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Positive chronotropic effect caused by transthoracic ultrasound in heart of rats

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Abstract: Pulsed ultrasound can produce chronotropic and inotropic effects on the heart with potential therapeutic applications. Fourteen 3-month-old female rats were exposed transthoracically to 3.5-MHz 2.0-MPa peak rarefactional pressure amplitude ultrasonic pulses of increasing 5-s duration pulse repetition frequency (PRF) sequences. An increase in the heart rate was observed following each PRF sequence: an ∼50% increase after the 4-5-6 Hz sequence, an ∼57% increase after the 5-6-7 Hz sequence, and an ∼48% increase after the 6-7-8 Hz sequence. Other cardiac parameters showed a normal or indicated a compensatory decrease at 3 and 15 min post-ultrasound compared to control.

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1. Introduction

More than half a century has passed since the implantation of the first pacemaker in 1958.[1] Since then, therapeutic cardiac stimulation has been in clinical practice. Pacemaker implantation has become the routine treatment for symptomatic bradycardia, and advances in technology have also led to the development of devices to terminate life-threatening ventricular tachyarrhythmias and restore synchronous ventricular function in patients with heart failure. Each year, more than 1×10^6 pacemakers are implanted worldwide, of which about 200 000 are implanted in the United States alone.[2,3] Despite the unquestionable benefits of pacemakers, potential complications and technical failures should be considered. Short-term complications (e.g., pneumothorax, cardiac perforation, lead dislodgement, and pocket infection or hematoma) have been reported to be as high as 12% in a nationwide Danish cohort.[4] Worldwide, long-term complications are related primarily to the pacing lead and subcutaneous pocket and include pocket infection, tricuspid regurgitation, venous obstruction, lead fractures, and insulation failure with mortality rates of 12%–31%.[5–7] These data indicate that the electrode is the weakest link in the pacemaker system, most often leading to complications. An answer to this obstacle may be the replacement of traditional electrode-based pacemakers with ultrasound (US)-derived pacing systems.

In 1929, E. Newton Harvey showed the possibility for US to effect cardiac pacing. Harvey applied ∼340-kHz US to reptile and amphibian hearts and observed cardiac vibration but no pacing. More than 60 years after this discovery, studies on frogs demonstrated modification of heart rate (HR) and aortic pressure from US pulses, indicating the feasibility of pulsed US in cardiac stimulation.[8,9] In 2017, a study published by Coiado and O'Brien[10] demonstrated in rat hearts the negative chronotropic effect as well as identifying marked arrhythmias. Additionally, negative chronotropic pacing may serve as a supplement or alternative therapy for cardiac arrhythmias. Considering the peculiar effectiveness of pulsed US in female rats and the increased incidence of atrial fibrillation in women, pulsed US could play a pivotal role in the future treatment of cardiac arrhythmias.

Previous studies[9] have shown that pulsed US results in a significant decrease in HR, fractional shortening, and cardiac output (CO) for young female rats as well as a significant decrease in HR for a group of male rats. The gender differences could be due to an interplay between various sex hormones or structural differences such as heart size and pericardial insulation. Another study[11] showed that increased duty factor (DF) values (0.25%, 0.5%, and 1%) during US

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application affected cardiac pacing (decreased the HR) and that US could potentially be used in cardiology as an alternative of leadless pacing of the heart.

The goal herein is to investigate the feasibility of well-controlled unfocused pulsed US to yield cardiac pacing using specific US exposure parameters [e.g., peak rarefaction pressure amplitude (PRPA), frequency, pulse repetition frequency (PRF), pulse duration (PD)]. This US therapy could potentially be used to treat cardiac abnormalities such as arrhythmias, bradycardia, tachycardia, or atrial fibrillation in older patients who can have surgery or implantable pacemakers.

2. Materials and methods

2.1 Animals, preparation, and measurement systems

The following experimental conditions were approved by the University of Illinois Institutional Animal Care and Use Committee (Protocol #10104). Fourteen F344 female rats (about 3 months old, weighing 150–200 g) were evenly allotted to one of four groups (Table 1). For ideal comparison, the control rats underwent the same anesthesia, preparation, measurement, and US techniques as the experimental rats; the sole difference was that in the control rats, the US equipment was turned off, while the US transducer was transthoracically pressed on the rats' chest.

The anesthesia, preparation, and measurement techniques were the same as those of our previous studies[10,12,13] (exception: arterial pressure was not recorded). In both experimental and control protocols, rats were anesthetized with isoflurane. The thoracic area of the rats was shaved and depilated to maximize transthoracic US transmission; ample gel was used for acoustic coupling. The rats were positioned in a dorsal recumbency position on a temperature-controlled platform and attached to a four-lead electrocardiogram (EKG) of the small animal Vevo 2100 high-frequency (13–24 MHz) US imaging system (VisualSonics, Toronto, ON, Canada). The Vevo 2100 system was used to monitor cardiac parameters (not for the application of experimental US) via echocardiograms by a registered diagnostic medical sonographer (RDMS). Additionally, the rectal temperatures of all rats were continuously monitored. Then, approximately 18 min after US exposure ceased, the rats were euthanized with 5 min of carbon dioxide inhalation, and the lungs and hearts were removed, fixed, and processed for evaluation by a board-certified pathologist. No signs of lungs or heart tissue damage were observed after the pathology evaluation.

