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A review on ultrasonic neuromodulation of the peripheral nervous system: enhanced or suppressed activities?

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Abstract

Ultrasonic (US) neuromodulation has emerged as a promising therapeutic means by delivering focused energy deep into the tissue. Low-intensity ultrasound (US) directly activates and/or inhibits neurons in the central nervous system (CNS). US neuromodulation of the peripheral nervous system (PNS) is less developed and rarely used clinically. Literature on the neuromodulatory effects of US on the PNS is controversy with some documenting enhanced neural activities, some showing suppressed activities, and others reporting mixed effects. US, with different range of intensity and strength, is likely to generate distinct physical effects in the stimulated neuronal tissues, which underlies different experimental outcomes in the literature. In this review, we summarize all the major reports that documented the effects of US on peripheral nerve endings, axons, and/or somata in the dorsal root ganglion. In particular, we thoroughly discuss the potential impacts by the following key parameters to the study outcomes of PNS neuromodulation by the US: frequency, pulse repetition frequency, duty cycle, intensity, metrics for peripheral neural activities, and type of biological preparations used in the studies. Potential mechanisms of peripheral US neuromodulation are summarized to provide a plausible interpretation to the seemly contradictory effects of enhanced and suppressed neural activities from US neuromodulation.

Keywords

ultrasound; neuromodulation; single-unit recording; pain; sciatic nerve; compound action potential

1. Introduction

Our nervous system consists of the central and peripheral nervous systems (CNS, PNS), which has similar ion channel/modulator compositions (1, 2). In the CNS, functional neural circuits implicated in different neurological diseases overlap significantly with one another

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Featured Application: The current review summarizes our recent knowledge in ultrasonic neuromodulation of peripheral nerve endings, axons and somata in the dorsal root ganglion. This review indicates that focused ultrasound at intermediate intensity can be a non-thermal and reversible neuromodulatory means for targeting the peripheral nervous system to manage neurological disorders.

(e.g. overlapping circuits for dementia, aphasia, and Alzheimer's disease (3)), and certain neural circuits are not restricted in one region but spread throughout the brain (e.g., the pain matrix (4)). Collectively, this has led to the challenges of developing selective and effective drugs that target certain neurological diseases with limited off-target side effects. Recent advancement of optogenetic neuromodulation offers the much-needed selectivity at the expense of invasive and permanent gene modification of neural tissues. On the other hand, neuromodulation with focal delivery of physical energy to affected area in patients has drawn growing attention as a non-drug alternative for managing neurological diseases and symptoms.

The most widely used stimulus modality in neuromodulation is electrical stimulation, including electrical stimulation of the brain for treating movement disorders, stroke, tinnitus, depression, and addiction (5), as well as, stimulation of the spinal cord and peripheral nerves for managing various types of chronic injuries and pain (6–8). Besides electrical stimulation, other modalities of stimuli have been implemented that allow no-contact delivery of physical energy deep into the neuronal tissue, including focused ultrasound (9), transcranial electromagnetic stimulation (TMS) (10, 11), and infrared light pulses (12). TMS uses a strong magnetic field of 2 to 3 T to evoke current pulses in the tissue, but TMS technique faces challenges in precise localization of activated areas in the brain due to the electrical and magnetic anisotropy of the brain and skull tissues. Infrared light pulses affect the neural activities by delivering a spatially precise thermal stimuli, but the local heating of the targeted region remains a concern to the method. On the other hand, ultrasound (US) as a mechanical wave operating at 250 KHz to 50 MHz allows spatially and temporally precise delivery of energy deep into the tissue with controllable heating. Hence, US can be considered as an ideal means for non- or minimally-invasive neuromodulation technique.

The effects of US on the CNS have been shown to disrupt the blood brain barrier and evoke excitatory and/or inhibitory responses in both the motor and sensory neurons (13–23). Outcomes of those researches have led to the successful translation of US stimulation, being approved by the U.S. Food and Drug Administration (FDA), to treat medical-refractory patients with essential tremor (24). Electrophysiological recording from the rat hippocampal dentate gyrus has been reported to simultaneously enhance (at fiber volley) and suppress (at dendritic layers) the compound action potentials (CAP) in response to US stimulation (25). In support, another ex vivo study based on calcium imaging, shows low-intensity focused pulsed US can evoke electrical activities in the mouse hippocampal slices (26). However, two most recent studies suggest that US neuromodulation of CNS does not directly activate brain regions in mice, but through an indirect auditory cochlear pathway (27, 28). Nonetheless, these recent results from whole organ and whole animal studies do not invalidate the prior studies in reduced systems showing apparent neuromodulation by focused US without a functioning auditory system, like C. elegans, tissue culture, retina (29), and brain slices (reviewed in (9)).

