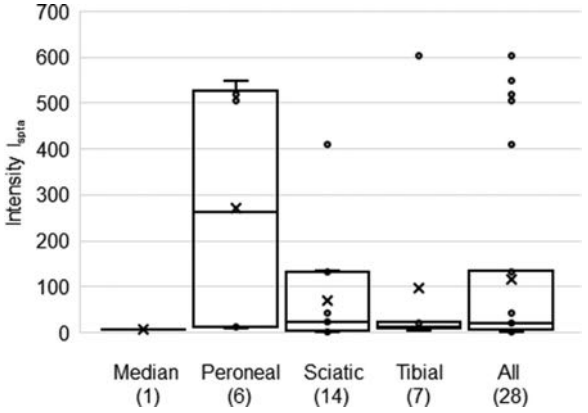


Fig. 6. Sensitivity of transected peripheral nerves to ultrasound stimulation as a function of nerve type. With regard to the transected nerves of amputee participants (N = 28), the intensity of ultrasound necessary to stimulate them did not vary as a function of the type of nerve. ($X^2 = 3.02$, $p < 0.35$). $I_{spta}$ = spatial peak, temporal average intensity.

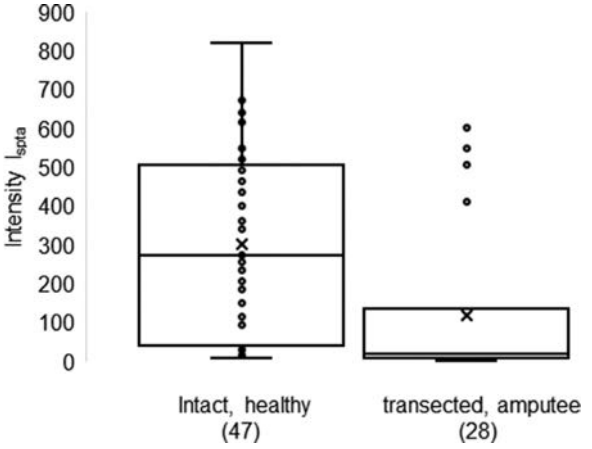


Fig. 7. Sensitivity of transected peripheral nerves to ultrasound stimulation relative to that of the intact nerves within healthy volunteers. Analysis revealed a significant difference between the ultrasound intensity value necessary to induce a sensation in the intact nerve of healthy volunteers (N = 47) relative to the transected nerves of amputees (N = 28); ($z = 4.29$, $p < 0.0001$). $I_{spta}$ = spatial peak, temporal average intensity.

value of an intact, contralateral nerve (Fig. 9a, 9b and Table 6). Analysis showed that transected nerves had a greater average sensitivity to iFU stimulation than the intact, contralateral nerves (Fig. 9a). We also observed three natural sub-groups within this data that differed from each other in a statistically significant way ($F = 61$, $p < 0.0001$). Group 1 demonstrated greater sensitivity of their transected nerve compared with their intact, contralateral nerve. Moreover, their transected/intact nerves had statistically significant greater/lesser sensitivity that that of the intact nerves of healthy volunteers. Group 2 exhibited comparable sensitivity of their transected and intact, contralateral nerves, across a wide range of iFU_t values. Indeed, group 2 itself divided naturally into two sub-groups. Group 2a had insensitive transected and intact nerves, each of which trended toward less sensitive than that of the intact nerves of healthy volunteers. Group 2b (Fig. 9b) had very sensitive transected and intact nerves, much more so than the intact nerves of healthy volunteers. Table 6 summarizes these data and statistical results.

Table 4. Basic statistics for iFU intensity threshold value (iFU_t) for transected nerves as a function of nerve type

| | All (W/cm$^2$) | Median (W/cm$^2$) | Peroneal (W/cm$^2$) | Sciatic (W/cm$^2$) | Tibial (W/cm$^2$) |
|---|---|---|---|---|---|
| Number (n) | 28 | 1 | 6 | 14 | 7 |
| Mean | 115 | 4.5 | 269 | 67.2 | 96.1 |
| Standard error | 37 | NA | 114 | 29.7 | 84.2 |
| Median | 19 | 4.5 | 262 | 22.1 | 11.7 |
| Standard deviation | 196 | NA | 280 | 111 | 223 |

iFU = intense focused ultrasound; iFU_t = iFU intensity threshold.

Table 5. Basic statistics for iFU intensity threshold value (iFU_t) for intact major nerves within healthy participants relative to the iFU_t values for the transected nerves within amputee participants

| | Intact nerve, healthy volunteer ($W/cm^2$) | Transected nerve, amputee volunteer ($W/cm^2$) |
|---|---|---|
| Number (n) | 47 | 28 |
| Mean | 300 | 115 |
| Standard error | 35 | 37 |
| Median | 272 | 18 |
| Standard deviation | 240 | 200 |

iFU = intense focused ultrasound; iFU_t = iFU intensity threshold.

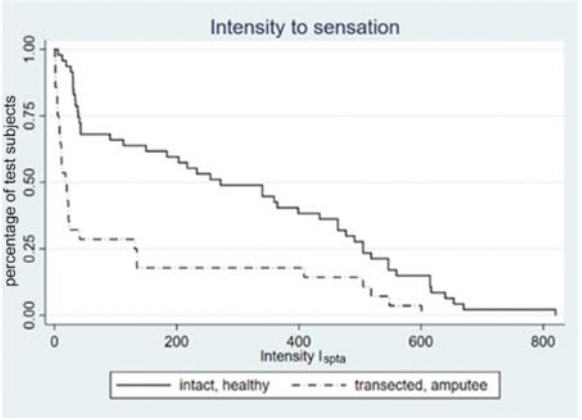


Fig. 8. Kaplan-Meier plots of intensity of ultrasound necessary to create a sensation for each of intact volunteers (intact nerves) and the transected nerves of participants with amputation. $I_{spta}$ = spatial peak, temporal average intensity.

## DISCUSSION

We documented the ability of iFU, under ultrasound image guidance (hence ig-iFU), to stimulate major, deep intact peripheral nerves within the limbs of healthy participants and of participants with amputation, as well as transected nerves from the same amputee participants. This extends the work of Mourad et al. (2018), who studied only (a smaller group of) amputees, without healthy volunteers as controls. We chose ultrasound consistent with parameters used in our prior work involving transected nerve stimulation (McClintic et al. 2013; Mourad et al. 2018): a 2 MHz carrier frequency with a pulse of length 0.1 s and with $I_{spta}$ stimulation values between 5−820 $W/cm^2$.

First, in all cases, the iFU necessary to generate a sensation when applied to a nerve—iFU_t—never generated a sensation when applied to nearby muscle. This supports the view that ig-iFU may also find value in locating a peripheral nerve. For example, ig-iFU−based stimulation may help locate a peripheral nerve, which clinicians can then inject with lidocaine to create regional anesthesia (*e.g.,* Marhofer et al. 2010; Zhou et al. 2015). Or, perhaps, ultrasound stimulation can locate a peripheral nerve in order then to use more intense ultrasound that by itself could generate regional anesthesia (Hong 1991). Stimulatory iFU guided by ultrasound imaging, applied to a peripheral nerve that enervates a major organ, may also activate the immune system in that organ (*e.g.,* Juan et al. 2014) or find other potential applications (Bobola et al. 2018; Mourad 2013).

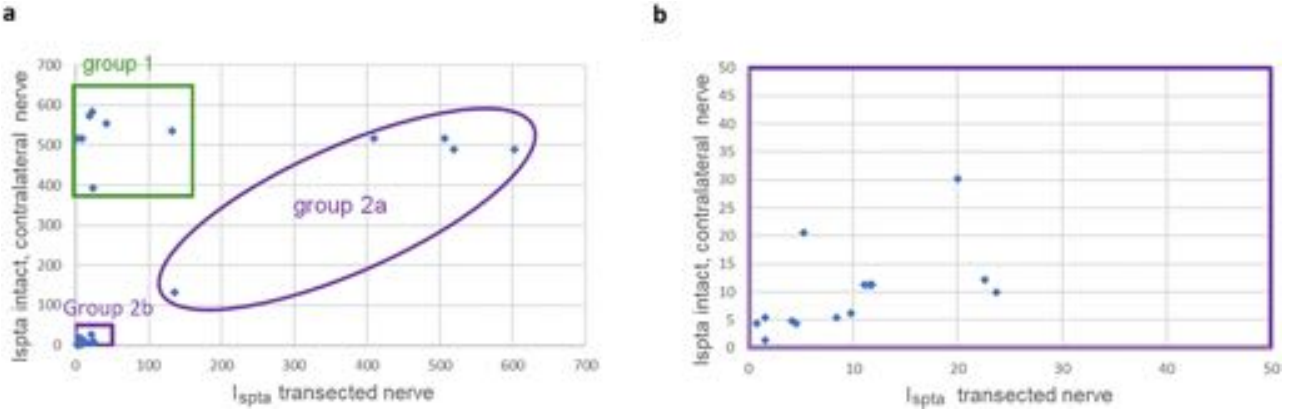


Fig. 9. For participants with amputation, paired intensity values necessary to generate a discernable sensation in transected (ipsilateral) and intact (contralateral) nerves for (a) all data; (b) a close up of the data associated with the nerve pairs most sensitive to ultrasound stimulation—group 2b. We observed a significant difference in the intensity necessary to create a sensation for the intact vs. paired transected nerve across all volunteers ($z = 2.136$, $p < 0.05$ $N = 26$). Within this data set we identified three distinct groups. Group 1 contained sensitive transected nerves and insensitive intact nerves, with a statistically significant difference between the two nerve types ($z = 2.366$, $p < 0.02$ $N = 7$). Group 2 contained comparably sensitive intact and transected nerves, itself dividing into two subgroups. Group 2a had intact and transected nerves relatively insensitive to iFU stimulation. Group 2b had intact and transected nerves relatively sensitive to iFU stimulation. In all cases, we define "relative" compared with the iFU_t values of intact nerves of healthy volunteers ($272 \pm 35$ $W/cm^2$) either with or trending toward statistical significance. See Table 6 for details. iFU = intense focused ultrasound; iFU_t = iFU intensity threshold; $I_{spta}$ = spatial peak, temporal average intensity.

Table 6. Basic statistics for iFU intensity threshold value (iFU_t) for transected major nerves within amputees and their corresponding intact, contralateral nerves, all relative to the iFU_t values of the intact nerves of healthy test patients, with groups defined in Figure 9a

| | Group 1 (sensitive transected, insensitive intact) ($W/cm^2$) | Group 2a (insensitive transected, insensitive intact) ($W/cm^2$) | Group 2b (sensitive transected, sensitive intact) ($W/cm^2$) |
|---|---|---|---|
| Number (n) | 6 | 6 | 14 |
| Mean | (18.6, 525) | (383, 449) | (9.7, 10.0) |
| Standard error | (6, 28) | (83, 63) | (2.0, 2.0) |
| Median | (19.7, 538) | (457, 505) | (9.0, 8.2) |
| Standard deviation | (15, 69) | (203, 154) | (7.5, 7.5) |
| (z, $p <$) values for each nerve compared with that of intact, healthy test patients | transected (3.341, 0.001)<br>intact (2.218, 0.03) | *transected (0.941, 0.3)<br>*intact (1.488, 0.1) | transected (5.403, 0.0005)<br>intact (5.395, 0.0005) |

iFU = intense focused ultrasound; iFU_t = iFU intensity threshold.

* Removal of the one outlier from the analysis of group 2a would increase the values of iFU_t such that each of their transected and intact nerves would have statistically significant less sensitivity to iFU stimulation than that of the intact nerves of healthy volunteers.Note that for two of our amputee participants, we found an iFU_t value for two transected nerves and for one contralateral, intact nerve. For each of these two amputee participants, we used in our analysis the iFU_t value for that single contralateral nerve to pair with the iFU_t value of each of the two transected nerves.

Also, we observed lower values of iFU_t for transected nerves (18 ± 37 $W/cm^2$) than for the intact nerves of healthy volunteers (272 ± 35 $W/cm^2$). On average, therefore, transected nerves exhibited hypersensitivity, namely, an unusual sensitivity to ultrasound stimulation compared with the intact nerves of healthy volunteers.