2.2 US application

A commercial US transducer was used, with a 19-mm-diameter aperture and a 3.5-MHz center frequency (Valpey Fisher, Valpey Corp., Hopkinton, MA). The transducer was driven by a function generator (33250A, Agilent Technologies Inc., Santa Clara, CA) and a radio frequency (RF) power amplifier (A150, Electronic Navigation Industries, Rochester, NY; 0.3–35 MHz; 55 dB). The US transducer was calibrated in a tank of distilled, degassed 22 °C water using a National Physical Laboratory (NPL)-calibrated polyvinylidene fluoride (PVDF) membrane hydrophone (Y-34–3598 EW295, GEC Marconi, Chelmsford, UK) with a 0.5-mm-diameter active element. During calibration, the transducer was fixed in place while a micropositioning system (2-μm translational accuracy) moved the PVDF hydrophone in a direction perpendicular to the beam axis and at a 1-cm distance from the transducer surface (in the near field).[14] All the aforementioned equipment and steps are identical to our prior experiments and between all the rats and groups in this study.[10,12,13]

The US transducer was placed externally in the chest of the animal, and prior to the exposure, the cardiac apex was marked using the Vevo2010 US imaging system. The *in situ* (in/at the heart) exposure parameters are estimated from a relatively complete experimental study to chest wall attenuation and thickness for the Sprague-Dawley rats for which the attenuation at 3.5 MHz was approximately 1.2 dB/cm MHz and the thickness was approximately 3.6 mm (Ref. 15). Thus, the *in situ* ultrasonic pressure is 0.84×2.0 MPa $= 1.7$ MPa, and the *in situ* ultrasonic spatial peak temporal peak intensity is 0.70×270 W/cm^2 $= 190$ W/cm^2.

The US protocol consisted of three consecutive 5-s intervals (15-s continuous pulsed exposure) with each of the three intervals differing only in its 5-s PRF groups (Fig. 1). For each experimental group, the PRFs were as follows: 4, 5, and 6 Hz (denoted "group 4-5-6"); 5, 6, and 7 Hz (denoted "group 5-6-7"); and 6, 7, and 8 Hz (denoted "group 6-7-8"). The US bursts had a 2.0-MPa peak rarefactional pressure amplitude, 2-ms PD, and DF range between 0.8% and 1.6%, which is equivalent to a mechanical index of 1.1 and *in vitro* spatial peak temporal peak intensities of ~270 W/cm^2. The PRF sequence started slightly below or above the HR of the rat (for rats, the HR is 300–350 beats/min or 5 Hz), and increasing by 1-Hz steps, this sequence was designed to achieve cardiac pacing while minimizing heat (total of 15-s US exposure).

Table 1. Assignment of the rats to different groups. All 14 rats were female, about 3–4 months in age, 150–200 g in weight, and of strain F344.

	Experimental	Control
Group with 4-5-6 Hz	$N = 4$	$N = 4$
Group with 5-6-7 Hz	$N = 3$	
Group with 6-7-8 Hz	$N = 3$	
Total	*$N = 10$*	*$N = 4$*

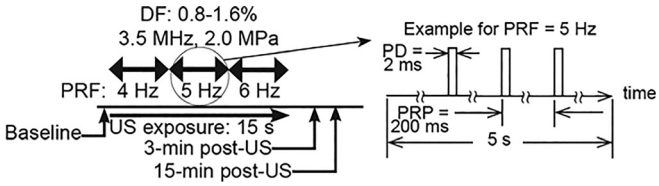

Fig. 1. Schematic of the group 4-5-6 experiment as an example. All individual pulses had a PD of 2 ms, a PRPA of 2.0 MPa, and a variable DF (0.8%–1.6%) that depends on PRF.

2.3 Statistical analyses

For each rat, the physiological parameters, HR, CO, etc., were recorded at baseline and at 3 and 15 min after the US exposure (or mock-US exposure for the control group), collectively called the post-US exposure time points. Additionally, HR was also measured at several time points during the PRF sequence. The notation of variables followed by time (i.e., HR3, SV0, EF15) was used to represent values at a time point [i.e., HR at 3 min post-US, stroke volume (SV) at baseline, ejection fraction (EF) at 15 min post-US]. All these data points were divided by the corresponding baseline (pre-US exposure, or pre-mock-US exposure in the control group; considered time = 0 min) value to obtain a relative change for each rat.

