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Intense focused ultrasound as a potential research tool for the quantification of diurnal inflammatory pain

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ABSTRACT

Quantifying pain through assay of a human's or animal's response to a known stimulus as a function of time of day is a critical means of advancing chronotherapeutic pain management. Current methods for quantifying pain, even in the context of etiologies involving deep tissue, generally involve stimulation by quantifiable means of either cutaneous (heat-lamp tests, electrical stimuli) or both cutaneous and subcutaneous tissue (von Frey hairs, tourniquets, etc.) or study of proxies for pain (such as stress, via assay of cortisol levels). In this study, we evaluate the usefulness of intense focused ultrasound (iFU), already shown to generate sensations and other biological effects deep to the skin, as a means of quantifying deep diurnal pain using a standard animal model of inflammation. Beginning 5 days after injection of Complete Freund's Adjuvant into the plantar surface of the rat's right hind paw to induce inflammation, the rats were divided into two groups, the light-phase test group (09:00–18:00 h) and the dark-phase test group (23:00–06:00 h), both of which underwent iFU application deep to the skin. We used two classes of iFU protocol, motivated by the extant literature. One consisted of a single pulse (SP) lasting 0.375 s. The other, a multiple pulse (MP) protocol, consisted of multiple iFU pulses each of length 0.075 s spaced 0.075 s apart. We found the night group's threshold for reliable paw withdrawal to be significantly higher than that of the day group as assayed by each iFU protocol. These results are consistent with the observation that the response to mechanical stimuli by humans and rodents display diurnal variations, as well as the ability of iFU to generate sensations via mechanical stimulation. Since iFU can provide a consistent method to quantify pain from deep, inflamed tissue, it may represent a useful adjunct to those studying diurnal pain associated with deep tissue as well as chronotherapeutics targeting that pain.

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1. Introduction

Many symptoms of inflammation-based pain diseases such as rheumatoid arthritis and fibromyalgia exhibit circadian rhythms [41,30,3]. As such, the diurnal variation of pain has received much scrutiny, through direct study of humans as well as via animal studies (human studies: [1,6,3,42]; animal studies: [32,38,35,39,8,15,24,7]). For example, numerous studies of the hypothalamic–pituitary–adrenal (HPA) axis have shown that many painful diseases exhibit circadian rhythms due to contributions from chronic stress, leading to a particularly active area of research in this field [29,5,40]. With an appreciation of the existence of diurnal pain rhythms comes the motivation for chronotherapeutic approaches to the treatment of pain, with particular attention paid to maximizing drug effects by administration at optimal times of day [21,4,22,28,26].

Current methods for quantifying pain in research, even in the context of etiologies involving deep tissue, generally involve application of a quantifiable source of stimulation of to cutaneous (heat-lamp tests) or to both cutaneous and subcutaneous tissue (von Frey hairs, tourniquets, etc.) or, by monitoring proxies for pain such as stress [6,31]. However, these methods are often inaccurate or incomplete tests for pain originating in deep tissue because they either do not specifically quantify the pain source of interest, or because they are only surrogate measures of pain.

Quantifying pain is also of clinical importance, and new techniques for this have recently emerged. These include biochemical sampling at trigger points to examine "near real-time" concentrations of inflammatory markers and pH as compared with normal muscle tissue, as well as making use of magnetic resonance elastography and sonography to quantify variations in tissue stiffness as it relates to deep pain (reviewed in [2]).

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Previous studies assessing diurnal pain variation in rats and mice through physical tests have utilized hot-plate or light tests [8,15,27,24,7] as well as mechanical compression and electrical stimulation of the base of a rat's tail (for example, [27]). These approaches provide a useful metric for general pain measurement, but are non-specific and have little relevance for clinical usage. Moreover, there is little consensus in the literature about the specific trend observed in rodents regarding timing of highest and lowest sensitivity to stimulation. For example, some studies whose focus is heat stimulation have found that shortest latency times occur during the light-phase [15,8] and others during the dark-phase [7].

With these challenges of experimental design and clinical practice in mind, we sought here to take the first steps towards testing the potential usefulness of intense focused ultrasound (iFU) as a quantifiable source of stimulation, capable of reliably interacting with deep, painful tissue without stimulating adjacent tissues.

iFU has already been shown to stimulate deep tissue, focusing its ultrasonic energy in a manner consistent with mechanically-based stimulation of tissue within a spot approximately the size and aspect of a grain of rice [34], with its focus at a prescribed depth below the surface of the skin [9,47,11,18,16,17]. (Used with greater power, high intensity focused ultrasound – HIFU – can destroy deep tissue such as tumors without affecting intervening tissue—through a combination of heat [44,37,23] and cavitation, by [20], who also established the threshold for HIFU-induction of cavitation *in vivo*). Because the output of the transducer can be characterized through standard means one can quantify the amount of iFU delivered to the tissue of interest [43,19]. In this way, iFU may be used to quantify thresholds for stimulation of deep tissue in anatomically specific way, giving iFU potential applicability for both researchers and clinicians.