Anatomic changes in the axons within nerves proximal to nerve transection could plausibly make them more susceptible to iFU stimulation. For example, Dyck et al. (1985) recount earlier literature and their own findings that demonstrate morphologic effects proximal to the transection point of the peripheral nerves, such as overall nerve atrophy. Lending detail to this observation, Oblinger and Lasek (1998) reported a reduction in the transportation of intracellular proteins from the cell body into individual axons within transected nerves, accompanied by a reduction in axonal cytoskeletal networks, all proximal to transection. Oliveira et al. (2018) used a chronic peripheral nerve transection model to document degeneration of nerves proximal to transection with their only partial replacement by histologically normal nerves. Moreover, Oliveira et al. reported that the majority of proximal nerves after transection had reduced axonal density without, however, providing a measure of individual axonal diameter.

Oliveira et al. (2018) is a rare study, since most peripheral nerve injury research has focused on the properties of regenerating peripheral nerves *distal* to injury, such as a crush injury, which, like in the stump of a residual limb, retain sufficient axonal support structure to allow regenerating peripheral nerves to follow the path of initially uninjured nerves. In the context of peripheral nerve regeneration distal to such an axonotmesis injury, Zellmer et al. (2018) and citations within show that individual axonal fiber diameter distributions weigh more heavily toward small values and have thinner myelin sheaths for regenerating axons distal to injury relative to uninjured axons. In addition, their mathematical modeling predicted that such nerves are more susceptive to electrical stimulation than larger-caliber, damaged axons. Zellmer et al. also cite extensive literature documenting a significant reduction in the distance between nodes of Ranvier for regenerating axons relative to healthy axons. These nodes represent gaps in the myelin sheath covering axons that are most susceptible to electrical stimulation; importantly here, they are where the vast majority of sodium and calcium ion channels reside (Carroll 2017). For the purposes this discussion, we will assume those results of distal nerve regeneration have relevance to proximal nerve regeneration after amputation, given the results of Oliveira et al. (2018).

How might these structural changes explain the differential receptivity to iFU stimulation between healthy and transected nerves? Recall that ultrasound stimulates central nerves through at least activation of calcium and sodium channels along their axons (reviewed in Bobola et al. 2018). These observations and those cited above suggest that transected nerves may have greater sensitivity to iFU stimulation than healthy nerves because of the structure of the axons within the transected nerves proximal to transection: smaller diameter axons with reduced myelin and more regions of high concentration of ultrasound-susceptible ion channels compared with intact nerves.

Results from the stimulation of intact, contralateral nerves of participants with amputation paired with their transected counterparts divided naturally into two groups. The first group had transected nerves much more sensitive to iFU stimulation than their contralateral, intact counterpart. The second group had comparable sensitivity between transected and contralateral intact nerve, with the majority of nerve pairs within this second group having greater sensitivity to iFU stimulation than

that of healthy test patients. Central sensitization at the level of the spinal cord that supports these peripheral nerves may explain at least part of this difference (*e.g.,* Hsu and Cohen 2013). For the first group, we speculate that the side of the spinal cord associated with the transected nerve has undergone central sensitization while its contralateral counterpart has not. For the second group, we speculate that central sensitization acts with comparable strength on each side of the spinal cord, very strongly in the case of very sensitive transected and contralateral, intact nerves.

*Limitations*

We assessed the sensitivity of the transected nerve endings by stimulating them in a way that induced nerve-related sensations, but not pain, thereby studying hypersensitivity, but neither allodynic nor hyperalgesic contributions (McMahon et al. 2013), an area of potential future study. Also, our analysis of the sensitivity of transected and intact, contralateral nerves for amputee volunteers identified three sub-groups of nerve pairs, each with different combinations of sensitivity to iFU stimulation relative to that of intact nerves of healthy volunteers. However, the small numbers within two of the three sub-groups warrants collection of additional data before we can make definitive conclusions from this sub-group analysis.

As noted above, scar tissue and edema often complicated our ultrasound imaging procedures. We therefore often stimulated proximal to the transection points on the amputee's nerves. We therefore have stimulated an abundance of sciatic nerves—always proximal to transection but not always the specific branch of peripheral nerve that underwent surgical transection. Future studies will place a greater emphasis on iFU stimulation within a few centimeters of the distal tip of the transected peripheral nerve.

## CONCLUSION

Our study demonstrated the ability of iFU under ultrasound image guidance to stimulate deep, healthy peripheral nerves as well as those whose sensitivity arises because of transection, which drove associated phenotypical changes proximal to the transection, and/or, central sensitization. In addition, we showed that, on average, iFU stimulation of intact peripheral nerves from healthy volunteers required more intense ultrasound than for transected nerves. Finally, we observed that intact, contralateral nerves in participants with amputations divided naturally into two groups—one less sensitive to iFU stimulation than their counterpart, transected nerve and another with comparable sensitivity.

Aided by ig-iFU, future work can target a more detailed analysis of the structure, function and sensitivity of peripheral nerves proximal to transection as a means of eventually addressing the pain experienced by patients with transected nerves. Our work also demonstrates that ig-iFU holds promise for guiding therapy to peripheral nerves, another possible avenue of applied research involving peripheral nerves.

*Acknowledgment*—This work received financial support from CMDRP, award # W81 XWH-15-1-0291 ("Localizing and Assessing Amputee Pain with Intense Focused Ultrasound").

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Elsevier Editorial System(tm) for BRAIN STIMULATION

Manuscript Draft

Manuscript Number: BRS-D-19-01053R1

Title: Case Study of an Amputee Regaining Sensation and Muscle Function in a Residual Limb after Peripheral Nerve Stimulation by Intense Focused Ultrasound

Article Type: Letter to the Editor

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UW Medicine

2 January 2020
Mark S. George, MD
Editor, Brain Stimulation.

Dear Dr. George

Please find enclosed a revision of our letter to the editor, submitted originally in late 2019, entitled "*Case Study of an Amputee Regaining Sensation and Muscle Function in a Residual Limb after Peripheral Nerve Stimulation by Intense Focused Ultrasound.*" We are grateful for your attention to our missive. We have responded positively to every reviewer comment, adding quite important material missing from the original version that we believe addresses all the concerns expressed by the reviewers.

Sincerely,

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***Detailed Response to Reviewers**

We thank the reviewers for their constructive criticism of our manuscript. We see in retrospect that our zeal to shorten the manuscript removed important material whose inclusion we believe addresses almost all of their quite reasonable questions. To summarize here, the return of function was cotemporaneous with application of sufficient intense focused ultrasound (iFU) and lasted for as long as we were able to observe the patient.

***Reviewer #1.***
*Please describe in detail the actual stimulation delivered, for how long.*

- We have provided those details in the revised version of the manuscript, lines 43-44.

*Please also better describe the time course of the recovery, over minutes, hours or days.*

- We have provided those details in the revised version of the manuscript, lines 47-51.

*And this last is most important, please document the longer term outcome of the patient. Were the changes permanent? What has happened since to the patient? If indeed this was a reconnection then it should be somehow stable.*

- Regrettably, we have lost this patient to follow up, despite attempts to communicate with him directly. All we can say is what we've added to the paper, namely that the patient reported motor and sensory function directly after sufficient iFU delivery, for up to the time he left, approximately 45 minutes after apparent reanimation of his tibial nerve – lines 51-56.
- We speculate here (but not in the revised manuscript, unless you would like us to put it in the revision) that he would have returned had he lost his regained function. Unfortunately we do not have any information to back up or refute this speculation.

***Reviewer #2.***
*The authors of this study stimulated the tibial nerve in an amputee using focused ultrasound and found functional improvement. They propose that the stimuli 'fixed' an unworking connection in the central nervous system. This is a rather far-fetched hypothesis that is hard to believe.*

- We wish we had more than one example of this finding!
- Also, we are open to alternative hypotheses and would happily represent them in the revised manuscript.

*One aspect that I am missing is EMG recording from the gastrocnemius muscle that the patient said he was unable to contract as well as nerve conduction studies if available.*

- We agree that this case study would have benefited from EMG and nerve conduction studies. Unfortunately we did not have EMG recording nor nerve conduction studies for any of our test subjects let alone the subject of our manuscript because the goal of the study was to generate diagnostic sensations in intact versus in transected nerves, not reanimate unfunctional nerves. We have direct reports of return of motor function by the patient and our observation of motor function with real-time ultrasound imaging simultaneous with our application of iFU – lines 48-51.
- In essence, we report here an incidental finding, one that arose in the context of a study targeting the ability of real-time ultrasound image guided iFU to stimulate a major nerve without stimulating intervening tissue.

*There is no information on the reason for the patient's amputation, nor where the patient's leg was amputated, neither on the function or the mechanism of the prosthesis that he could not effectively use.*

- We have now provided this important information, lines 30-40.

*The authors focus their study in the tibial nerve without explaining why this could be important. Were there other functioning nerves in the leg?*

- We have now provided this important information, lines 32-40.

*The construction of some sentences is not acceptable. The authors state that iFU '…. produced phantom limb sensations, then, in rapid succession, involuntary movement without sensation, followed by voluntary movement and an ability to detect cutaneous stimulation.' This statement is unsupported as there is no proof that the changes were produced by iFU. At the most, they occurred after iFU. There is no clear reference to the timing of the events, except for an imprecise '..rapid succession'. Furthermore, there is no documentation of any real change between before and after iFU. The only result is that the 'non-functioning nerve recovered function'. Were there any objective measurements of such functional recovery?*

- We have provided in the revised manuscript important details of the study that address these important concerns. We have added what we believe is the extra information you require, including the timing of the iFU delivery relative to the recovery of function (in essence, simultaneous), our observation of muscle movement during real-time imaging of the tissue of interest, along with other information that we believe addresses these quite important concerns – lines 48-51.

*It comes as a surprise that the first hypothesis that the authors bring up as an explanation for their results is that the stimulus travelled with the peripheral nerve to the thalamus to activate thalamic connections to the motor and sensory cortices. If this hypothesis is correct, what would make ultrasound different from other types of stimuli? This patient had probably been having rehabilitation therapy for the entire previous year, with supposedly various forms of stimulation of the transected nerve, including prosthesis-related stimulation. What makes ultrasound different from these more natural forms of stimulation?*

- This was the only hypothesis we have generated to date, despite a great deal of thought applied to generating any explanation of our observation. Again, we are open to discussing others.
- As we note briefly in the revised version of this manuscript, our generation of phantom limb sensations with ultrasound was the first sensation the participant had felt since his TMR surgery, *perhaps* because iFU represents the only direct stimulation of *sufficient* intensity that the nerve had experienced after the TMR surgery, lines 44-46.

# *Case Study of an Amputee Regaining Sensation and Muscle Function in a Residual Limb after Peripheral Nerve Stimulation by Intense Focused Ultrasound*

Ezeokeke CK [1], Bobola MS [1], Selby M [1], Ko JH [2], Friedly JL [3], Mourad PD [1,4].

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*Under revision as a letter to the editor for Brain Stimulation.*

Standard amputation surgery places the distal transected nerve ending in soft tissue to minimize pain from external pressure. Despite this, nerve-related pain often occurs due to a variety of peripheral and central sources [1]. Targeted muscle reinnervation (TMR) connects the distal transected nerve to a neuromuscular junction in the residual limb during amputation surgery in order to facilitate myoelectric prosthesis use and to reduce the incidence and severity of neuroma-related pain [2]. During a study to determine the relative sensitivity to external stimulation of transected nerves after standard amputation versus TMR, we encountered a single participant who recovered motor and sensory function of their tibial nerve after TMR surgery during ultrasound stimulation of the nerve.

We used intense focused ultrasound (iFU), delivered under real-time ultrasound image guidance, to stimulate at or near the distal tip of major transected nerves in amputated limbs following a previously described protocol [3,4]. In this way we determined the minimum iFU intensity capable of generating a first discernable sensation through use of a ramp-up paradigm that started at low intensity values and increased until we achieved that aim or reached the maximum intensity value of our device.