Using the relative change values, one-way ANOVA tests were used to compare the three experimental groups and the control group (that is, the factor is US protocol and has four levels) for a certain physiological parameter and time point. For example, an analysis of variance (ANOVA) test of HR15/HR0 was done to compare control, 4–5-6 Hz US, 5–6-7 Hz US, and 6–7-8 Hz US. Additionally, if a statistical difference ($p \leq 0.05$) was noted, then a Tukey honestly significant differences (HSD) test was done to perform multiple pairwise-comparison between the means of the groups and determine which specific group comparisons were significant. Statistical analyses and figures were generated in the software R (version 3.6.1, "Action of the Toes," R Foundation for Statistical Computing, Vienna, Austria). Results were expressed as mean ± standard error of the mean (SEM).

3. Results and discussion

Prior studies have shown that pulsed US can cause a significant decrease in HR in young female and male rats, using a decreasing PRF sequence.[10,12] The main goal herein was to investigate the feasibility of well-controlled unfocused US pulses to yield cardiac pacing based on careful analysis of the increasing PRF and US exposure parameters. Another goal was to determine an US protocol that would yield a positive chronotropic effect in rats while maintaining hemodynamic stability. Finding such a protocol would allow for the identification of a threshold (or thresholds) for cardiac pacing, to "control" the heart via increasing or decreasing the HR. The HR data before, during, and after the US application are presented for each group in Fig. 2 (relative to baseline as a bar chart).

The other physiological parameters of the rats at baseline and at 3 and 15 min after US exposure are listed for each of the groups in Table 2.

The corresponding magnitudes of change are plotted for the most relevant parameters in Fig. 3 (HR and CO) and Fig. 4 (ESV and EDV). Histological examination of the hearts and lungs of all animals exposed to US did not show lesions after the procedure.

Figure 2 shows that the HR increased in all groups as pulsed US exposure was applied and decreased after the exposure was terminated. ANOVA tests showed significant differences at each of three pulse time points as well as

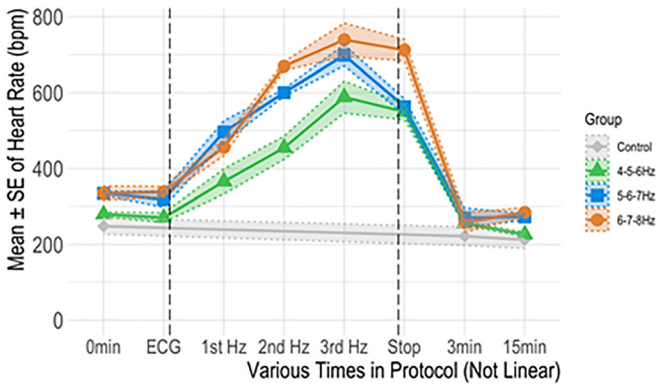

Fig. 2. HR at various points before, during, or after US application (points are not linearly spaced). An increase in HR was observed during each increased PRF sequence (1st, 2nd, and 3rd PRF). ANOVA showed $p \leq 0.05$ at these times.

Table 2. Measured physiological parameters at baseline and at 3 and 15 min after US exposure (US and control).

	Control group ($N=4$)			Group with 4-5-6 ($N=4$)		
Parameter (units)	Baseline	3 min	15 min	Baseline	3 min	15 min
HR (bpm)	248.20 ± 19.10	221.80 ± 22.07	212.00 ± 20.04	286.30 ± 1.96	260.30 ± 4.72	230.00 ± 5.66
CO (ml/min)	28.51 ± 4.39	27.73 ± 3.45	25.34 ± 3.37	35.27 ± 3.54	27.11 ± 2.64	25.31 ± 3.28
Systolic/stroke volume (μl)	116.00 ± 13.25	114.50 ± 10.09	118.50 ± 10.56	123.10 ± 12.25	104.10 ± 9.75	109.40 ± 12.75
Ejection fraction (%)	79.74 ± 3.30	82.30 ± 2.11	82.58 ± 0.33	80.82 ± 2.44	77.64 ± 1.25	76.36 ± 2.61
EDV[a] (μl)	140.70 ± 17.14	151.00 ± 5.76	143.20 ± 13.59	153.80 ± 18.58	134.80 ± 14.61	143.70 ± 16.79
ESV[b] (μl)	27.26 ± 4.38	26.98 ± 3.80	25.14 ± 2.77	30.73 ± 6.74	30.69 ± 4.88	34.31 ± 6.26
Respiratory rate (breaths/min)	51.75 ± 4.80	49.25 ± 5.50	44.75 ± 5.25	55.00 ± 4.08	31.00 ± 0.82	30.00 ± 0.00
Rectal temperature (K)	308.60 ± 0.27	307.30 ± 0.10	305.90 ± 0.33	8.90 ± 0.28	307.00 ± 0.22	306.30 ± 0.24
Fractional shortening (%)	49.54 ± 3.50	52.03 ± 2.33	51.84 ± 0.27	50.29 ± 2.45	46.67 ± 1.07	45.70 ± 2.37
	Group with 5-6-7 Hz ($N=3$)			Group with 6-7-8 Hz ($N=3$)		
Parameter (units)	Baseline	3 min	15 min	Baseline	3 min	15 min
Heart rate (bpm)	334.70 ± 4.39	270.30 ± 19.91	272.70 ± 7.08	334.70 ± 14.56	258.00 ± 20.24	284.70 ± 9.61
CO (ml/min)	42.78 ± 0.81	27.02 ± 5.22	31.08 ± 0.61	39.38 ± 2.26	24.54 ± 3.97	36.96 ± 3.25
Systolic/stroke volume (μl)	127.90 ± 3.58	96.63 ± 16.44	114.50 ± 3.83	117.30 ± 1.77	91.92 ± 8.21	128.70 ± 6.97
Ejection fraction (%)	83.66 ± 1.72	77.22 ± 5.07	86.59 ± 2.47	87.23 ± 1.08	75.19 ± 5.39	89.46 ± 0.32
EDV (μl)	153.50 ± 7.43	121.60 ± 15.93	133.20 ± 7.36	134.70 ± 3.63	121.90 ± 5.05	144.10 ± 8.11
ESV (μl)	25.71 ± 3.95	24.98 ± 3.33	18.69 ± 3.93	17.39 ± 1.86	29.96 ± 6.69	15.24 ± 1.13
Respiratory rate (breaths/min)	55.67 ± 3.06	48.67 ± 3.29	39.00 ± 2.90	48.67 ± 3.75	35.33 ± 5.18	38.67 ± 4.97
Rectal temperature (K)	309.60 ± 0.15	307.70 ± 0.24	306.50 ± 0.07	309.20 ± 0.13	307.90 ± 0.15	306.70 ± 0.13
Fractional shortening (%)	53.47 ± 1.92	47.48 ± 4.74	57.61 ± 3.54	57.61 ± 1.47	46.28 ± 5.86	60.64 ± 0.43