Peripheral neuromodulation targets the PNS to preclude off-target CNS effects, and thus is even more selective than the CNS neuromodulation. PNS neuromodulation is particularly appealing to treat chronic pain, as the pain circuitry in the CNS is complex and wide spread. State-of-the-art peripheral neuromodulatory strategies to treat chronic pain include 1) spinal

cord stimulation that targets peripheral nerve entry to the spinal cord (8, 30), 2) peripheral nerve field stimulation that targets a region of tissue (31, 32), 3) direct peripheral nerve stimulation (33), and recently, 4) dorsal root ganglion (DRG) stimulation of sensory neural somata (34, 35). The US neuromodulation of the PNS is far less advanced compared to the CNS counterpart (see (36) for a review), and its mechanisms of action remain unclear. In this paper, we comprehensively review the methods and outcomes of studies on US effects in altering neural activities in the peripheral nerve axons, endings, and DRG. The seemly contradictory effects of US on peripheral nerves are discussed in the context of the different study designs and methods. In addition, we summarize the existing theories that account for the effects of US neuromodulation on peripheral nerves and DRG.

2. Peripheral ultrasonic neuromodulation – technical specifications

US waves are acoustic waves caused by mechanical vibrations at frequencies above 20 kHz (the upper hearing range of the human ear). The frequency (f_0) of the mechanical vibration source determines the frequency of the propagating US wave. US waves propagate through both the liquid and solid media, in the form of vibrating media particles, with a governing equation as follows:

$$\nabla^2 p - (\rho\kappa)\frac{\partial^2 p}{dt^2} = 0 \quad (1)$$

In which p is the media pressure, ρ is the media density, and κ is the media compressibility. The US wave speed c is equal to $1/\sqrt{\rho\kappa}$. For a simple monochromatic vibration source, the pressure of the US wave derived from Eq. (1) takes the form of a harmonic plane wave:

$$p(r,t) = A\cos(2\pi f_0 - kr) \quad (2)$$

In which r is the distance vector from the source, A is the amplitude of the wave, and k is the wave number and equal to $2\pi / \lambda$.

Our current understandings of PNS neuromodulation are limited by the controversial outcomes from different experimental studies, which are most likely affected by several critical parameters relevant to the US itself, as well as, the metrics to evaluate the US neuromodulation. Accordingly, the following parameters will be discussed in details in this review: US frequency, pulse repetition frequency, duty cycle, intensity, metrics for peripheral neural activities, and type of biological preparations.

2.1. US frequency

The frequency of the US wave is determined by the central frequency of the acoustic vibration source, generally a US transducer. The US frequency used in the biomedical field ranges from 0.25 to 50 MHz. The intensity of US beam attenuates exponentially with the propagation distance due to both absorption and scattering processes. Bones, especially cancellous bones cause more severe US scattering than soft tissues due to their material heterogeneity. Also, the US absorption coefficient is much higher in bones than in the soft

tissues. In addition, higher frequency components are prone to relatively rapid attenuation. Hence, to penetrate the bony skull and skin, non-invasive US neuromodulation generally operates at a lower frequency range from 0.5 to 3 MHz (36). In contrast, high frequency US (>3 MHz) is used in invasive surgeries to ablate tissues by implementing its high absorption coefficients to heat the local tissues (37). In addition, high frequency US at low intensity is widely used in US imaging to enhance resolution via reduced wavelength in sub-millimeter range. One exception for the use of high frequency US in neuromodulation (up to 43 MHz) is the activation of the retina with high spatial resolution for vision restoration (38) in which the penetration of the skull is not required.

2.2. Pulse repetition frequency

US neuromodulation usually does not use continuous wave, but burst of waves with certain pulse-width as shown in Figure 1. The pulse repetition frequency reflects the frequency of the burst which can be orders of magnitude lower than the US frequency. The pulse width can be as short as a few US cycles and as long as the pulse repetition period. The pulse repetition frequency adds an additional frequency component in the spectrum of the US wave and can be critical in activating auditory nerve endings and other low-threshold mechanoreceptors.

2.3. Duty cycle

The duty cycle is defined as the ratio between the pulse width and the pulse repetition period, which is generally less than 2% for diagnostic US imaging devices (39), but can be as high as 100% in US neuromodulation.

2.4. Intensity

The strength of an ultrasonic wave is characterized by its intensity usually in the unit of watts per centimeter square (W/cm^2), i.e., the average power per unit cross-sectional area evaluated over a surface perpendicular to the propagation direction. For acoustic plane waves, the intensity is related to the pressure amplitude by:

$$I\left(W/\mathrm{cm}^2\right) = \frac{p^2}{2}\sqrt{\frac{\kappa}{\rho}} = \frac{p^2}{2\rho c}$$

As shown in Figure 1A, the spatial distribution of the US intensity peaks at the focal location and attenuates quickly outside the focus. To evaluate the US intensity in neuromodulation, spatial peak (SP) intensity is more widely used than the spatial average (SA) intensity. In the time domain, the instantaneous intensity can be calculated from the pressure plot as shown in Figure 1B. The temporal peak (TP) intensity is the maximum US intensity, the pulse average (PA) intensity is the average intensity within the pulse width, and the temporal average (TA) is the average intensity for several pulse repetition cycles. In experimental and clinical studies, the US intensity is generally quantified as spatial peak temporal peak- (SPTP), spatial peak pulse average- (SPPA), and spatial peak temporal average- (SPTA) intensities. In order to compare between studies, it is worth emphasizing that, for the same US wave, the magnitude for I_{SPTP}, I_{SPPA} and I_{SPTA} is in descending order, and I_{SPTP} can be orders of magnitude higher than I_{SPTA} for US pulses with low duty cycle.