We obtained University of Washington Institutional Review Board (IRB) and military Human Research Protection Office (HRPO) approvals for our study. All participants in the study provided informed consent. The participant in question had a below-knee amputation in March of 2003 due to posttraumatic arthritis, then a surgical revision in February of 2016 using TMR to address three painful neuromas in his residual limb, one for each of the peroneal, tibial and sural nerves. Prior to his participation in our study in February of 2017, our participant reported his inability to contract his lateral gastrocnemius muscle to which the tibial nerve was connected via the lateral motor branch of the gastrocnemius nerve using the TMR procedure (Figure 1a). He also could not detect sensations from the posterior portion of the leg – that associated with the site of tibial nerve implantation. This lack of motor and sensory function of the tibial nerve persisted for the entire twelve months after TMR surgery until the day of our study. Together, this impaired his ability to effectively use his standard, below-knee prosthesis. The participant reported normal motor and sensory function associated with the other transected nerves. We verified these self-reports through palpation of muscle during voluntary movement by the patient and our formal, single-blinded cutaneous stimulation of the residual limb.

iFU stimulation of his non-functioning tibial nerve under ultrasound image guidance (Figure 1b) with sufficient spatial peak temporal average intensity (71.5 $W/cm^2$ , 2.0 MHz, for each of five individual pulses of 0.1 second in duration, spaced 1-2 seconds apart) produced corresponding transient pulses of phantom limb sensations, the first time the participant had felt sensations of any sort associated with his tibial nerve since his TMR surgery. We continued the study but, because of his surprise, we used a lower iFU intensity value (66.5 $W/cm^2$), doing so within one minute of the previous stimulation that generated phantom limb sensations. By the third of five iFU pulses at that intensity, we directly observed with ultrasound imaging involuntary movement of the lateral gastrocnemius muscle. Within approximately ten seconds and without additional stimulation,

there followed voluntary movement of that muscle by the participant that we directly observed along with his reported ability to detect cutaneous stimulation which we verified as above. During the next 45 minutes we continued the study, for example successfully stimulating his transected peroneal nerve at a comparable intensity value as for the tibial nerve (66.5 W/cm$^2$). Up to and including the time the participant left our facility, he reported voluntary control of and sensations associated with his lateral gastrocnemius muscle. Regrettably we have lost the participant to follow up, so do not know the long-term outcome of this apparent reanimation of his tibial nerve.

Several published reports document the ability of ultrasound to stimulate already functioning peripheral nerves [3–7] and activation (as well as inhibition) of the brain with ultrasound [8]. One case study [9] reported substantial activation of a patient's brain associated with ultrasound application after prolonged minimal consciousness. Specifically, Monti et al [9] directed transcranial ultrasound to the thalamus of a patient whose traumatic brain injury led to 19 days of prolonged loss of consciousness. At the time of ultrasound delivery the patient had attained a minimally conscious state [10]. Three days after ultrasound delivery, the patient demonstrated significantly increased voluntary behavior consistent with emergence from a minimally conscious state; by five days post-ultrasound the patient tried to walk. In our case and theirs, at most minimally functional but structurally connected nervous system tissue started to function after delivery of ultrasound. In our case, this occurred moments after delivery of sufficient ultrasound to a major peripheral nerve, which feeds via the thalamus into the motor and sensory cortices. In their case, this occurred days after delivery of sufficient ultrasound directly to the thalamus. Monti et al [9] may have derived inspiration for their effort by Yoo et al [11], who observed acceleration out of an anesthetized state by rodents caused by ultrasound delivered to the rodent's thalamus. Yoo et al [11] and Monti et al [9] directly stimulated thalamus using non-invasively delivered ultrasound with results analogous to those achievable by deep brain stimulation of the thalamus of patients with disordered consciousness, as discussed in Yoo et al [11]. Referring to the mesocircuit hypothesis of Schiff [10] and the discussion of Yoo et al [11], we hypothesize that we activated a previously dormant thalamus/cortex circuit via our stimulation with iFU of the mixed motor/sensory tibial nerve.

We report here the first observation known to us of ultrasound stimulation causing a non-functioning nerve to recover its function. Through direct activation of a major peripheral nerve with iFU we hypothesize that we stimulated the thalamic/cortical circuit thereby entraining central function that supported tibial nerve function. Our observation may therefore have conceptual overlap with that of Monti et al [9] and of Yoo et al [11] in that all three studies demonstrated activation of a previously non-functioning nervous system circuit, through direct (as in Monti et al [9] and Yoo et al [11]) or indirect (our case) stimulation of the thalamus.

Our case report joins a burgeoning field demonstrating that ultrasound can activate the peripheral as well as the central nervous systems, with clinical applications of this phenomena in the early stages of exploration.

**Author contributions.**

Ezeokeke CK [validation, formal analysis, investigation, visualization, data curation, writing], Bobola MS [validation, formal analysis, investigation, resources], Selby M [validation, formal analysis, investigation, data curation], Ko J [methodology, investigation], Friedly JL [investigation, methodology, writing, funding acquisition], Mourad PD [conceptualization, methodology, validation, investigation, writing, supervision, project administration, funding acquisition].

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## Conflict of Interest Statement

All authors declare that they have no financial interests or any other conflict of interest with other people or organizations that could inappropriately influence this work.


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***Revised Manuscript (with highlighted changes)**

*Case Study of an Amputee Regaining Sensation and Muscle Function in a Residual Limb after Peripheral Nerve Stimulation by Intense Focused Ultrasound*

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*For submission as a case study to Brain Stimulation.*

Standard amputation surgery places the distal transected nerve ending in soft tissue to minimize pain from external pressure. Despite this, nerve-related pain often occurs due to a variety of peripheral and central sources [1]. Targeted muscle reinnervation (TMR) connects the distal transected nerve to a neuromuscular junction in the residual limb during amputation surgery in order to facilitate myoelectric prosthesis use and to reduce the incidence and severity of neuroma-related pain [2]. During a study to determine the relative sensitivity to external stimulation of transected nerves after standard amputation versus TMR, we encountered a single participant who recovered motor and sensory function of their tibial nerve after TMR surgery during ultrasound stimulation of the nerve.

~~In this study, we~~We used intense focused ultrasound (iFU), delivered under real-time ultrasound image guidance, to stimulate at or near the distal tip of major transected nerves in amputated limbs following a previously described protocol [3,4]. ~~We sought to~~In this way we determined the minimum iFU intensity capable of generating a first discernable sensation through use of a ramp-up paradigm that started at low intensity values and increased until we achieved that aim or reached the maximum intensity value of our device.

We obtained University of Washington Institutional Review Board (IRB) and military Human Research Protection Office (HRPO) approvals for our study. All participants in the study provided informed consent. The participant in question had a below-knee amputation in March of 2003 due to posttraumatic arthritis, then a surgical revision in February of 2016 using TMR to address three painful neuromas in his residual limb, one for each of the peroneal, tibial and sural nerves. Prior to his participation~~,~~ in our study in February of 2017, ~~one~~ our participant reported ~~an~~ his inability to contract his lateral gastrocnemius muscle to which the tibial nerve was connected via the lateral motor branch of the gastrocnemius nerve using the TMR procedure (Figure 1a). ~~In addition to compromised motor function, this participant~~He also could not detect sensations from the posterior portion of the leg – that associated with the site of tibial nerve implantation. This lack of motor and sensory function of the tibial nerve persisted for the entire twelve months after TMR surgery until the day of our study. Together, this impaired his ability to effectively use his ~~his~~ standard, below-knee prosthesis. ~~. For example, he often lost his balance during activities such as bowling. In addition to compromised motor function, this participant could not detect sensations from the posterior portion of the leg associated with the site of tibial nerve implantation.~~ The participant reported normal motor and sensory function associated with the other transected nerves. We verified these self-reports through palpation of muscle during voluntary movement by the patient and our formal, single-blinded cutaneous stimulation of the residual limb~~This lack of motor and sensory function of the tibial nerve persisted for the entire twelve months after TMR surgery until the day of our study~~.

iFU stimulation of his non-functioning tibial nerve under ultrasound image guidance (Figure 1b) with sufficient spatial peak temporal average intensity (~~66.5 –~~71.5 W/cm$^2$ , 2.0 MHz, for each of five individual pulses of 0.1 second in duration, spaced 1-2 seconds apart) ~~first~~ produced corresponding transient pulses of phantom limb

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sensations, the first time the participant had felt sensations of any sort associated with his tibial nerve since his TMR surgery. We continued the study but, because of his surprise, we used a lower iFU intensity value (66.5 W/cm$^2$), doing so within one minute of the previous stimulation that generated phantom limb sensations. By the third of five iFU pulses at that intensity, we directly observed with ultrasound imaging~~, then, in rapid succession,~~ involuntary movement of the lateral gastrocnemius muscle~~without sensation~~. Within approximately ten seconds and without additional stimulation, there~~,~~ followed ~~by~~ voluntary movement of that muscle by the participant that we directly observed along with~~and~~ his ~~an~~ reported ability to detect cutaneous stimulation which we verified as above. During the next 45 minutes we continued the study, for example successfully stimulating his transected peroneal nerve at a comparable intensity value as for the tibial nerve (66.5 W/cm$^2$). Up to and including the time the participant left our facility, he reported voluntary control of and sensations associated with his lateral gastrocnemius muscle. Regrettably we have lost the participant to follow up, so do not know the long-term outcome of this apparent reanimation of his tibial nerve.

Several published reports document the ability of ultrasound to stimulate already functioning peripheral nerves [3–7] and activation (as well as inhibition) of the brain with ultrasound [8]. One case study [9] reported substantial activation of a patient's brain associated with ultrasound application after prolonged minimal consciousness. Specifically, Monti et al [9] directed transcranial ultrasound to the thalamus of a patient whose traumatic brain injury led to 19 days of prolonged loss of consciousness. At the time of ultrasound delivery the patient had attained a minimally conscious state [10]. Three days after ultrasound delivery, the patient demonstrated significantly increased voluntary behavior consistent with emergence from a minimally conscious state; by five days post-ultrasound the patient tried to walk. In our case and theirs, at most minimally functional but structurally connected nervous system tissue started to function after delivery of ultrasound. In our case, this occurred moments after delivery of sufficient ultrasound to a major peripheral nerve, which feeds via the thalamus into the motor and sensory cortices. In their case, this occurred days after delivery of sufficient ultrasound directly to the thalamus. Monti et al [9] may have derived inspiration for their effort by Yoo et al [11], who observed acceleration out of an anesthetized state by rodents caused by ultrasound delivered to the rodent's thalamus. Yoo et al [11] and Monti et al [9] directly stimulated thalamus using non-invasively delivered ultrasound with results analogous to those achievable by deep brain stimulation of the thalamus of patients with disordered consciousness, as discussed in Yoo et al [11]. Referring to the mesocircuit hypothesis of Schiff [10] and the discussion of Yoo et al [11], we hypothesize that ~~in a similar way~~ we activated a previously dormant thalamus/~~motor~~ cortex circuit via our stimulation with iFU of the mixed motor/sensory tibial nerve~~, which then facilitated function of the entire circuit.~~

We report here the first observation known to us of ultrasound stimulation causing a non-functioning nerve to recover its function. Through direct activation of a major peripheral nerve with iFU we hypothesize that we stimulated the thalamic/cortical circuit thereby entraining central function that supported~~s~~ tibial nerve function. Our observation may therefore have conceptual overlap with that of Monti et al [9] and of Yoo et al [11] in that all three studies demonstrated activation of a previously non-functioning nervous system circuit, through direct (as in Monti et al [9] and Yoo et al [11]) or indirect (our case) stimulation of the thalamus.

Our case report joins a burgeoning field demonstrating that ultrasound can activate the peripheral as well as the central nervous systems, with clinical applications of this phenomena in the early stages of exploration.

**Author contributions.**

Ezeokeke CK [validation, formal analysis, investigation, visualization, data curation, writing], Bobola MS [validation, formal analysis, investigation, resources], Selby M [validation, formal analysis, investigation, data curation], Ko J [methodology, investigation], Friedly JL [investigation, methodology, writing, funding acquisition], Mourad PD [conceptualization, methodology, validation, investigation, writing, supervision, project administration, funding acquisition].

**Acknowledgements**

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**Conflict of Interest Statement**

All authors declare that they have no ~~known~~ financial interests or any other conflict of interest with other people or organizations that could inappropriately influence this work.