[a]End-diastolic volume (EDV).
[b]End-systolic volume (ESV).

immediately after the US was stopped. Tukey's HSD test showed that all of the PRF sequences were statistically different from control, although none of the differences among the PRF sequences reached statistical significance until the end of the US sequence (at the US stop, 5-6-7 Hz was different from 6-7-8 Hz by $p \leq 0.01$). In contrast, ANOVA results at 3 and 15 min post-US were generally not significant among any of the experimental or control groups. However, the variables still exhibited trends consistent with prior studies[10] that used a decreasing-then-increasing PRF US protocol.

The significance of investigating the biological effects triggered by US pulses arises from the fact that they can potentially serve various therapeutic approaches. The impact is determined by the existence of US variable interactions, which must be assessed empirically.[16,17] Ultrasonic *in vivo* heart studies of frogs,[8,9] pigs,[17] guinea pigs,[18] mice,[14,19] and dogs[20] have shown that different PRPAs, frequencies, and PDs can cause premature cardiac contractions, inotropic, lusitropic, or chronotropic effects.

Rat normal HR is roughly 300–350 bpm (~5–6 Hz). In our studies, we observed a consistent increase in the rat HR with the PRF sequence that was slightly above the HR of the rat (5-6-7 Hz and 6-7-8 Hz groups). This PRF-dependent HR suggests the positive chronotropic threshold should be observed when the PRF sequence started slightly greater than the animal's HR (for rats 5 Hz). Our results suggest that 6-7-8 Hz seems to provide the best outcome in increasing HR, indicating that potentially we would be able to trigger the heart for pacing, starting greater than the HR that could allow for control and maybe training the heart. Another study[21] showed that 3.33 Hz PRF (heart rat varying from 40 to 90 beats/min) caused an increase in beat frequency of 25% in isolated neonatal rat ventricular cardiomyocytes. Marquet *et al.*[22] showed that high intensity focused ultrasound (HIFU) can be used to perform remote pacing using reversibility of electromechanical coupling of cardiomyocytes. Cardiac pacing was also observed in *in vivo* rat experiments using HIFU,[23] suggesting that US can be used as temporary, non-invasive, painless, and reliable therapy.

Future research has multiple avenues that can be productively explored including US variable optimization, understanding the underlying US-induced pacing physiologically and with translational studies and moving toward humans. Given the effect US has on damping HR parameter, the technology shows promise for future use as an alternative or adjunct therapy for cardiac arrhythmias and other pacing heart abnormalities. One study[13] has shown that transthoracic cardiac US stimulation at an approximately 1% DF triggered a negative chronotropic response in rat hearts without impairing the hemodynamic system. Another report indicated that 0.5% DF and 1.0% DF sequences affected cardiac pacing. Both sequences revealed a significant negative chronotropic effect relative to baseline.[11] Another study[24] showed that