While already shown to stimulate deep, healthy tissue, iFU has not been used to differentially stimulate inflamed tissue, nor has that stimulation been shown to vary in a diurnal way. We demonstrate both, here. Specifically, we have tested the hypothesis that stimulation generated by each of a single acoustic pulse as well as a series of iFU pulses preferentially stimulates inflamed tissue relative to contralateral tissue in an animal model of inflammatory pain, and that the amount of iFU necessary to stimulate inflamed tissue exhibits a diurnal pattern. As such, our results suggest that researchers could use iFU as a way to quantify diurnal pain patterns from deep tissue by providing a consistent method by which researchers can accurately and objectively stimulate deep inflamed tissue.

2. Materials and methods

2.1. Animals

All animal procedures were approved by the Institutional Animal Care and Use Committees (IACUC) of both the University of Washington and the Veterans Administration of Puget Sound as well as conformed to relevant national guidelines.

Adult male Fischer rats (Charles River) weighing approximately 180 g were housed 3 per cage under housing conditions of 12 h light:12 h dark (light on at 06:00 h and light off at 18:00 h) and temperature of 20–22 °C. Animals were kept at this standardized light/dark regimen for at least 1 week to establish synchronization. The animals had free access to food and water.

2.2. Animal model of peripheral inflammatory pain

Thirty-two adult male Fischer rats (approximately 180 g, Charles River) were used for the study. The rats were deeply anesthetized with a 5% isoflurane (Pitman-Moore, Mundelein, IL) and oxygen mixture via nose cone for induction and 2% isoflurane for maintenance of the anesthetic plane. Inflammation was induced using methods adapted from Nagakura et al. 0.2 ml of Complete Freund's Adjuvant (CFA, Sigma Aldrich) was injected subcutaneously over 45 s into the plantar surface of the right hind paw at the base of the toes using a 25 g 5/8" needle. This produced significant inflammation throughout the right hind paw—from skin to periosteum—relative to the left [35].

2.3. Ultrasound devices and acoustic protocols

In order to apply ultrasound for stimulation we used the inner element (22.6 mm inner diameter, 48.5 mm outer diameter) of a two-element, 2 MHz annular array transducer (H-106 S/N-01, Sonic Concepts, Inc., Woodinville, WA), placed within a brass housing that facilitated hand-held deployment of the device. The radius of curvature of the device measured 62.6 mm. We quantified the focus of the device (Fig. 1) using numerical simulations [25,36], using MATLAB, The Mathworks, Inc, Natick, MA), appropriate at the relatively high intensities given off by our transducer [43,19]. The transducer had its focus at 7.0 mm beyond the proximal surface of the device, with less than 20% of the intensity of the ultrasound at the focus found at that surface.

The transducer was driven by two function generators (33120A, Hewlett Packard/Agilent, Palo Alto, CA) and an amplifier (A150 RF Power Amplifier, ENI, Chesnut Ridge, NY). The first generator gated the pulse to a specific duration. The second generator, in series with the first, modified the acoustic output and ensured that the pulse was emitted at a specific frequency. The amplifier increased the signal from the function generators and sent it to the solid cone device. An oscilloscope (Wave Runner LT 322, LeCroy, Chesnut Ridge, NY) measured the duration of the pulse, its carrier frequency and the voltage delivered to the iFU device by the amplifier during each experiment. This voltage was correlated to acoustic intensity emitted by the iFU device via a 'force balance' technique [43,19]. In particular, the displacement of a scale produced by ultrasound energy emitted by the device, along with mathematical calculations of the spatial distribution of ultrasound energy (the half-maximum-pressure contour), is translated mathematically into a measure of intensity (I_{SATA}). Specifically, I_{SATA} is the spatially and temporally averaged intensity over the area enclosed by the half-pressure-maximum contour in the focal plane, a standard measure of ultrasound intensity. We have also measured the peak positive and peak negative pressures associated with representative intensity values (Table 1) using a calibrated hydrophone (Onda, Sunnyvale CA), using linear extrapolation at large values of pressure and intensity.

The paw withdrawal data were collected for two acoustic protocols. One protocol consisted of multiple pulses (MPs) made up of five 0.075-s pulses spaced by 0.075 s, similar to those previously used by other researchers [9,47]. The other acoustic protocol consisted of a single pulse (SP) with length of 0.375 s, motivated by protocols explored by Gavrilov, Wright, Dalecki and colleagues [47,10,11,18,45,46].

2.4. iFU application to rats

Beginning 5 days after CFA injection, the rats were divided into two groups, the light-phase group and the dark-phase group. The testing was done in the time between 10:00 h and 16:00 h for the light-phase group and between 23:00 h and 04:00 h for the dark-phase group.

We habituated sets of three rats to their free-ranging presence within individual cages containing three separate enclosures, each with a mesh bottom whose individual holes were large enough to

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