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**Figure**

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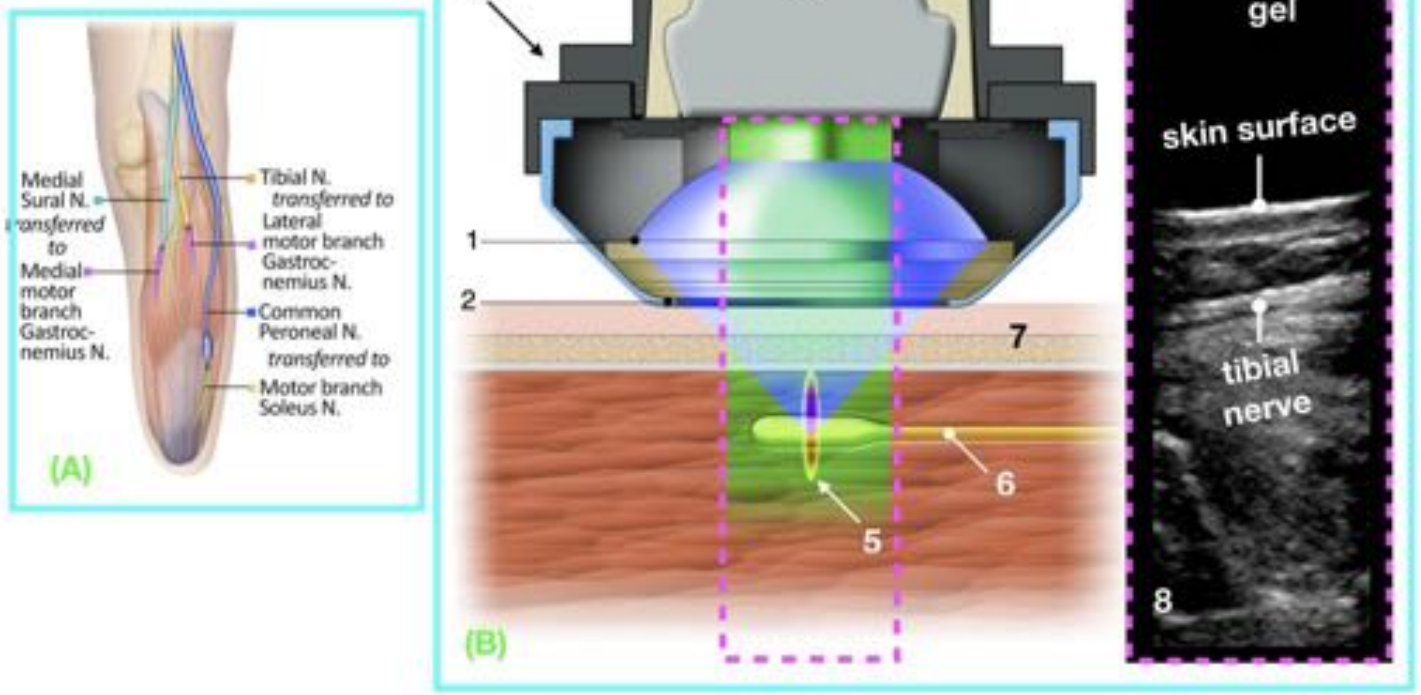


**Experimental target and setup. (A)** Schematic of the test subject's major peripheral nerves in his residual limb. We targeted the distal aspect of the tibial nerve for stimulation by intense focused ultrasound. This schematic also shows the other nerves that had undergone the TMR procedure, with a neuroma at the distal end of the common peroneal nerve. **(B)** Schematic of the experimental procedure using an ultrasound system (#1 {iFU transducer}, 2 {gel pad, which couples the transducer to skin}, 3 {mounting system}, 4 {diagnostic ultrasound scan head}) that puts the ultrasound focus (#5) on the tibial nerve (#6) below the skin (#7) using ultrasound image guidance (#8).

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**Highlights**

Intense focused ultrasound activated a once dormant peripheral nerve in an amputee's residual limb.

This case study has conceptual overlap with that of Monti et al [9], who used transcranial ultrasound delivered to the thalamus of an unconscious patient to 'awaken' that patient.

Interim report for W81XWH-15-1-0291 “Localizing and Assessing Amputee Pain with Intense Focused Ultrasound”

Mourad PD, Bobola M, Ezeokeke CK, Selby M, Lahti AC, Loeser JD, Olmstead TA, Ko J, Friedly JL.

**Abstract**

People with amputation often experience residual limb pain as well as phantom limb sensation and pain. However, it is often difficult to distinguish if pain is originating from transected nerves or from surrounding limb tissues. Using intense focused ultrasound (iFU) stimulation, we sought to determine if pain sensitivity thresholds varied between intact nerves, standardly transected nerves and nerves re-implanted into a neuromuscular junction via targeted muscle re-innervation (TMR) surgery and control tissues. We applied iFU to three cohorts of participants: standard amputees with standardly transected nerves, TMR amputees and health volunteers. As controls, we stimulated ipsilateral muscle, contralateral and intact nerves in the two amputation cohorts, and intact nerves in healthy volunteers. Our iFU sources, guided with diagnostic ultrasound, emitted individual bursts of 2 MHz ultrasound lasting 0.1 seconds. We started at low intensity values, increasing the intensity until either the test subjects experienced a sensation associated with successful stimulation of the nerve or reached the upper bound on the intensity emitted by our device (820 W/cm^2 spatial peak temporal average intensity - $I_{spta}$), defined as the iFU threshold intensity value or iFU_t. All successfully stimulated (all transected and 58/76 of intact nerves across all test subjects. Also, all nerves were more sensitive than surrounding, muscular tissue. Intact nerves across all cohorts had comparable iFU_t values, on average: 318 +/- 44 W/cm^2 $I_{spta}$ (mean+/-SE) for intact volunteers, 223+/-70 $I_{spta}$ for standard amputees and 402+/-102 $I_{spta}$ for TMR amputees. Standard amputees had lower iFU_t values for their transected nerves (37+/-25 W/cm^2 $I_{spta}$) than TMR amputees (92+/-51 W/cm^2 $I_{spta}$). 5/13 (38%) of the standard amputees had substantially lower (by an order of magnitude) ipsilateral iFU_t than contralateral iFU_t, while the rest had comparable values of iFU_t between ipsilateral and contralateral nerves. This stands in contrast to the TMR amputees, for whom 5/6 (88%) had much smaller ipsilateral versus contralateral iFU_t values. For the TMR test subjects, their iFU_t value varied significantly with the presence/absence of neuropathy.

This study demonstrates the feasibility of using high intensity focused ultrasound to distinguish between painful tissue and surrounding non-painful tissue. In addition, test subjects who have undergone TMR surgery show a trend towards less residual and phantom limb pain along with reduced sensitivity of their the transected nerves than test subjects who have undergone standard surgical nerve transection. Given these encouraging results and confirmation of our original power analysis, we find support for further data collection and analysis.

**KEYWORDS**

- Image-guided intense focused ultrasound (ig-iFU)
- Intense focused ultrasound (iFU)
- Targeted muscle Reinnervation surgery (TMR surgery)
- Limb amputation

**SUPPORT**
This work received financial support from DoD award # W81XWH-15-1-0291 (“Localizing and Assessing Amputee Pain with Intense Focused Ultrasound”).

**INTRODUCTION.**

After amputation, people often experience residual limb pain as well as phantom limb pain. Transected nerve endings or neuromas, adhesions, muscle, bone, and other soft tissues (citation) can act as direct peripheral sources of an amputee's pain through 'peripheral sensitization'. Central sensitization (citation) - abnormal processing of peripheral signals within one or both of the spinal cord and brain - often contributes to the experience of pain after amputation (citation). Delineating the contribution of peripheral versus central sensitization to the experience of phantom limb pain remains problematic given the complexity of these processes, the overlap, and because current diagnostic methods can only image tissue, not identify it as a candidate contributor to a patient's pain (citation). Understanding the degree to which central versus peripheral sensitization contributes to phantom limb pain is important to better tailor treatments to target the pain generators.

In standard amputation surgery, nerves are transected and allowed to retract into soft tissue. However, neuroma formation, ectopic nerve firing and errant phantom limb sensations and pain after standard amputation is extremely common (CITE). "Targeted Muscle Reinnervation" or TMR surgery, is a newer surgical procedure originally developed to facilitate more intuitive use of myoelectric prostheses in upper extremities. In TMR, transected nerves are implanted into muscle, typically a neuromuscular junction, rather than allowing the severed nerve to retract into soft tissue. (Kuiken citations). TMR has also been used as an alternative surgical treatment for neuroma-related pain given anecdotal findings of decreased neuropathic pain following TMR, but no studies have directly compared TMR to standard amputation to determine if there is decreased neuroma formation following TMR and reduced nerve pain compared to standard nerve transection. In this study, we test the hypothesis that TMR test subjects experience less residual and phantom limb pain than do standard amputees, in part because their transected nerve endings are less sensitive to external stimuli than those of standard amputees.

To test this hypothesis, we used two tools: test subject reported outcomes assessing residual limb and phantom limb pain intensity and quality, and image-guided intense focused ultrasound (ig-iFU) stimulation. We used a previously described technique using ig-iFU to assess the sensitivity of the transected nerve endings by stimulating them in a way that induced nerve-related sensations, but not pain, and did not stimulate the surrounding tissue (Mourad et al, 2017).

Testing our primary hypothesis required addressing the following intermediate hypotheses.

(1) Transected nerve endings have greater sensitivity to iFU stimulation than intact nerves and muscle, re-affirming the ability of ig-iFU to locate and stimulate deep and tender nerves relative to surrounding and healthy tissue.

(2) The transected nerves of TMR test subjects are less sensitive to iFU stimulation than the transected nerves of standard amputees, consistent with the view that TMR surgery can produce transected nerves less sensitive to external stimulation than standard amputation.

(3) TMR test subjects experience less phantom and residual limb pain than do standard amputee test subjects in a way that scales inversely with their sensitivity to iFU stimulation, consistent with the view that their re-approximated nerves contribute less to a test subject's allodynic pain than the free, transected nerves of standard amputees.

**Methods.**

***Test-Subject Population***

The University of Washington Institutional Review Board and the military Human Research Protection Office approved this study. We recruited participants aged 18 or older from cohorts of intact volunteers, patients that have received standard lower limb amputation surgery and patients that have received targeted muscle reinnervation in which the nerve was implanted into a neuromuscular junction or a variant in which the nerve was implanted into the muscle (i.e. targeted nerve implantation (TNI)) (Pet MA, Ko JH, Friedly JL, Mourad PD, Smith DG. Does targeted nerve implantation reduce neuroma pain in amputees? Clin Orthop Relat Res 2014; 427:2991–3001.).

Our amputee test subjects had to have undergone their last surgical procedure six months or more before our study. Exclusion criteria were presence of pressure ulcers, rashes, or open skin over the residual limb, a history of skin grafting or burns on residual limb, a history of diabetes mellitus, cognitive or communication impairments that would impede participation in the consenting and testing procedures.

***Pain Questionnaires***

After providing written informed consent, all study participants completed three validated pain questionnaires. The Leeds Assessment of Neuropathic Symptoms and Signs (LANSS - citation) was administered to asses pain quality (neuropathic versus non-neuropathic pain) and is rated on a scale of 0 to 24, with scores higher than 12 indicating likely neuropathic pain during the prior week. Each participant also reported the intensity of phantom limb pain and of residual limb pain associated over the last 24 hours (numeric pain rating scale from 0 = “no pain” to 10 = “pain as bad as you can imagine”).

***iFU system***

Our iFU system (Figure 1) is a portable commercial diagnostic ultrasound imaging machine connected to a custom-made intense focused ultrasound stimulation transducer. This image-guided iFU (ig-iFU) system (Gellhorn et al. (2015) has previously been tested for feasibility (Mourad et al (2017)). Refinements yielded a device capable of distributing iFU stimulation at a greater range of depths, guided by an imaging system with greater sophistication and image fidelity. As in Gellhorn et al and Mourad et al, we calibrated the iFU transducers using a needle hydrophone in a water tank, producing intensity values given throughout this paper in terms of their spatial peak, temporal-averaged value ($I_{spta}$).


**Figure 1. Image-guided intense focused ultrasound (ig-iFU) stimulation system.** **(A)** Phillips EPIQ L12-3 imaging probe (left) next to its housing (center) and an iFU transducer (right) whose central aperature allows transmission of diagnostic ultrasound in a manner co-located with the iFU focus**.** Assembled proximal aspect of the ig-iFU device in **(B)** axial view and **(C)** side view. **(D)** iFU transducer with gel standoff to control depth of stimulation. **(E)** Side view of iFU transducer with gel standoff placed on the transducer. **(F)** Electronic setup for the iFU device including oscilloscope, function generators, amplifier and power regulator/surge protector. **(G)** Diagnostic ultrasound imaging machine with a completely assembled iFU transducer plus imaging probe. **(H)** A close up of the assembled probe for simultaneous ultrasound imaging and stimulation.

***Ultrasound imaging and iFU stimulation of targets***

We first imaged the target area using ultrasound imaging transducer in order to identify the nerves of interest: for the amputee participants, these included the transected nerve ending with a possible neuroma, and the proximal intact nerve (Figure 2A,B), as well as contralateral intact nerve. For the healthy volunteers, we imaged the median, fibular, tibial and sciatic nerves. We noted the location and depth of the nerves to facilitate subsequent iFU stimulation with the appropriate iFU transducer under ultrasound image guidance. We then used the ig-iFU device with the appropriate gel standoff to control the depth of stimulation to relocate the target nerve (Figure 2A, right).