Physically, I_{SPTP} reflects the highest spatial intensity in the US beam and is closely related to potential mechanical effects and cavitation in the tissue. I_{SPTA} measures the highest spatial intensity averaged over the pulse repetition period and is related to the magnitude of thermal effect. US neuromodulation generally operates at low intensity levels to avoid cavitation, and is more concerned with the local thermal effect. Thus, I_{SPTA} appears to be a more suitable intensity indicator for peripheral neuromodulation studies to avoid any prominent thermal effectors.

US stimulation of I_{SPTA} below 1 W/cm^2 is generally considered as low-intensity, and the FDA has approved the application of US in patients with a maximum I_{SPTA} of 0.72 W/cm^2 in diagnostic purpose which is, presumably, safe enough for therapeutics (40). Several studies have reported neuromodulatory effects on CNS with US intensity < 1 W/cm^2 (15, 20–22, 26). So far, there seems to be no studies reporting appreciable neuromodulatory effects on PNS with US intensity < 1 W/cm^2 (41). On the other hand, I_{SPTA} over 200 W/cm^2 is generally considered as high-intensity US, which has been tested in a number of clinical trials (42, 43), after being approved by the FDA for the ablation of cancer cells in patients via local elevation of temperature up to 85 °C (42, 44). High intensity focused US is also an approved tool by the FDA for coagulative necrosis in the brain to create stereotactic lesions, also an irreversible ablation process (24).

We, along with some other research groups have demonstrated that, US stimulation on the PNS, between I_{SPTA} of 1 and 200 W/cm^2, is unlikely to induce sufficient temperature change in the target region to elicit temperature-driven neuromodulation(41, 45). This intermediate intensity range has been explored by several neuromodulation studies on PNS, which have been systematically reviewed in the subsequent sections.

2.5. Metrics for peripheral neural activities

Assessment of the neuromodulatory effects of US requires a reliable metric of peripheral neural activities, which includes direct and indirect measurements of neural action potentials from peripheral nerves or neurons. In addition, secondary effects of neural activities were also used to indirectly infer the neuromodulatory effects on PNS, including altered organ functions (i.e., bladder contraction, urethral sphincter relaxation), electroencephalogram recordings in the brain, and behavioral signs (e.g., whisker movement, freeze in motion, toe-pinch response). Detection of secondary effects tends to lag the US stimulation by hundreds of milliseconds to seconds, a time frame much slower than the direct neuronal effects of milliseconds (36). This review will mostly focuse on the direct assessment of neural action potentials and omit metrics using secondary effects.

Nerve axons in the PNS are generally protected by soft connective tissues stacked in multiple layers, in contrast to the neurons and processes in the CNS with the protection of bony structures like the skull or the vertebrae. These tightly wrapped tissue layers in the PNS, functioning as electrical insulators, pose a great challenge in recording electrophysiological activities from individual nerve axons, i.e., single-unit recordings (46); recordings from individual neurons or axons in the CNS are straightforward when the electrodes are placed inside the skull or vertebrae. Consequently, the major metrics to assess the neuromodulatory effects on the PNS are either compound action potentials (CAP) as a

summation of action potentials from a bulk nerve bundle (45, 47–50), or evoked muscle forces as an indicator of motor nerve functions (40, 51, 52). However, the characteristics of a CAP (peak amplitude, temporal location and spread) depends on the temporal summation of a population of action potentials from axons with various spatial locations, morphologies and insulation environment. The CAP-characteristics can also be affected by the changes in recording conditions, e.g., relative position of the electrode sites and axons, change of access impedance of recording electrodes due to altered moisture conditions and multiple/chronic use of electrodes, etc. (46). Thus, neither the changes in CAP amplitude nor the assumed changes in conduction delay (Figure 2A) can appropriately be representative of effects of US neuromodulation. In addition, the signal strength in a CAP record can be misleading as shown in Figure 2A : the large peak contributed to by fast-conducting A-fibers usually overshadows the small volleys by slow-conducting C-fibers despite significantly higher proportions of C-fibers than A-fibers in the PNS (53, 54). Collectively, CAP appears to be an inappropriate metric for PNS neuromodulation. Further, muscle forces, evoked by US neuromodulation of a nerve, are indirect metrics of neve activities and limited to the study of motor axons innervating specific muscles.