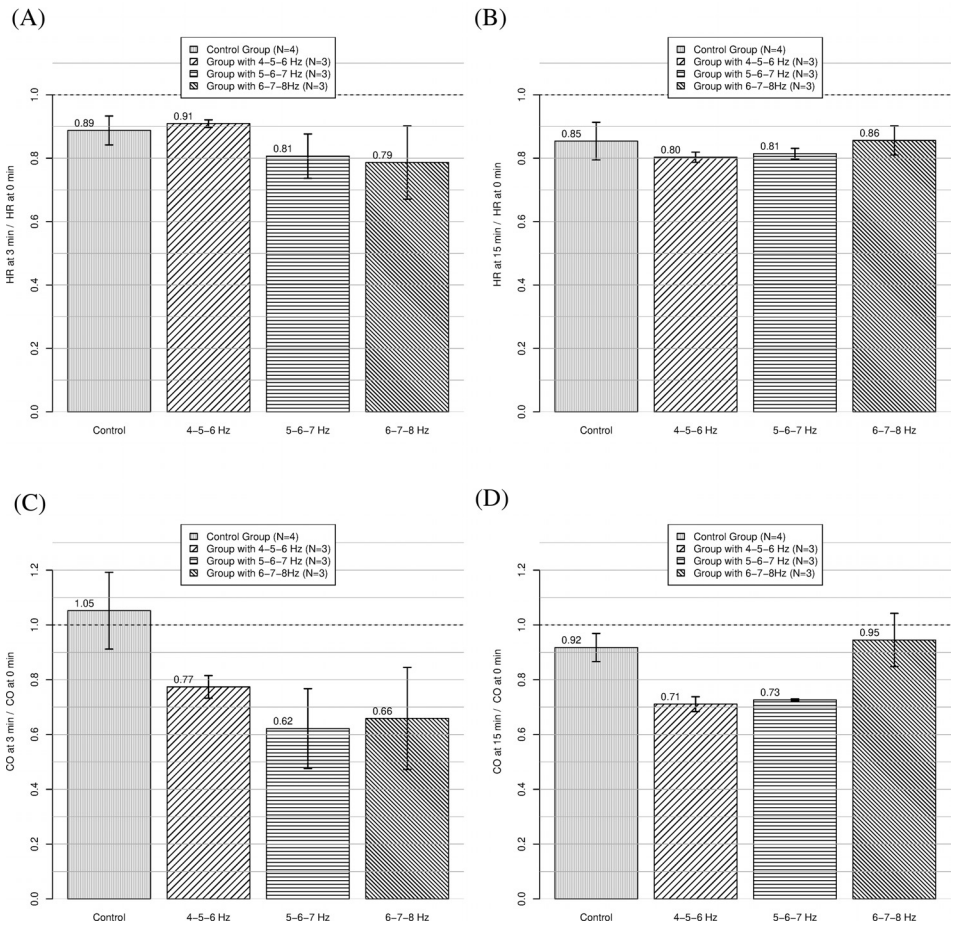

Fig. 3. HR and CO, relative to pre-US baseline, at the post-US times of 3 min [(a) and (b)] and 15 min [(c) and (d)].

shock waves of 64 MPa induced arrhythmia in piglet hearts; we eliminated the cavitation effect in our studies using a much lower PRPA (2 MPa).

Another area of significant work is understanding the US mechanism of altering inotropy. It is important to note that after pulsed US exposure was terminated, we still observed a significant decrease in HR. A similar phenomenon was noted *in vivo* by Dalecki *et al.* where high intensity US pulses modified the HR; however, the rhythm of the heart and aortic pressure returned to normal shortly after exposure discontinued.[25] Whatever the true interaction mechanism, it needs to be reversible with no lasting tissue damage.

The inotropic changes could be related to the direct but temporary effect of the pulsed US on the calcium channels. Studies indicated that US exposure might expedite calcium release or accumulation in the cardiac tissue. Reports suggest that US utilization can decrease the threshold for cardiac electrical excitation and yield a positive inotropic effect by augmenting the influx of calcium into cardiac cells.[16,26,27] Recently, another ion channel activity modification by US has been proposed.[28,29] Researchers found that focused US modulates K^+ currents of two-pore-domain potassium family channels (K2P) and Na^+ currents of Na^+-selective voltage-activated ion channel (NaV1.5).[30] Both channels K2P and NaV1.5 are expressed in neurons, cardiomyocytes, and other cells.[31,32] Moreover, this discovery importantly suggests a potential explanation for the contradictory findings that US may promote both inhibitory[33,34] and excitatory[29,35] effects. For example, if cells express primarily Na^+ or Ca^{++} ion channels, US application may lead to cellular activation. On the other hand, US exposure may result in inhibition in cells that express mostly K^+ US-receptive ion channels. Furthermore, in cells that show a fine balance of excitatory and inhibitory ion channels, pulsed US effects may be neutralized. Other studies have proposed mechanisms such as localized cavitation, baroreceptor sensitivity changes, and parasympathetic influence.[36]