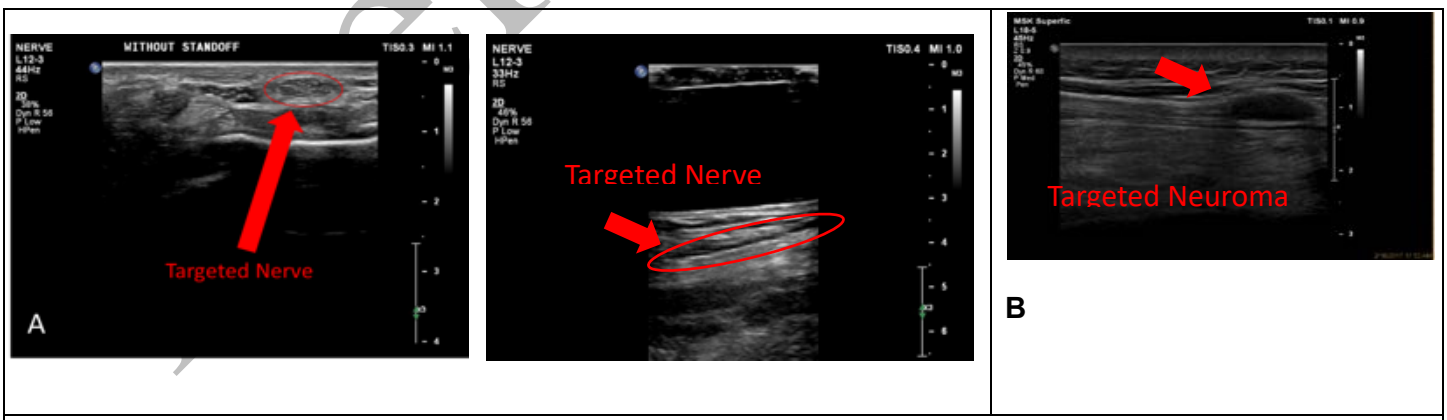



**Figure 2. Ultrasound images of nerves targeted by iFU (A - left)** Cross sectional image featuring the nerve of interest using B-mode ultrasound imaging. **(A - right)** Longitudinal image of that same nerve imaged with ig-iFU system before application of iFU. **(B)** We have comparable images for transected nerve endings, here a peroneal nerve with a neuroma at its distal tip.

We then applied iFU in five individual 0.1-second bursts, at approximately 0.5-1 Hz, to the target nerve. We started by using a low iFU intensity value, approximately 16 W/cm2. After each application of five iFU bursts,

study participants reported the presence or absence of sensations either deep below the skin beneath the device or distal to the point of application and consistent with the enervation pattern of that nerve, sometimes below the device but not on the skin surface. The iFU intensity threshold value (iFU_t) was identified as the intensity at which the participant either felt a sensation deep to the skin or the maximum intensity of the device was reached (820 W/cm2). If we induced a sensation with iFU, we then applied iFU stimulation with the same intensity to nearby muscle within the same limb and recorded whether or not sensation occurred at the nerve ifU_t. For amputee participants, we repeated the iFU threshold determination process for the corresponding nerve in the intact, contralateral limb. For intact volunteers, we stimulated several major nerves, including median nerve in the arm and the sciatic nerve in the leg and when feasible, the fibular and tibial nerves.

When iFU_t thresholds were obtained from multiple transected nerves in an individual participant, the lowest value was used to when determining the relationship between iFU_t and pain scores and when comparing ipsilateral and contralateral iFU_t values for amputee test subjects.

***Statistical analysis***

Basic data analysis was performed using Excel (Office 365, Microsoft, Redmond WA) and nonparametric statistical analysis was performed using Stata 15.1 (StataCorp LLC). The Kruskal-Wallis equality-of-populations rank test was used to measure between-group differences. The Wilcoxon rank-sum test was used to determine if two sets of data were statistically significantly different from one another. The Wilcoxon matched pairs signed-rank test was used to examine differences in paired data.

The a priori power analysis was based on *in vivo* data from McClintic et al, (2014) with a 20% standard deviation and determined that 45 participants per cohort would be needed to detect a difference in the intensity of ultrasound necessary to induce a sensation in intact versus transected nerves via each of the standard amputation method or TMR method, with greater than 80% power to detect an effect size of 0.6 at $p<0.05$. With our new data we have re-assessed our original power analysis, using Stata.

**Results**

At this intermediate stage in our study (winter 2018), we successfully consented and tested nerve sensitivity in 23 intact volunteers, 18 standard amputees, two amputees that received the TNI surgery and nine amputees that received the TMR surgery. We studied a large subset of this amputee population, with dropouts arising due to due technical issues such as difficulty in locating the target nerve (typically due to extensive scarring or edema) or test-subject fatigue. Of those consented, we collected data and report the findings for 23 intact volunteers,16 standard amputees, 2 amputees that received the TNI surgery and 6 amputees that received the TMR surgery. The two TNI test subjects displayed data consistent with the TMR test subjects – not a surprise given our small test-subject numbers to date. Given this intermediate result, we combined their data with that from the TMR test subjects in our final analysis.

Note that we found a iFU threshold value, below 820 W/cm^2, for all transected nerves and for 58/76 intact nerves across all test subjects. For cases for which we did not induce a sensation in an intact nerve, we represented that value as 820 W/cm^2 in our analysis, again, the maximum intensity value of our device.

***iFU stimulation of intact nerves.***

We first examined the iFU intensity threshold value required to induce a discernable sensation (iFU_t) for intact nerves, testing for differences between nerve type regardless of cohort source - (Figure 3) and Table 1. Across all test cohorts, no statistically significant differences in iFU_t were observed between the four intact nerves.

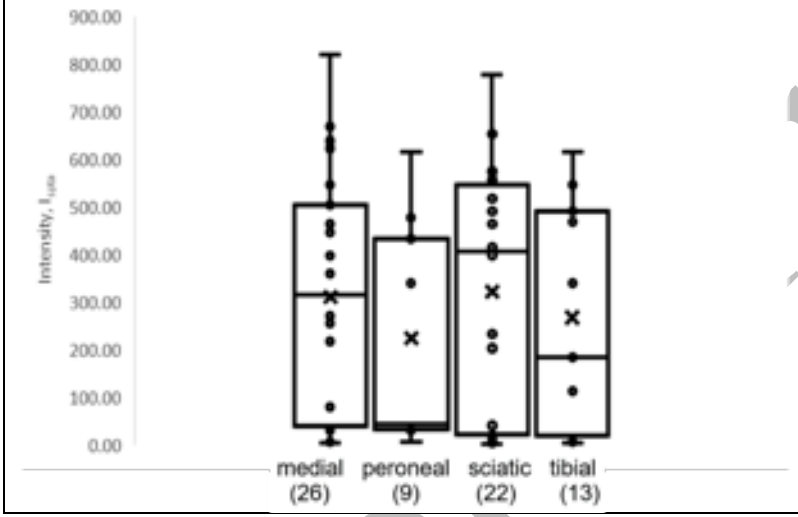



**Figure 3: Sensitivity of intact peripheral nerves to ultrasound stimulation pooled across test cohorts.** Intensity of ultrasound necessary to stimulate intact peripheral nerves of healthy participants and from the contralateral limb of standard and TMR amputee participants. (Kruskal-Wallis equality-of-populations rank test, Chi-squared=9.8 p> 0.27).

| | medial-$I_{spta}$ | peroneal-$I_{spta}$ | sciatic-$I_{spta}$ | tibial-$I_{spta}$ |
|---|---|---|---|---|
| Number (n) | 26 | 9 | 21 | 13 |
| Mean | 310 | 224 | 322 | 268 |
| Standard Error | 50 | 80 | 57 | 67 |
| Median | 316 | 43 | 407 | 184 |
| Standard Deviation | 255 | 241 | 265 | 241 |

**Table 1. Basic statistics for iFU intensity threshold value (iFU_t) for intact major nerve types, pooled across all participants**.

We then measured the iFU_t value of the intact nerves of amputee test subjects from each of standard and TMR test subject, to compare those values with that of the intact nerves of the healthy test subjects. We found no statistically significant differences in the intensity of stimulation of intact nerves between our three cohorts (Figure 4A and Table 2B). We note, however, the wide distribution of iFU_t values within the intact test subjects, with some iFU_t values comparable to that of transected nerves, but others much less sensitive. We also note the bimodal distribution of iFU_t values for the intact nerves of standard amputees, with some much more sensitive to iFU stimulation than the intact nerves of healthy test subjects. When separated out, iFU_t values for the 'sensitive' intact nerves from the standard amputees had an average value of 11.6+/-3.2 W/cm^2 while the iFU_t value for the remainder equaled 541+/-15 W/cm^2. This sub-analysis yielded a statistically significant difference among the groups (Figure 4B and Table 2B).

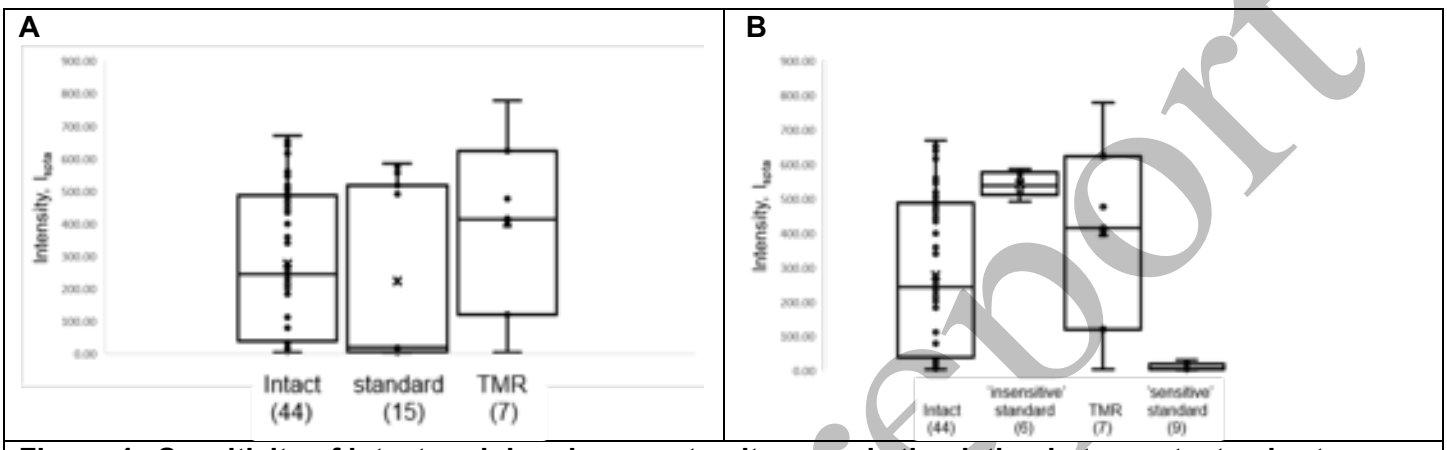



**Figure 4. Sensitivity of intact peripheral nerves to ultrasound stimulation between test cohorts.** **(A)** Intensity of ultrasound necessary to stimulate intact peripheral nerves within each of the three test subject cohorts (intact, healthy controls; standard amputees; TMR amputees), specifically pooled iFU_t values of intact nerves of healthy test subjects, pooled iFU_t values of the nerves in the intact limb contralateral to the residual limb of each of the standard and TMR amputee test subjects. Analysis revealed no significant differences in sensitivity between these three cohorts (Kruskal-Wallis equality-of-populations rank test, Chi-squared=3.28 $p \geq 0.19$). **(B)** When data from the 'sensitive' (N = 9) and 'insensitive' (N = 6) intact nerves from the standard amputee cohort were separated into their own groups, their iFU_t values differed in a statistically significant fashion from that of the other groups. (Kruskal-Wallis equality-of-populations rank test, Chi-squared=24.5 $p \leq 0.00001$).

| | Intact nerve, healthy test subject ($I_{spta}$) | Contralateral nerve, standard amputee ($I_{spta}$) | Contralateral nerve, TMR amputee ($I_{spta}$) |
|---|---|---|---|
| Mean | 318 | 223 | 402 |
| Standard Error | 44 | 70 | 102 |
| Median | 300 | 21 | 415 |
| Standard Deviation | 211 | 269 | 269 |
| Count | 23 | 15 | 7 |

| | Intact nerve, healthy test subject ($I_{spta}$) | Less sensitive, contralateral nerve, standard amputee ($I_{spta}$) | Contralateral nerve for TMR amputee ($I_{spta}$) | More sensitive, contralateral standard amputees ($I_{spta}$) |
|---|---|---|---|---|
| Mean | 318 | 541 | 402 | 12 |
| Standard Error | 44 | 15 | 102 | 3 |
| Median | 300 | 538 | 415 | 7 |
| Standard Deviation | 211 | 37 | 269 | 10 |
| Count | 23 | 6 | 7 | 9 |

**Table 2. Basic statistics for iFU intensity threshold value (iFU_t) for intact major nerve types (A) pooled within test subjects and (B) with a sub-population analysis for standard amputee test subjects.**

***iFU stimulation of transected nerves.***

We observed lower average iFU_t values for transected nerves relative to the intact nerves of healthy test subjects (Figure 5 and Table 3). Specifically, our data show a statistically significant difference between the ultrasound intensity value necessary to induce a sensation for intact controls (318 +/- 44 W/cm^2 $I_{spta}$) versus the transected nerves for each of the standard and TMR amputees (37+/-25 W/cm^2 and 92+/-51 W/cm^2 $I_{spta}$, respectively) (Figure 5 and Table 3). Our analysis did not, however, reveal a statistically significant difference in iFU_t values between standard and TMR amputees, although with a trend towards greater sensitivity of the standard versus TMR amputees.