On the other hand, single-unit recording can record action potentials from individual nerve axons (Figure 2B), capable of capturing relatively fine variations of neural responses to mechanical, chemical, and/or thermal stimuli (55–57). Single-unit recording relies solely on the temporal information of the spike, which is mainly determined by the action potential transmission and not affected by experimental artifacts like changes in electrode impedance. Thus, compared with CAP, single-unit recordings are more robust and provide much higher sensitivity to allow the detection of subtle changes of conduction delay in individual axons. However, single-unit recordings are technically challenging and involve microdissection of nerve fiber bundles until action potentials from a single nerve axon are isolated (41, 46).

In general, the CAP recordings and evoked muscle activities are “macroscopic” detections of a large population of peripheral neural activities, and thus may not serve as reliable and sensitive metrics for assessing the subtle changes of individual neural activities. The single-unit recordings possess a much higher sensitivity able to detect neuromodulatory effects within individual nerve axons, i.e., at “microscopic level”. However, single-unit recordings are technically challenging and have only been implemented by a handful of studies (41, 58–66), whereas CAP recordings were widely used. Among the above single-unit studies, neuromodulation by ultrasound was assessed only by a recent study from us (41).

2.6. Biological preparations to assess peripheral US neuromodulation

Peripheral US neuromodulation has been studied both in human and preclinical animal models. Human studies benefit from the direct verbal feedback from the subjects while preclinical animal studies allow mechanistic investigation *in vivo* as well as with reduced and isolated *in vitro* or *ex vivo* systems. Studies that implement whole animal *in vivo* preparations require to maintain anesthesia, the level of which can directly affect the detection of US neuromodulation (20). In addition, interpretation of the results from *in vivo* studies can be confounded by indirect effects of US on surrounding muscles, blood vessels and immune cells. *In vitro* cultures of dissociated sensory neurons from the dorsal root

ganglion (DRG) have also been used as a model to study the effects of US neuromodulation (67). Although the sensory cell somata in the DRG differ significantly from the nerve axons and endings in fundamental electrophysiological properties (57), the neurite outgrowth in cultured DRG has similar dimensions as axons and nerve endings and thus could potentially be an adequate model for studying peripheral neuromodulation. Nonetheless, cultured neurites in the absence of Schwann cells lack clustering of sodium channels (68) and thus differ significantly from the bundled axons in peripheral nerves in the physiological situation. In contrast, the *ex vivo* studies on isolated peripheral nerves offer a physiologically relevant model for direct assessment of the US neuromodulatory effects while avoiding the potential confounding factors in the *in vivo* or *in vitro* studies. It is worth mentioning that, many studies documented isolated peripheral nerves as *in vitro*, which will be considered as *ex vivo* in this review to separate from the *in vitro* cultured DRG preparations.

3. Ultrasonic neuromodulation of the peripheral nervous system

The effect of US stimulation to alter tissue activities was reported as early as in 1929 by a study on frogs and turtles (69). The ability of low-frequency low-intensity US to modulate the CNS neural activities was elegantly demonstrated both *in vivo* in whole animal and *in vitro* in reduced systems of brain tissue slices (see (9) for a review). Recent studies indicate that US neuromodulation of the CNS might take an indirect route through the auditory cochlear pathway (27, 28). In stark contrast, the mechanisms of US neuromodulation of the PNS are still under debate partly due to the contradictory experimental outcomes, which are systematically summarized and discussed below.

As summarized in Table 1, the US appears to directly activate peripheral sensory nerve endings as evidenced by studies of US stimulation of human hand, skin, soft tissues, bones, joints, ears, acupuncture points (51, 70–73) as well as cat ear (74) and frog Pacinian corpuscles (75). CAP activities were evoked by US stimulation in animal preparations. More convincing evidences have been demonstrated by the clinical studies from which direct verbal reports showed that US stimulation was able to evoke virtually all the somatosensory modalities: tactile, warm, cold, itch, Deqi, hearing and pain. Unlike in the CNS, low-intensity US (<1 W/cm^2) was unable to activate mammalian nerve endings. In non-mammals, dissociated frog Pacinian corpuscle can be activated by US with intensity as low as 0.4 W/cm^2. US with intermediate intensity (1 – 200 W/cm^2) activates only low-threshold mechanoreceptors, e.g., tactile receptors and auditory nerve endings. Activation of other sensory modalities like temperature and pain generally requires high-intensity US stimulation (>1000 W/cm^2). However, it remains unclear whether US inhibits sensory nerve endings, which unlike neurons in the CNS generally do not fire spontaneously. Further experimental studies are required to investigate whether US application to the sensory nerve endings could lead to the loss of sensation.