At an anatomical level, EKG showed areas of depolarization outside of sinus rhythm during the experiment when pulsed US was applied. This pattern was seen throughout the rats in all groups except the control groups. This signifies that pulsed US did not cause the sinoatrial (SA) node to intrinsically speed up but caused depolarizations outside of the normal conduction system. One hypothesis would be that the US-induced mechanical pressure waves caused ion channel flux changes resulting in immediate ventricular contraction. Even with these extra beats, cardiologic functional

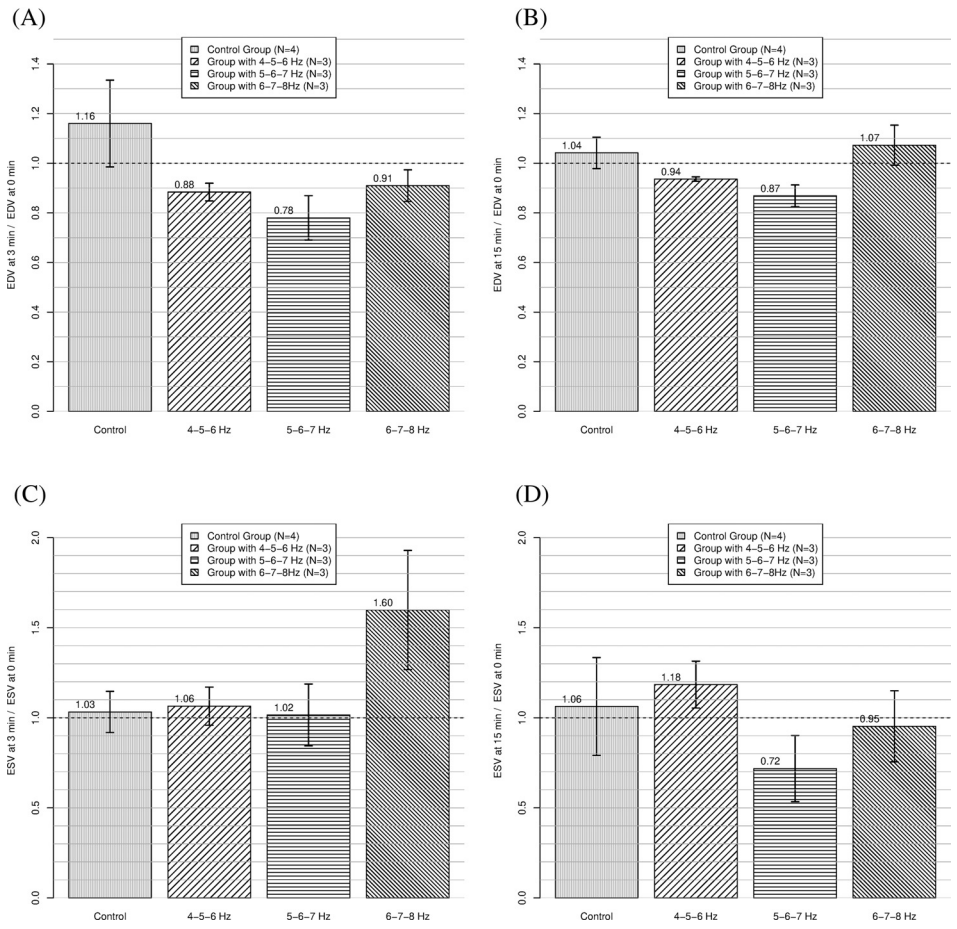

Fig. 4. ESV and EDV, relative to pre-US baseline, at the post-US times of 3 min [(a) and (b)] and 15 min [(c) and (d)].

parameters and hemodynamic stability were maintained. The control rats remained in sinus rhythm throughout the experiment as the US was off. In any medical US pacing technique, it would be vital to maintain a controlled rhythm to avoid deadly tachyarrhythmias that can arise with rapid ventricular depolarization outside of sinus rhythm.[37]

This experiment did not maintain sinus rhythm, but this does not mean it is impossible. The normal conduction travels from the SA node to the atrium, AV node, and ventricles. Given the relatively large footprint of the transducer to rat heart, it is unlikely the waves were focused specifically on the SA node to initiate the heartbeat. If the true mechanism involves the changing of ion channel flux, it is likely this experiment occurred throughout the heart and was not localized to one region. This experiment likely caused immediate ventricular contractions. A future study in an animal with a larger heart could be used to explore the conduction system further by focusing the pulsed US on specific heart regions. Specifically, one could focus on pacing the SA node alone in the diastole period to attempt to generate a P-wave and maintain sinus rhythm. Additionally, aiming at different regions of the heart can help localize areas of specific ion channels and narrow down the cause of changing inotropy. The physiological variables of CO, EDV, ESV, and EF should also be recorded during this future experiment. Overall, studying localized regions in a larger heart will be essential for translation to human application.

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References and links

[1]N. M. van Hemel and E. E. van der Wall, "8 October 1958, D Day for the implantable pacemaker," Neth. Heart J. **16**(Suppl 1), 1–2 (2008).