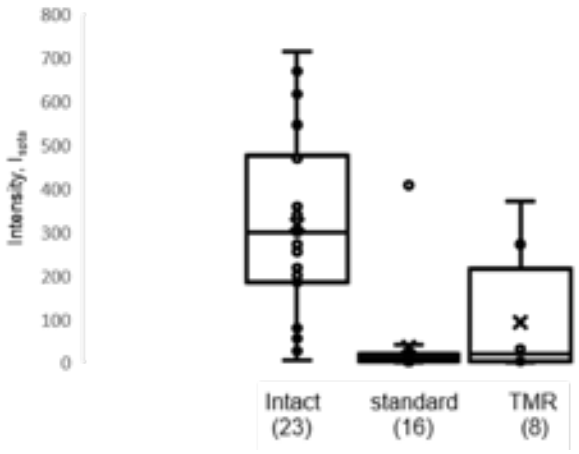



**Figure 5. Sensitivity of transected peripheral nerves to ultrasound stimulation relative to that of the intact nerves within healthy test subjects.** Analysis revealed a significant difference between the ultrasound intensity value necessary to induce a sensation in the intact nerve of healthy test subjects relative to standard amputees (Wilcoxon rank-sum, z=4.139 p<0.001) and relative to TMR amputees (Wilcoxon rank-sum, z=2.311 p<0.021), without, however a significant difference between iFU_t values for the transected nerve endings of standard versus TMR amputees (Wilcoxon rank-sum, z=0.391 p>0.35). Removal of the outlier from the standard amputee population did not change the results of this analysis.

| | Intact ($I_{spta}$) | Standard ($I_{spta}$) | TMR ($I_{spta}$) |
|---|---|---|---|
| Mean | 318 | 36 | 92 |
| Standard Error | 44 | 24 | 51 |
| Median | 300 | 9 | 19 |
| Standard Deviation | 211 | 99 | 144 |
| Count | 23 | 16 | 8 |

**Table 3. Basic statistics for iFU intensity threshold value (iFU_t) for pooled, intact major nerves within healthy test subjects relative to the iFU_t values for the transected nerves within each of standard and TMR amputees.**

Figure 5 partially obscures an interesting distribution of iFU_t values of the TMR test subjects relative to the nerves of intact healthy test subjects, displayed here through use of a Kaplan-Meier plot (Figure 6). For all intact test subjects and except for one standard amputee test subject, their iFU_t values distribute fairly evenly through a range of values (though neither distributions are Gaussian). In contrast, we note that a few TMR test subjects had iFU_t values substantially higher than that of the majority of the TMR and standard amputees.

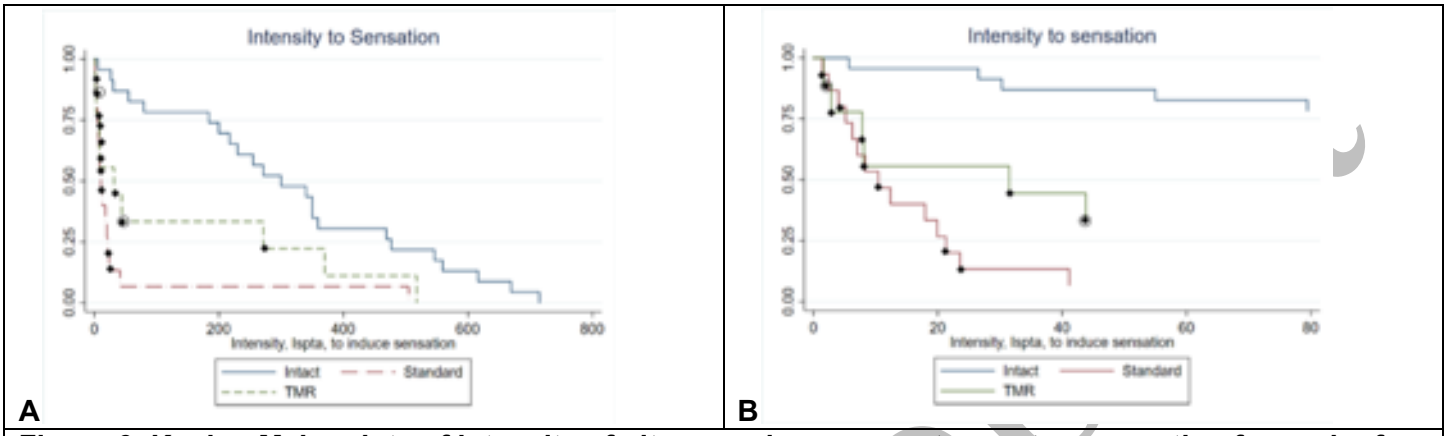



**Figure 6: Kaplan-Meier plots of intensity of ultrasound necessary to create a sensation for each of intact test subjects (intact nerves) and the transected nerves of standard amputees and TMR amputees. (A)** Shows a display of all of the data while **(B)** displays the data associated with intensity values below 80 W/cm^2. Circles identify test subjects with observable neuromas. Data points with black diamonds indicate test subjects deemed neuropathic via the LANNS pain score.

Next we developed a pair-wise comparison of the sensitivity to iFU stimulation of the ipsilateral, transected nerve and contralateral, intact nerve for each of the standard and TMR amputee cohorts. With regard to standard amputee test subjects, analysis of all the data showed that their transected nerves had greater average sensitivity to iFU stimulation than the contralateral, intact nerve (Figure 7A), even after removing the one outlier (Figure 7B). These figures highlight, however, the high sensitivity to iFU stimulation of a majority (9/15) of these test subjects (Figure 7C). Specifically, for this subset of standard amputee test subjects, their transected and intact nerves showed comparable and high sensitivity to iFU stimulation (Figure 7C). Of note, for this subset of test subjects, these iFU_t values show these nerves have much greater sensitivity to iFU stimulation than the intact nerves of almost all of the healthy test subjects (e.g., Figures 4-6).

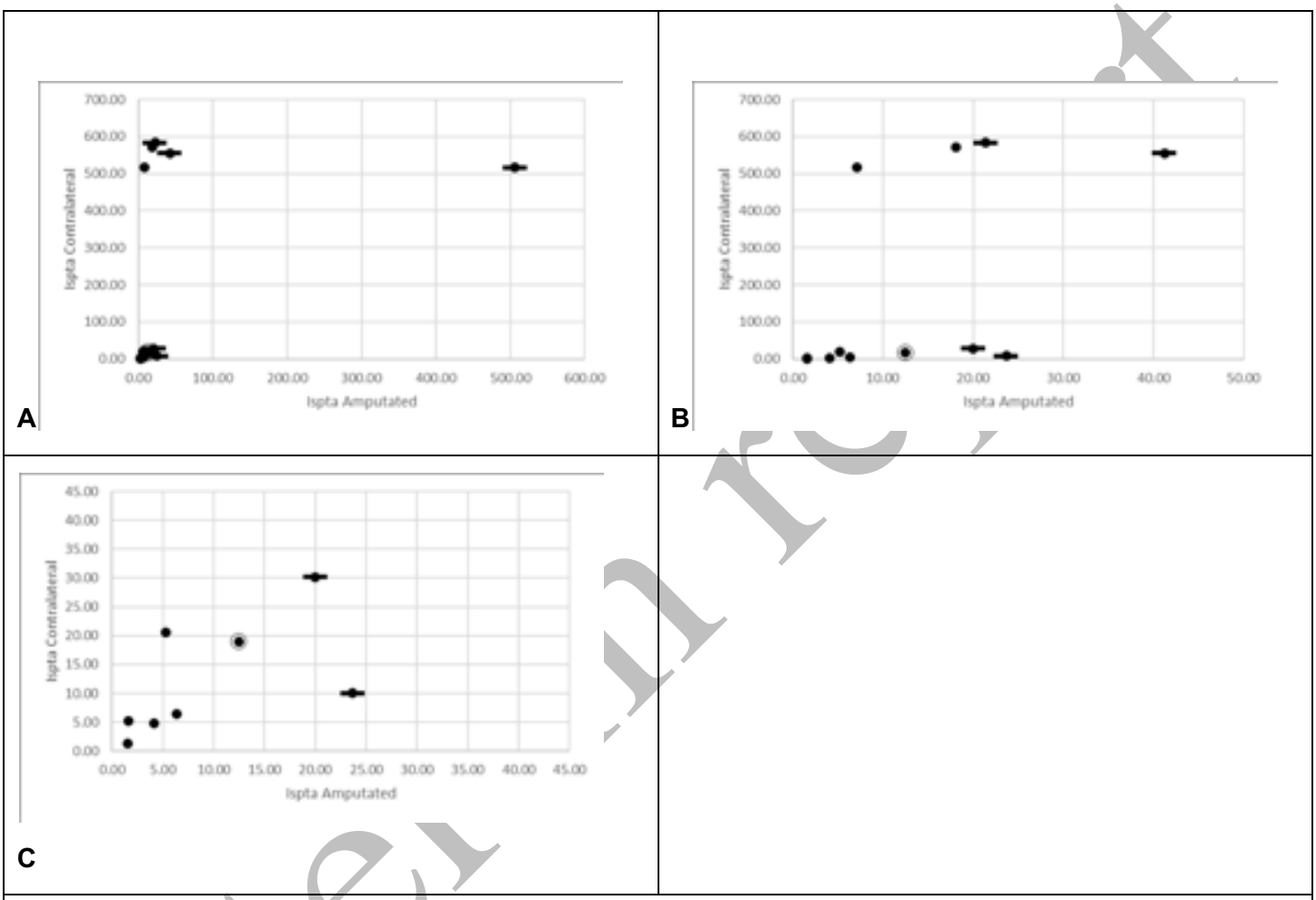



**Figure 7. For standard amputee test subjects, paired intensity values necessary to generate a discernable sensation (or maximum of the device) in transected (ipsilateral) and intact (contralateral) nerves, (A) for all test subjects, (B) for all data points save one, and (C) a close up of the data associated with the standard amputees most sensitive to ultrasound stimulation.** There was a significant difference in the intensity necessary to create a sensation for the intact versus paired transected nerve across all test subjects (Wilcoxon matched pairs signed-rank test, $z=2.587$ $p<0.01$ $N=13$). Removing the outlier, the paired comparison of ipsilateral and contralateral remain did not change these results. There exist a sub-set (C) of standard amputees that have comparable levels of sensitivity to iFU stimulation between their ipsilateral and contralateral limb (Wilcoxon matched pairs signed-rank test, $z=1.187$, $p>0.25$, $N=8$). Data points with a line through them indicate test subjects deemed neuropathic via the LANNS pain score. Data point with a circle denotes data from a test subject with a neuroma. Note the large difference in range of the ipsilateral versus contralateral data points in Figure 7B.

The same pair-wise comparison for the TMR test subjects showed a statistically significant difference, with their transected nerves much more sensitive to iFU stimulation than the paired, contralateral nerve (Figure 8).