US neuromodulation of peripheral nerve trunks and axons has been investigated by a handful of studies as summarized in table 2, which gives seemly contradictory results with reports of enhanced nerve activities, suppressed activities and mixed effects. Nonetheless, there are two consistent observations. First, all studies documented no direct activation of peripheral nerves by US stimulation alone except a recent abstract report lacking technical

details (76). Second, high-intensity US stimulation (>200 W/cm^2) causes nerve conduction block likely from a local thermal effect, and the nerve blocking effect can last for days to weeks and even be completely irreversible (48, 52, 77). Lee et al., did report reversible conduction block with proper selection of the stimulus parameters (78). The contradictory results occur with US intensity at the intermediate range, with enhanced nerve conduction velocity by some reports (41, 49, 79) and suppressed conduction velocity by others (45, 80); Mihran also reported that US enhanced the CAP amplitude, thus providing mixed outcomes (80). The difference in US neuromodulation effects could be attributed to the difference in the types of nerves studied (e.g., sciatic vs. vagus), different preparations (e.g., *ex vivo* vs. *in vivo*), and different animal species (e.g., mammals vs. non-mammals). The CAP recordings were used to evaluate the US effects except for one study in which single-unit recordings from individual axons were implemented (41). As discussed earlier, single-unit recordings are more sensitive in detecting neuromodulatory effects than the CAP recordings. Collectively, the study by Ilham et al. conducted on harvested nerve *ex vivo* with single-unit recordings has the least confounding factors and thus provides the most convincing results: US stimulation of intermediate intensity enhances the peripheral nerve activity by increasing the conduction velocity in both A- and C-type axons (41). This is further supported with a clinical study showing increased conduction velocity in human median nerves following US stimulation (79).

The sensory afferent somata in the DRG has emerged as a promising target for neuromodulation (81–83). To the best of our knowledge, all existing DRG neuromodulations implement electrical stimulation, and the modality of US has yet to be investigated on DRG. A recent pilot report with patch-clamp and calcium imaging recordings on dissociated DRG neurons showed that US evokes action potentials in 33–40% DRG neurons, which may involve the activation of sodium, calcium and non-selective ion channels (67).

4. Mechanisms of peripheral US neuromodulation

Mechanisms of action for US to modulate neural activities in the CNS have been systematically reviewed previously (9, 36). Here we focus on the US neuromodulation of peripheral nerve endings, axons and somata in the DRG, which unlike the bone-protected CNS are tightly wrapped by multiple layers of connective tissues, e.g., the epineurium, perineurium, endoneurium, and nerve-extracellular matrix. This might explain why low-intensity US (<1 W/cm^2) directly activates brain neurons (9) but cannot activate the peripheral nerve endings or axons. Another difference between the CNS and PNS is the lack of inhibitory neurons in the peripheral sensory afferents. In the CNS, both activation and suppression effects were reported by US stimulation (36) which can be attributed to the selective activation of excitatory (e.g., glutamatergic) and inhibitory (e.g., GABAergic and glycinergic) neurons, respectively. In the periphery, the suppression effect is generally reflected as inhibition of action potential generation or transmission (48).

The sensory nerve endings can be directly activated by US stimulation as evidenced by a series of classical clinical studies by Gavrilov et al., which revealed that virtually all the sensory modalities can be activated by the US at the intermediate and high intensity range (70, 71). Activation of the low-threshold mechanoreceptors in the skin and cochlear hair cell

appears to require the least US intensity and energy (70, 73, 74), indicating that US likely evokes action potentials via mechanotransduction intrinsic to those nerve endings, i.e., opening of mechanosensitive ion channels by the local mechanical force leading to action potential generation in the spike-initiation zone (57). To evoke other sensory modalities requires high-intensity focused US, which can lead to significant local heating (>5 °C) and inertial cavitation with sudden collapse of the bubble (87). How high-intensity US activates sensory nerve endings remains undetermined. Putative mechanisms include 1) temperature gating of voltage-sensitive sodium and potassium ion channels, 2) mechanical gating of other transducer molecules like the TRP channels, and 3) indirect effects on surrounding non-neuronal tissues.

US neuromodulation to block action potential transmission in the peripheral nerve axons was extensively studied, which appears to require the local thermal effect from high-intensity US stimulation (47, 48). Rise of local temperature reportedly induces conduction block in the peripheral axon by changing the kinetics of voltage-sensitive sodium channels leading to their inactivation; temperature has a much greater impact on the inactivation kinetic than the activation kinetic (88). Non-thermal mechanisms could also contribute to the peripheral nerve block by high-intensity US, e.g., the inertia cavitation with strong acoustic forces that directly “bombs” the fibers leading to irreversible disruption (47). Despite the invasiveness, clinical applications of high-intensity US neuromodulation on peripheral nerves showed beneficial effects on pain management in painful amputation stump neuromas, phantom limbs (89), and spasticity (90).