[2]H. G. Mond and A. Proclemer, "The 11th world survey of cardiac pacing and implantable cardioverter-defibrillators: Calendar year 2009—A world society of Arrhythmia's project," Pacing Clin. Electrophysiol. **34**(8), 1013–1027 (2011).

[3]A. J. Greenspon, J. D. Patel, E. Lau, J. A. Ochoa, D. R. Frisch, R. T. Ho, B. B. Pavri, and S. M. Kurtz, "Trends in permanent pacemaker implantation in the United States from 1993 to 2009: Increasing complexity of patients and procedures," J. Am. Coll. Cardiol. **60**(16), 1540–1545 (2012).
[4]R. E. Kirkfeldt, J. B. Johansen, E. A. Nohr, O. D. Jorgensen, and J. C. Nielsen, "Complications after cardiac implantable electronic device implantations: An analysis of a complete, nationwide cohort in Denmark," Eur. Heart J. **35**(18), 1186–1194 (2014).
[5]M. P. Brunner, E. M. Cronin, O. Wazni, B. Baranowski, W. I. Saliba, J. F. Sabik, B. D. Lindsay, B. L. Wilkoff, and K. G. Tarakji, "Outcomes of patients requiring emergent surgical or endovascular intervention for catastrophic complications during transvenous lead extraction," Heart Rhythm **11**(3), 419–425 (2014).
[6]K. G. Tarakji, O. M. Wazni, S. Harb, A. Hsu, W. Saliba, and B. L. Wilkoff, "Risk factors for 1-year mortality among patients with cardiac implantable electronic device infection undergoing transvenous lead extraction: The impact of the infection type and the presence of vegetation on survival," Europace **16**(10), 1490–1495 (2014).
[7]A. Polewczyk, W. Jacheć, A. Tomaszewski, W. Brzozowski, M. Czajkowski, G. Opolski, M. Grabowski, M. Janion, and A. Kutarski, "Lead-related infective endocarditis: Factors influencing early and long-term survival in patients undergoing transvenous lead extraction," Heart Rhythm **14**(1), 43–49 (2017).
[8]D. Dalecki, "Mechanical bioeffects of ultrasound," Annu. Rev. Biomed. Eng. **6**, 229–248 (2004).
[9]D. Dalecki, B. B. Keller, E. L. Carstensen, D. S. Neel, J. L. Palladino, and A. Noordergraaf, "Thresholds for premature ventricular contractions in frog hearts exposed to lithotripter fields," Ultrasound Med. Biol. **17**(4), 341–346 (1991).
[10]O. C. Coiado and W. D. O'Brien, "The negative chronotropic effect in rat heart stimulated by ultrasonic pulses: Role of sex and age," J. Ultrasound Med. **36**(4), 799–808 (2017).
[11]O. C. Coiado and W. D. O'Brien, "The role of the duty factor in ultrasound-mediated cardiac stimulation," J. Acoust. Soc. Am. **136**(3), EL231–EL235 (2014).
[12]O. Coiado, E. Buiochi, and W. O'Brien, "Ultrasound-induced heart rate decrease: Role of the vagus nerve," IEEE Trans. Ultrason. Ferroelectr. Freq. Control **62**(2), 329–336 (2015).
[13]E. B. Buiochi, R. J. Miller, E. Hartman, F. Buiochi, R. A. Bassani, E. T. Costa, and W. D. O'Brien, "Transthoracic cardiac ultrasonic stimulation induces a negative chronotropic effect," IEEE Trans. Ultrason. Ferroelectr. Freq. Control **59**(12), 2655–2661 (2012).
[14]C. Rota, C. H. Raeman, S. Z. Child, and D. Dalecki, "Detection of acoustic cavitation in the heart with microbubble contrast agents *in vivo*: A mechanism for ultrasound-induced arrhythmias," J. Acoust. Soc. Am. **120**(5), 2958–2964 (2006).
[15]R. T. Towa, R. J. Miller, L. A. Frizzell, J. F. Zachary, and W. D. O'Brien, "Attenuation coefficient and propagation speed estimates of rat and pig intercostal tissue as a function of temperature," IEEE Trans. Ultrason. Ferroelectr. Freq. Control **49**(10), 1411–1420 (2002).
[16]N. N. Petrishchev, T. D. Vlasov, M. M. Galagudza, and Y. N. Makov, "Effect of low-frequency low-intensity ultrasound on contractile function of isolated heart," Bull. Exp. Biol. Med. **133**(4), 327–329 (2002).
[17]B. C. Towe and R. Rho, "Ultrasonic cardiac pacing in the porcine model," IEEE Trans. Biomed. Eng. **53**(7), 1446–1448 (2006).