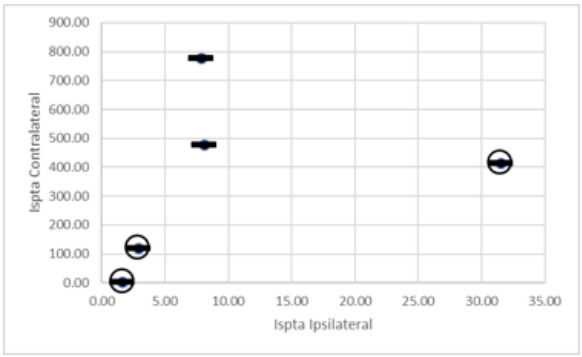
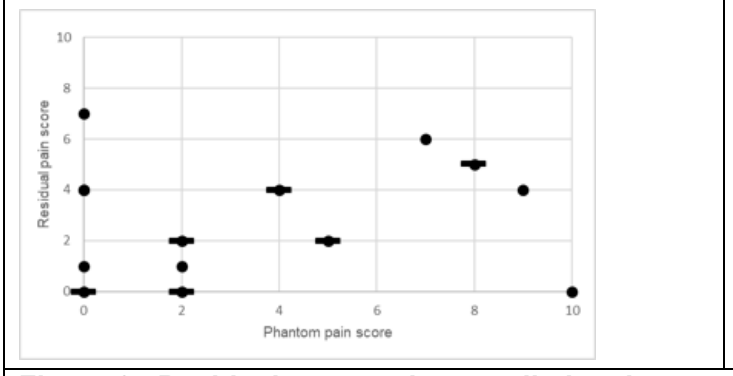



**Figure 8 For TMR amputee test subjects, paired intensity values necessary to generate a discernable sensation (or maximum of the device) in transected (ipsilateral) and intact (contralateral) nerves**. There was a significant difference in the intensity necessary to create a sensation with ultrasound for the intact versus paired transected nerve (Wilcoxon matched pairs signed-rank test, $z=2.023$ $p<0.05$ $N=5$). Data points with a line through them indicate test subjects deemed neuropathic via the LANNS pain score. Circled data points indicate test subjects with an observable neuroma. Note the large difference in range of the ipsilateral versus contralateral data points.

When broken down by amputation type, the standard amputees reported a wide range of values for their phantom and residual limb pain scores, without obvious pattern between those scores across all standard amputees (Figure 9A) other than an increasing average pain score for neuropathic standard amputees. Pain scores for TMR test subjects also showed a wide range of scores, without obvious pattern (Figure 9B). Finally, we did not observe a difference in the likelihood that amputee test subject would develop neuropathy as a function of their surgery type (Person Chi-squared test, z=1.73, p>0.15) with a trend, however, that favored the TMR procedure as less likely to produce neuropathy than the standard amputation procedure.



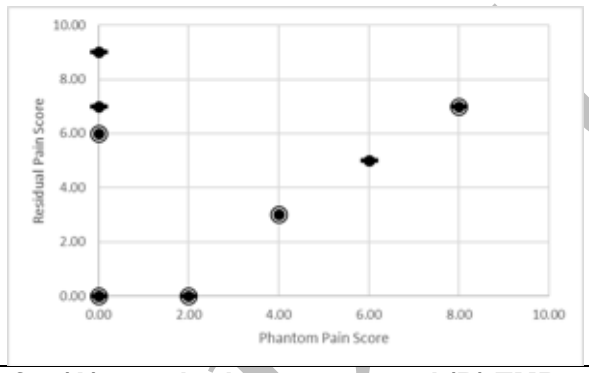
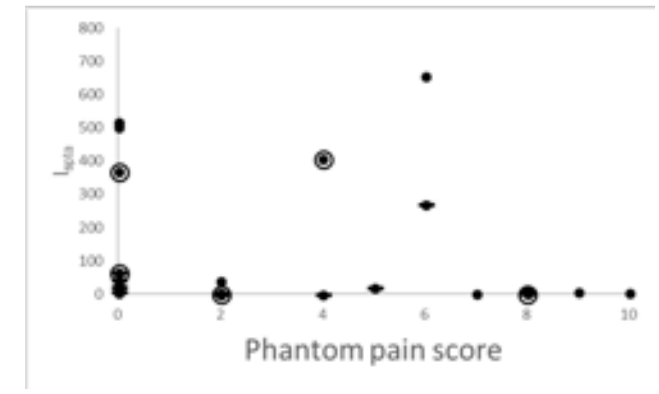




**Figure 9. Residual versus phantom limb pain score for (A) standard amputees and (B) TMR amputees.** *With regard to standard amputee test subjects,* there was no meaningful relationship between residual limb pain score and phantom limb pain score ($R^2$=0.078, f=0.943 p>0.3 N=13). This remained true for the non-neuropathic test subjects ($R^2$<0.0001, F<0.0001, p>0.95 N=8). However, an linearly increasing relationship does exist between residual pain score and phantom pain score within the neuropathic test subjects ($R^2$=0.729, F=10.78, p<0.035 N=6). *With regard to TMR amputee test subjects,* analysis revealed no linear relationship for all TMR amputation test subjects ($R^2$=0.064 F=0.549 p>0.4 N=10), nor for neuropathic TMR test subjects only ($R^2$=0.141 F=0.82 p>0.4 N=5). Data points with a line through them indicate amputee test subjects deemed neuropathic via the LANNS pain score. Data points with circles identify individuals with neuromas. Note that there exists two data points that overlap at the origin of Figure 9B.

**Add mean values here.** There does not exist a significant difference in residual pain score for standard amputees (median value = 2.0) versus those receiving the TMR procedure (median value = 3.0) – Wilcoxon rank-sum, z=0.694 p>0.45. Likewise, the difference in phantom limb pain score did not differ significantly between standard amputees (median value = 2.0) and TMR amputees (median value = 0), although the association is close (Wilcoxon rank-sum, z=1.331 p>0.18.)

With regard to pain experienced by *all* the amputees test subjects and its relationship to iFU stimulation, we did not observe a statistically significant linear trend in their iFU_t scores – Figure 10A,B.



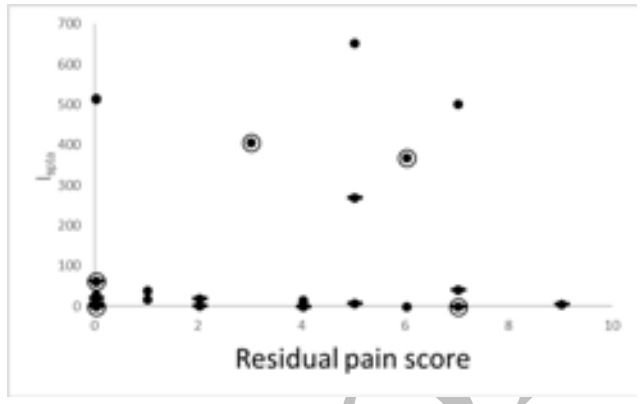




**Figure 10. Intensity value necessary to create a sensation in a transected nerve versus (A) phantom and (B) residual limb pain score, across all amputees.** Regression analysis examining phantom limb pain score versus intensity necessary to generate a sensation revealed no relationship across all standard amputation test subjects ($R^2$=0.012 F=0.33 p>0.6 N=28). Regression analysis examining residual limb pain score versus intensity necessary to generate a sensation revealed no relationship across all standard amputation test subjects ($R^2$=0.011 F=0.59 p>0.6 N=28). Data points with a line through them indicate test subjects deemed neuropathic via the LANNS pain score. Circled points highlight test subjects with observable neuromas.

We then compared the amount of iFU necessary to generate a sensation in the transected nerves of *standard* amputee test subjects with each of the phantom- and residual-limb pain scores, finding no linear trends (Figures 11,12, respectively).

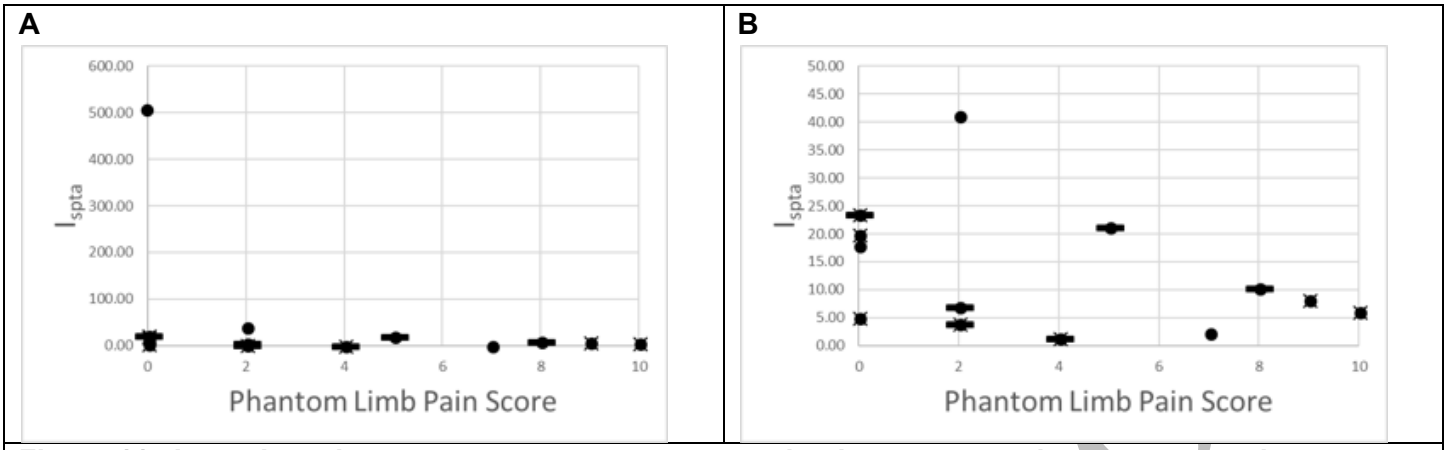



**Figure 11. Intensity value necessary to create a sensation in a transected nerve versus phantom limb pain score, (A) for all standard amputees and (B) for all standard amputees with outlier removed.** Regression analysis examining phantom limb pain score versus intensity revealed no relationship across all standard amputation test subjects ($R^2$=0.146 F=2.229 p>0.15 N=14) nor for neuropathic standard amputee test subjects alone ($R^2$=0.023 F=0.093 p>0.7 N=13). Repeating the analysis removing the outlier did not alter these relationships. Data points with a line through them indicate test subjects deemed neuropathic via the LANNS pain score.

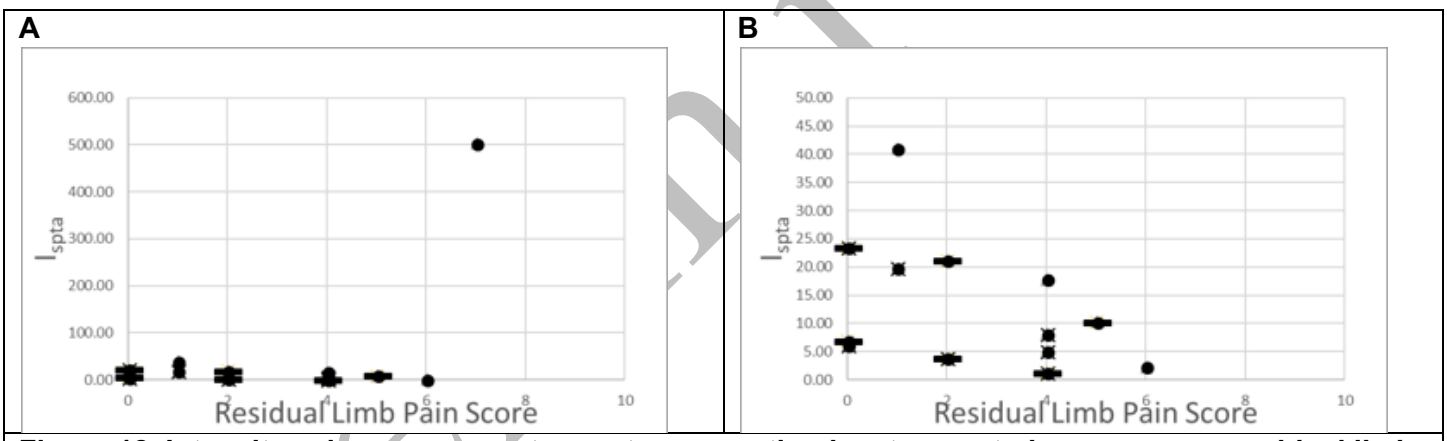



**Figure 12. Intensity value necessary to create a sensation in a transected nerve versus residual limb pain score, (A) for all standard amputees and (B) for all standard amputees but one.** Regression analysis examining residual limb pain score versus intensity revealed no meaningful linear relationship across all standard amputation test subjects ($R^2$=0.013 F=0.17 p>0.6 N=14), nor for the same analysis skipping the obvious outlier test subject ($R^2$=0.0228 F=0.0933 p>0.75 N=13), nor for neuropathic standard amputee test subjects alone ($R^2$=0.168 F=0.807 p>0.4 N=6). Data points with a line through them indicate test subjects deemed neuropathic via the LANNS pain score. An 'x' through a data point denotes those test subjects who had comparable values of iFU_t for their ipsilateral and contralateral limbs – Figure 7C.