Reversible peripheral neuromodulation on the peripheral nerve axons implements low- and intermediate-intensity US which does not directly evoke action potentials (41, 45). Intermediate-intensity US generally causes negligible thermal effect (<1°C) and produces harmless stable cavitation (41, 45), and thus its neuromodulatory effects on peripheral nerve axons are likely through local acoustic radiation forces. From analyzing the existing experimental reports in table 2, intermediate-intensity US likely enhances the neural activities in mammalian peripheral nerves by increasing the nerve conduction velocity. Unlike the nerve endings, the axon may lack the mechanosensitive ion channels tuned to transduce micromechanical forces. Thus, it is likely that other mechanically gated ion channels may participate to collectively enhance the neural activity, which include but are not limited to voltage-sensitive sodium (91–93), K2P (94), ASIC (94), TRP (95), and Piezo channels (96, 97).

Virtually no study has been conducted to assess the US neuromodulation on intact DRG, the clustering of sensory afferent somata. A recent study indicates that action potentials can be evoked by focused US in dissociated DRG neurons (67), opening new avenues of research on US DRG neuromodulation in future preclinical and clinical studies. The underlying mechanisms of US activation of DRG neurons are unclear, which likely involve sodium, potassium and non-selective cation channels (67).

The non-thermal and non-cavitation bio-effect of the focused US at low and intermediate intensity is of central interests for reversible US neuromodulation, which likely induces local acoustic forces below the harmful range. Potential mechanisms of neuromodulation include

mechanical gating of transmembrane ion channels as discussed earlier. In addition, several theories have attributed the US neuromodulatory effect to altered properties of lipid bilayer membrane at the nerve endings, axons and somata, including the soliton model (98), the flexoelectricity hypothesis (99), the neuronal intramembrane cavitation excitation (NICE) model (100), and more recently the theory of direct transmembrane pore formation (101). The main assumption of the soliton model is that, the transmission of signal through nerve occurs as an electromechanical soliton wave packet rather than a complete electrical phenomenon. However, the model could not mathematically explain the role of voltage gated ion channels in action potential generation. The flexoelectric effect hypothesizes that mechanical energy of the curved lipid bilayer membrane leads to electrical membrane polarization to depolarize the neural membrane, but the relevant mathematical formulation to account for the action potential generation has yet to be established. In the NICE model, it is hypothesized that US stimulation with sufficient intensity (> 0.10 W/cm^2 I_{SPTA}) causes nanobubble formation in the intramembrane space which subsequently changes transmembrane capacitance. Hence, the NICE model suggests the membrane capacitive current caused by the change of transmembrane capacitance as the source of US neuromodulation, and appears to explain the increased conduction velocities by US modulation from our recent study with single-unit recordings (41). The recent experimental finding on an expression system indicates that US as low as 0.4 W/cm^2 can form pores in the lipid bilayer membrane large enough to allow the passage of large dye molecule calcein (101), indicating the size of pore sufficiently large for passage of sodium and potassium ions to excite neurons. Both the NICE model and transmembrane pore formation can explain the enhanced excitability of peripheral nerve axons by US, consistent with the recent *ex vivo* study with single-unit recordings from individual axons (41). Further experimental and theoretical studies are required to advance our mechanistic understanding of peripheral US neuromodulation.

5. Conclusions

Peripheral US neuromodulation is capable of both enhancing and suppressing the neural activities which are likely dependent upon the range of US intensity and strength. Unlike the neurons in the brain, low-intensity US (<1 W/cm^2) is unable to evoke action potentials in the peripheral nerve endings or axons. US of intermediate intensity (1 to 200 W/cm^2) exerts mainly acoustic radiation force on tissues with no apparent thermal or inertia cavitation effects. US of intermediate intensity activates low-threshold mechanosensitive nerve endings likely through the regular mechanotransduction process by opening the mechanosensitive ion channels to evoke action potentials. US of intermediate intensity also enhances the neural activity of peripheral nerve axons leading to increased nerve conduction velocities in both A- and C-type fibers, which is likely caused by mechanical gating of other ion channels like the NaV, K2P, ASIC, TRP, and Piezo channels. In addition, enhanced neural activity could be attributed to direct effect of acoustic radiation force on the lipid bilayer neural membrane. Plausible mechanisms include a transient capacitive current from rapid changes of local membrane capacitance (the NICE model) and transmembrane pore formation to allow sodium and potassium ions to pass through. High-intensity US (>1000 W/cm^2) consistently inhibits the action potential transmission in peripheral nerves (i.e., nerve block)

likely from a thermal effect. In addition, inertia cavitation from high-intensity US could lead to irreversible damage of peripheral nerve axons. In conclusion, the US neuromodulation of the PNS has profound therapeutic potential especially for the non-thermal non-cavitation bio-effect in the intermediate intensity range, which is capable to non-invasively and reversibly enhance the peripheral neural activities.