[18]F. Kuma, N. Ueda, H. Ito, T. Maruyama, Y. Kaji, T. Fujino, and M. Harada, "Effects of ultrasound energy application on cardiac performance in open-chest Guinea pigs," Circ. J. **70**, 1356–1361 (2006).
[19]A. G. Macrobbie, C. H. Raeman, S. Z. Child, and D. Dalecki, "Thresholds for premature contractions in murine hearts exposed to pulsed ultrasound," Ultrasound Med. Biol. **23**(5), 761–765 (1997).
[20]A. Smailys, Z. Dulevičius, K. Muckus, and K. Daukša, "Investigation of the possibilities of cardiac defibrillation by ultrasound," Resuscitation **9**(3), 233–242 (1981).
[21]A. Fleischman, C. Vecchio, Y. Sunny, C. R. Bawiec, P. A. Lewin, J. Y. Kresh, and A. R. Kohut, "Ultrasound-induced modulation of cardiac rhythm in neonatal rat ventricular cardiomyocytes," J. Appl. Physiol. **118**(11), 1423–1428 (2015).
[22]F. Marquet, P. Bour, F. Vaillant, S. Amraoui, R. Dubois, P. Ritter, M. Haïssaguerre, M. Hocini, O. Bernus, and B. Quesson, "Non-invasive cardiac pacing with image-guided focused ultrasound," Sci. Rep. **6**, 36534 (2016).
[23]A. Livneh, E. Kimmel, A. R. Kohut, and D. Adam, "Extracorporeal acute cardiac pacing by high intensity focused ultrasound," Prog. Biophys. Mol. Biol. **115**(2), 140–153 (2014).
[24]M. Delius, E. Hoffmann, G. Steinbeck, and P. Conzen, "Biological effects of shock waves: Induction of arrhythmia in piglet hearts," Ultrasound Med. Biol. **20**(3), 279–285 (1994).
[25]D. Dalecki, B. B. Keller, C. H. Raeman, and E. L. Carstensen, "Effects of pulsed ultrasound on the frog heart: I. Thresholds for changes in cardiac rhythm and aortic pressure," Ultrasound Med. Biol. **19**(5), 385–390 (1993).
[26]H. Salz, E. H. Rosenfeld, and M. Wussling, "Effect of ultrasound on the contraction of isolated myocardial cells of adult rats," Ultrasound Med Biol. **23**(1), 143–149 (1997).
[27]N. N. Petrishchev, T. D. Vlasov, M. M. Galagudza, Y. N. Makov, and C. M. Minasyan, "Frequency-dependent effects of low-intensity ultrasound on activity of isolated heart," Bull. Exp. Biol. Med. **136**(3), 239–241 (2003).
[28]W. J. Tyler, "The mechanobiology of brain function," Nat. Rev. Neurosci. **13**, 867–878 (2012).
[29]W. J. Tyler, Y. Tufail, M. Finsterwald, M. L. Tauchmann, E. J. Olson, and C. Majestic, "Remote excitation of neuronal circuits using low-intensity, low-frequency ultrasound," PLoS One **3**, e3511 (2008).
[30]J. Kubanek, J. Shi, J. Marsh, D. Chen, C. Deng, and J. Cui, "Ultrasound modulates ion channel currents," Sci. Rep. **6**, 24170 (2016).
[31]A. Beyder, J. L. Rae, C. Bernard, P. R. Strege, F. Sachs, and G. Farrugia, "Mechanosensitivity of Nav1.5, a voltage-sensitive sodium channel," J Physiol. **588**, 4969–4985 (2010).
[32]A. H. Christensen, F. C. Chatelain, I. G. Huttner, M. S. Olesen, M. Soka, S. Feliciangeli, C. Horvat, C. F. Santiago, J. I. Vandenberg, N. Schmitt, S.-P. Olesen, F. Lesage, and D. Fatkin, "The two-pore domain potassium channel, TWIK-1, has a role in the regulation of heart rate and atrial size," J. Mol. Cell. Cardiol. **97**, 24–35 (2016).
[33]T. Deffieux, Y. Younan, N. Wattiez, M. Tanter, P. Pouget, and J. F. Aubry, "Low-intensity focused ultrasound modulates monkey visuomotor behavior," Curr. Biol. **23**(23), 2430–2433 (2013).

[34]S. S. Yoo, A. Bystritsky, J. H. Lee, Y. Zhang, K. Fischer, B.-K. Min, N. J. McDannold, A. Pascual-Leone, and F. A. Jolesz, "Focused ultrasound modulates region-specific brain activity," NeuroImage **56**, 1267–1275 (2011).
[35]Y. Tufail, A. Yoshihiro, S. Pati, M. M. Li, and W. J. Tyler, "Ultrasonic neuromodulation by brain stimulation with transcranial ultrasound," Nat. Protoc. **6**, 1453–1470 (2011).
[36]O. C. Coiado, J. Lowe, and W. D. O'Brien, "Therapeutic ultrasound in cardiovascular medicine," J. Ultrasound Med. **40**, 1061–1076 (2021).
[37]P. Kohl, "Sudden cardiac death by *Commotio cordis*: Role of mechano–electric feedback," Cardiovasc. Res. **50**(2), 280–289 (2001).