The ipsilateral, transected nerve of seven standard amputees had comparably sensitive, ipsilateral nerves to iFU stimulation (Figure 7C). When analyzed as a separate cohort, we found trends towards a linear relationship between $I_{spta}$ and pain for each of residual pain ($R^2$=0.51 F=5.15 p>0.075 N=8) and phantom pain ($R^2$=0.31 F=2.28 p>0.2 N=8). Analyzing the remaining 4, skipping the obvious outlier, yielded no evidence of a relationship for phantom pain ($R^2$=0.005 F=0.01 p>0.95 N=4) or for standard pain ($R^2$=0.001 F=0.002 p>0.97 N=4).

These results for standard amputee test subjects did not change when taking into account the presence or absence of neuropathic pain as assessed by LAANS score (Figure 13).

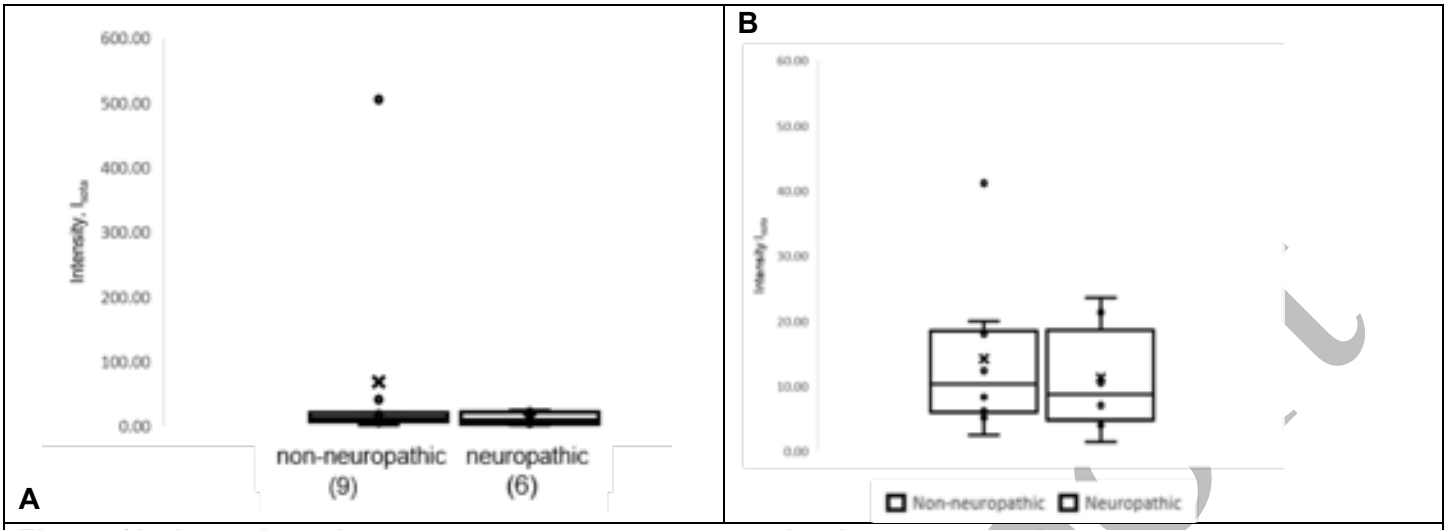
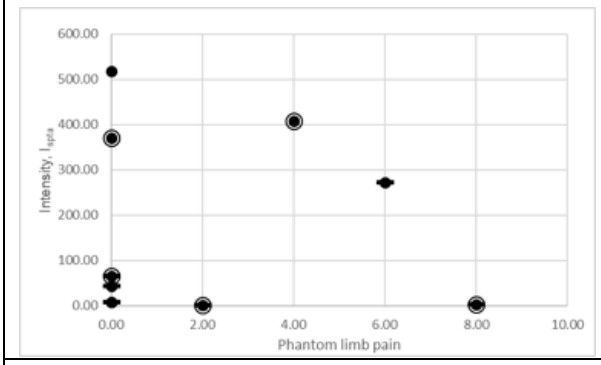



**Figure 13. Intensity value necessary to create a sensation in a transected nerve versus presence or absence of neuropathic symptoms as assessed by LANNS score, (A) for all standard amputees and (B) for all standard amputees but one. (A)** Analysis showed no significant difference between the intensity at sensation for neuropathic (N=6) versus non-neuropathic (N=9) test subjects. (Wilcoxon rank-sum, z=-0.0586 p>0.5). **(B)** This remained true after removing the obvious outlier.

We also compared the amount of iFU necessary to generate a sensation in the transected nerves of TMR amputee test subjects with each of the phantom and residual limb pain scores, finding no linear trends (Figures 14A,B, respectively).



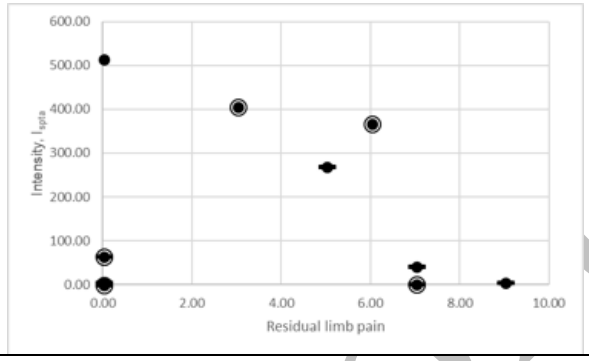




**Figure 14. Intensity value necessary to create a sensation in a transected nerve versus (A) phantom limb pain score and (B) residual limb pain score for all TMR amputees. (A)** Linear regression analysis examining *phantom limb pain* and intensity revealed no relationship across all TMR amputation test subjects ($R^2$=0.0004 F=0.003 p>0.9 N=10), nor for neuropathic TMR test subjects only ($R^2$=0.141 F=0.82 p>0.4 N=7). Similarly, **(B)** linear regression analysis examining *residual limb pain* and intensity revealed no relationship across all TMR amputation test subjects ($R^2$=0.029 F=0.236 p>0.6 N=10), nor for neuropathic TMR test subjects only ($R^2$=0.004 F=0.021 p>0.8 N=7). Data points with a line through them indicate test subjects deemed neuropathic via the LANNS pain score. Data points with circles identify individuals with neuromas.

Contrary to the results of the standard amputee test subjects (Figure 13), however, we observed a significant difference in iFU_t sensation between between non-neuropathic and neuropathic TMR test subjects (Figure 15).

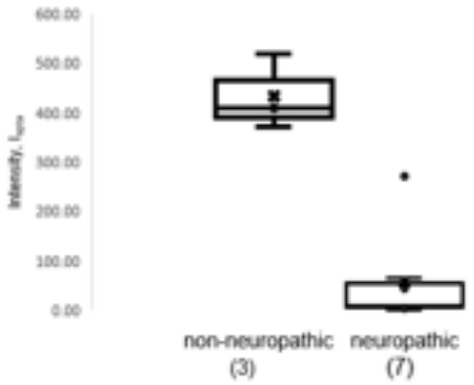



**Figure 15. Intensity value necessary to create a sensation in a transected nerve versus presence or absence of neuropathic symptoms as assessed by LANNS score, for all TMR amputees.** We detected a significant difference between the intensity value necessary to induce a sensation for neuropathic (N=7) versus non-neuropathic (N=3) TMR amputees (Wilcoxon rank-sum, z=-2.39 p<0.02). Removing the outlier maintained this statistically significant difference.

**Revised power analysis**

As of Winter 2018 we have successfully studied 23 intact volunteers, 16 standard amputees and 8 TMR amputees, a subset of the 45 test subjects from each cohort we have estimated we need to test the hypothesis that governs our study. With this preliminary data in hand, we have updated these estimates. We find that we still require 45 test subjects per cohort to detect a difference in the intensity of ultrasound necessary to induce a sensation in intact versus transected nerves via each of the standard method or TMR methods, with greater than 80% power to detect and an effect size of 0.6 at $p<0.05$. We now also estimate that this sample size would also provide 80% power to detect a correlation of 0.41 at $p<0.05$, e.g. in the relationship between and among phantom limb pain score, residual limb pain score and intensity of ultrasound to induce a sensation.

**Discussion.**

Anecdotal evidence suggests that targeted muscle reinnervation (TMR) surgery produces less pain than does standard amputation and that this occurs through production of a de-sensitized transected peripheral nerve. To test this idea, we assessed the phantom and residual limb pain, as well as likely presence of neuropathy, of two populations of amputees (standard and TMR); we also assessed the sensitivity of their major transected nerves to stimulation through use of image-guided intense focused ultrasound (ig-iFU) stimulation.

In contrast to the hypothesis governing this work to date, analysis of our preliminary data thus far does not show a difference in average phantom and residual limb pain between the two amputee populations. In addition, we have not yet found a difference in the average value of intense focused ultrasound necessary to induce a sensation between these two populations – on average their transected peripheral nerves have comparable sensitivity to iFU stimulation. Finally, based upon this new data we have confirmed that we need to collect more data from more standard and amputee test subjects to complete this analysis.

These results, while preliminary, point towards encouraging signs. We note, for example, that the peripheral nerves treated via the TMR method trended towards less sensitivity to iFU stimulation as compared to those who experienced a standard amputation. Also, 8/14 of standard amputees demonstrated comparable iFU_t values, hence comparable sensitivity to stimulation, for their ipsilateral and contralateral nerves. They suffer from allodynia. This stands in contrast to 6/15 of the standard amputees: heir iFU_t value for the transected nerve was substantially less than that of the contralateral nerve, hence, the intact nerve demonstrating much less sensitivity to stimulation compared to the transected nerve. This held true for 5/6 of the TMR test subjects. For those of 8/14 'sensitive' standard amputees, this comparable sensitivity may arise due to bilateral central sensitization within their spinal cord, while the others may have central sensitization in only the ipsilateral portion of their spinal cord. (While they may simply have very sensitive contralateral intact nerves, this seems unlikely, since there were very few intact test subjects with comparable sensitivity to iFU stimulation.) In addition, those TMR test subjects without neuropathy as given by the LAANS score had insensitive transected nerves relative to those TMR test subjects with neuropathy. This did not hold true for standard amputee test subjects. Finally, four out of 14 (28%) standard amputees did not report phantom limb pain while five out of nine (56%) of TMR test subjects reported no phantom limb pain – consistent with the larger median value in phantom limb pain score for standard versus TMR amputees. If these trends continue, they support the possibility that TMR surgical method may, indeed, reduce amputee's pain relative to a standard amputation, through desensitization of the transected nerve itself (a reduction in peripheral sensitization of the transected nerve ending) and/or reduction of central sensitization in the spinal cord ipsilateral to that nerve.

**Limitations.**

We assessed the tenderness of the transected nerve endings by stimulating them in a way that induced nerve-related sensations, but not pain, thereby studying allodynic contributions to these test subject's central sensitization but not hyperalgesic contributions, an area of potential future study.

We observed that a few healthy test subjects were sensitive to iFU stimulation in a way comparable to that of the intact *and* transected nerves in amputee test subjects. We also did not identify a useful trend in iFU_t value versus any pain score. Together, these results show it unlikely that the numerical value of iFU_t may one-day serve as useful *absolute* measure of test-subject's peripheral sensitization or allodynia. We note, however, that Garcia et al, demonstrated a diurnal variation in sensitivity to iFU stimulation of inflamed tissue, *in vivo*. While the absolute value of iFU_t may not relate to amputee's pain, perhaps one day ig-iFU may find utility in tracking the treatment of the pain of individual test subjects, another area of potential future study.

**Conclusion**

We have produced encouraging signs that TMR surgery reduces the pain experienced by amputees relative to standard amputation. Given our results to date and our power analysis, completion of our study should definitively test the governing hypotheses of our study, namely: TMR test subjects experience less residual and phantom limb pain than do standard amputees, in part because their transected nerve endings are less sensitive to external stimuli than those of standard amputees.