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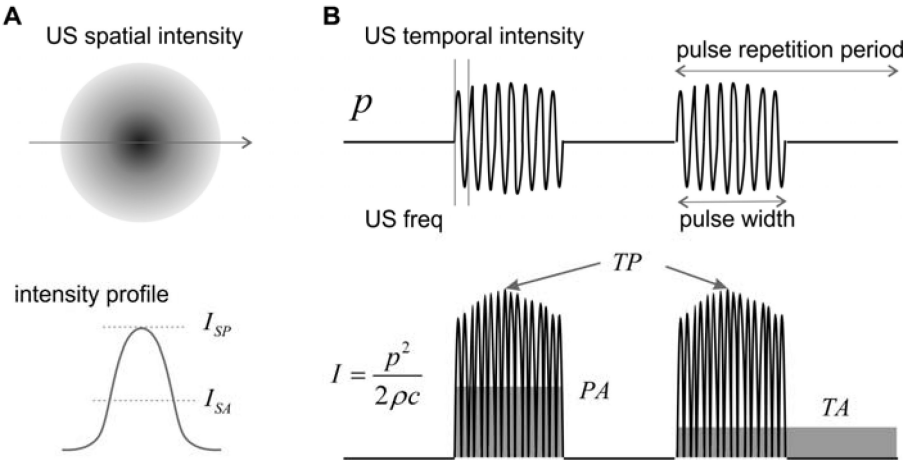

Figure 1.

Schematics of spatial and temporal US intensity. (A) The spatial intensity distribution peaks at the focal location and attenuates quickly outside the focus. (B) Three different temporal averages of the US intensity. *p*: pressure; *I*: intensity; SP: spatial peak; SA: spatial average; TP: temporal peak; PA: pulse average; TA: temporal average.

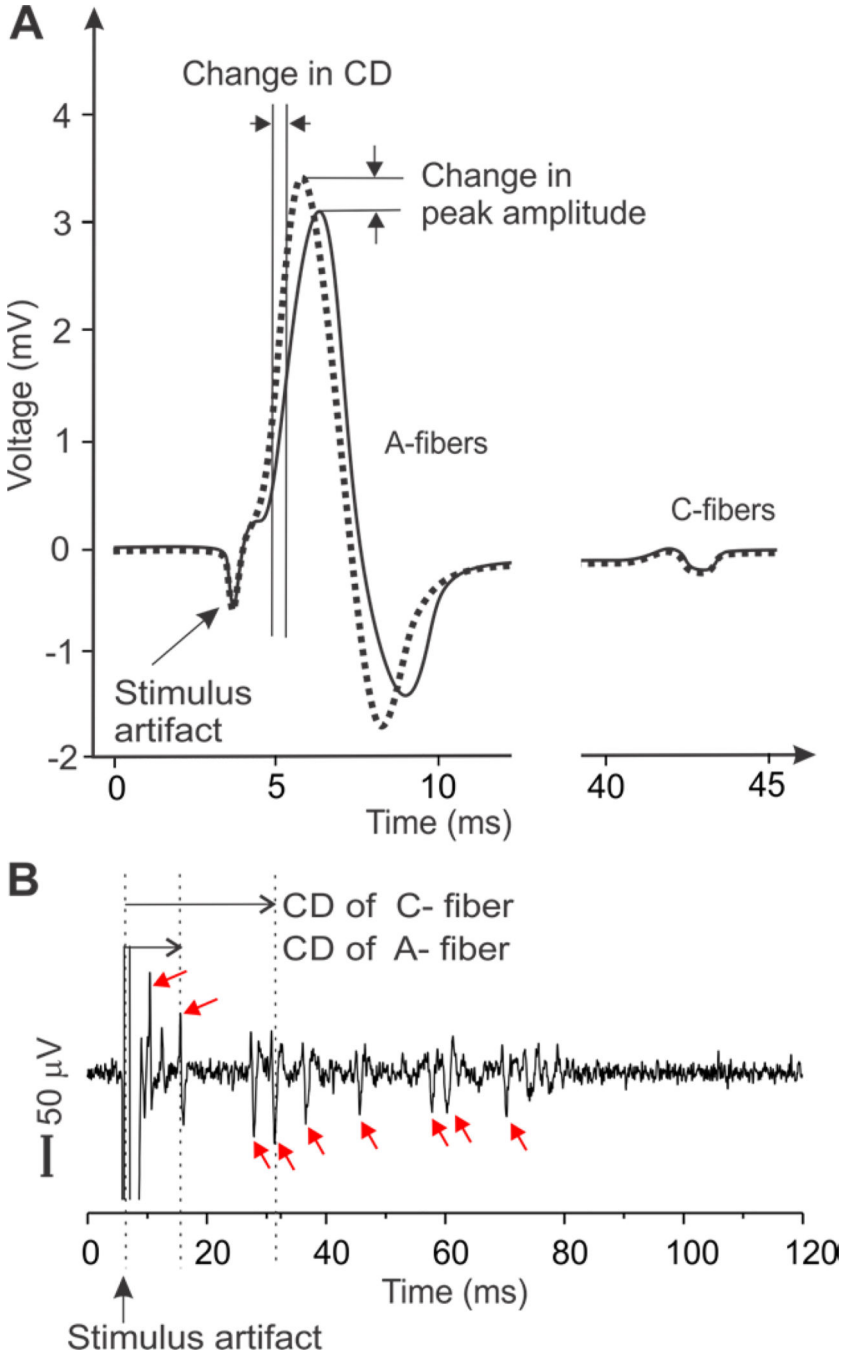

Figure 2.

Metrics of peripheral neural activity via electrophysiological recordings of the compound action potentials (CAP) in (A) and the single units in (B), respectively. CAP represents the temporal summation of multiple action potentials in the nerve trunk. Action potentials from individual nerve axons are recorded as distinct peaks in the single-unit recordings (red arrows), allowing precise determination of the conduction velocities of individual axons for both myelinated A-fibers and unmyelinated C-fibers. CD: conduction delay.

Table 1.

US neuromodulation of peripheral nerve endings

PNS endings (research)	Effect	Freq (MHz)	PRF (Hz)	DC (%)	Intensity (W/cm^2)	Duration (mSec)	Metric for modulation	Preparation type
Human hand nerve endings (70) (Gavrilov et al., 1977)	Tactile, warm, cold, itch, and pain sensation	0.48 0.887 2.67		100%	160 – 30,000	1, 10, 100	Verbal report	Clinical study
Human skin, soft tissue, bone, joint (71) (ab Ithel Davies et al., 1996)	Pain sensation	0.48–2.67			12 –15,000	1–100	Verbal report	Clinical study
Human fingers and upper forearm (72) (Dalecki et al., 1995)	Tactile sensation	2.2	50– 1000	50%	150	0.1 to 100	Verbal report	Clinical study
Human ear (73) (Tsirulnikov et al., 1988)	Activate acoustic nerve fibers	2.5	125–8000	50%	1 – 5	0.05–0.1	Verbal report	Clinical study
Human acupuncture point (51) (Yoo et al., 2014)	Deqi sensation	0.65	50	10%	1–3 (SPPA)	1000	Verbal report	Clinical study
Cat ear (74) (Foster and Wiederhold, 1978)	Activate of auditory nerve	5		100%	30	0.068	CAP	*In vivo*
Frog Pacinian corpuscle (75) (Gavrilov et al., 1977)	activation	0.48		100%	0.4 – 2.5	0.1 to 100	CAP	*Ex vivo*

Table 2.

US neuromodulation of peripheral nerve axons

PNS axons (research)	Effect	Freq (MHz)	PRF (Hz)	DC (%)	Intensity (W/cm^2)	Duration (mSec)	Metric for modulation	Preparation type
Enhanced activities								
Human median nerve (79) (Moore et al., 2000)	Increase conduction velocity	1–3		50–100%	1	480,000	Sensory and motor latency	Clinical study
Mouse sciatic nerve (41) (Ilham et al., 2018)	Increase conduction velocity	1.1	200,000	20–40%	0.91–28.2	40,000	Single-unit	*Ex vivo*
Rat posterior tibia nerve (40) (Casella et al., 2017)	Inhibit rhythmic bladder contraction	0.25	2000		0.9 (MPa)	300	bladder contraction	*In vivo*
Bullfrog sciatic nerve (49) (Tsui et al., 2005)	Enhance conduction, increase conduction velocity.	3.5	2	<1%	1–3 W		CAP	*Ex vivo*
Crab leg nerve (76) (Saffari et al., 2017)	Direct activation							*Ex vivo*
Suppressed activities								
Rabbit sciatic nerve (52) (Foley et al., 2007)	Nerve conduction block	3.2		58%	1930 (SATA)	10,000	Flexion Muscle force	*In vivo*
Rat sciatic nerve (77) (Foley et al., 2008)	Nerve conduction block	5.7			390– 7890 (SPTP)	5,000	Muscle activities	*In vivo*
Rat sciatic nerve (78) (Lee et al., 2015)	Nerve conduction block	2.68			2290–2810 (SATA)	3,000–7,000	CAP	*Ex vivo*
Rat vagus nerve (45) (Juan et al., 2014)	Inhibit conduction, reduce conduction velocity	1.1	20–1000		18.7–93.4	15,000	CAP	*In vivo*
Earth worm giant axon (84)–(Wahab et al., 2012)	Inhibit conduction, reduce conduction velocity	0.825	100	10%	0.1 – 0.7 (MPa)	15,000–75,000	CAP	*In vivo*
Frog sciatic nerve (85, 86)–(Young and Henneman, 1961)	Nerve conduction block	2.7	0.33–0.5	11–30%	1150 (SATA)	9,800	CAP	*Ex vivo*
Bullfrog sciatic (48)– (Colucci et al., 2009)	Nerve conduction block	0.66–1.98	10, 20	1–20%	370	30,000	CAP	*Ex vivo*
Mixed effects								
Frog sciatic nerve (80)–(Mihran et al., 1990)	Enhance and suppress excitability	2–7	3–20kHz		100–800 (SPTP)	0.5	CAP	*Ex vivo*