

## Stochastic resonance in osteogenic response to mechanical loading

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### ABSTRACT

Stochastic resonance, in which noise enhances the response of a nonlinear system to a weak signal, has been observed in various biological sensory systems. We speculated that bone formation in response to mechanical loading could be enhanced by adding noise (vibration) to a standard exercise regimen. To test this hypothesis, three different loading regimens were applied to the ulnae of mice: (1) high amplitude, low frequency sinusoidal loading at 2 Hz with an amplitude of 3 N to simulate exercise; (2) low amplitude, broad frequency vibration with frequency components 0-50 Hz and 0.3 N of mean amplitude; (3) the sinusoidal wave combined with vibration (S+V) to invoke stochastic resonance. The simulated exercise regimen induced new bone formation on the periosteal surface of the ulna, however the addition of vibration noise with exercise enhanced the osteogenic response by almost 4-fold. Vibration by itself had no effect on bone formation. It was concluded that adding low magnitude vibration greatly enhanced bone formation in response to loading, suggesting a contribution of stochastic resonance in the osteogenic response.

Key words: biomechanics • bone formation • osteoporosis • mechanotransduction

A phenomenon called stochastic resonance, in which noise vibration enhances the response of a nonlinear system to a weak signal, might affect mechanosensitivity in osteoblasts. Stochastic resonance has been reported in a wide range of systems. This phenomenon was originally proposed by Benzi (1) and applied to a theoretical explanation to the periodic recurrences of the Earth's ice ages (2, 3). In biology, stochastic resonance has been demonstrated experimentally in various sensory neural systems, including crayfish (4), shark (5), cricket (6), and human (7, 8). Collins (7) showed that the tactile sensation of human fingertip can be enhanced by mechanical vibration, which had Gaussian distribution and broad-frequency components up to 30 Hz. Collins' finding suggests that mechanoreceptors can be influenced through stochastic resonance.

Our previous work (9) has shown that low-amplitude, broad-frequency vibration enhanced the expression of osteocalcin mRNA when combined with high-amplitude, low-frequency, sinusoidal loading of osteoblastic (MC3T3-E1) cells cultured in a collagen gel. These results suggest that stochastic resonance might enhance mechanosensation in bone tissue. If so,

stochastic resonance could be used to maximize the bone-building effect of exercise. Potentially one could design new exercises for elderly people to build their bone mass and help prevent osteoporosis.

In this study, we investigated the effect of low-amplitude, broad-frequency vibration combined with simulated exercise on bone formation *in vivo*. We hypothesized that low-amplitude, broad-frequency vibration enhances new bone formation in response to exercise through stochastic resonance.

## MATERIALS AND METHODS

### Animals

Thirty-six female C57BL/6 mice (16-weeks-old) with a mean body weight of  $22.0 \pm 0.2$  g were used for this study. The mice were divided into four groups, which included a non-loaded group (control,  $n=8$ ) and three loaded groups: haversine wave to simulate exercise (sine;  $n=10$ ); broad-frequency vibration (vibration;  $n=10$ ); and vibration added to the sine to invoke stochastic resonance (S+V;  $n=8$ ). Four to five mice in the same group were housed together. We performed all procedures throughout the experiment following the guidelines of the Indiana University Animal Care and Use Committee.

### Mechanical stimulation

[Figure 1](#) shows a schematic diagram of mechanical stimulator and control system used in this study. Right forearm was held between a loader and a nylon screw ([Figs. 1](#) and [2](#)). Mechanical loading was applied axially to the ulna across the flexed carpus and olecranon process. Four bimorph-type piezoelectric actuators (LPD12060X, Megacera Inc., Saitama, Japan) were utilized to mechanically load the ulna. A voltage-signal was sent from a computer to the actuators based on a programmed loading waveform, via an AD-DA board (aISA-A57, Adtek-system Science, Kanagawa, Japan) and a piezo-driver with a frequency response of 3 kHz (E470.00, Physik Instrumente (PI) GmbH & Co., Waldbronn, Germany). Applied load to mouse ulna was monitored by using a strain gauge on a cantilever. The signals from the strain gauge were amplified by a strain gauge conditioner (2120B, Measurements Group, Inc., Raleigh, NC) and were collected by the AD-DA board. Errors between the desired and the measured waveforms were minimized by the feedback control at 250  $\mu$ s intervals, which were performed by using a program written by Visual Basic (Microsoft, Bellevue, WA). Three loading waveforms shown in [Figure 3](#) were programmed on a personal computer by using LabVIEW<sup>R</sup> (National Instruments Co., TX) and Visual Basic (Microsoft): 1) sine (haversine waves at 2 Hz with 3 N peak-to-peak amplitude); 2) vibration (Gaussian quasi-white noise with standard deviation of 0.3 N and frequency components 0–50 Hz; and (3) S+V (the vibration was superimposed on the sinusoidal waves, so that both waves were applied to the ulnae simultaneously). The loading was given to ulna of a mouse under general anesthesia for 30 s per day on two consecutive days.

## Strain gauge analysis

The ulna was loaded through the carpal joint and overlying soft tissues. Therefore, we anticipated a damping effect of the joint and the soft tissues that would reduce high-frequency components of mechanical load into an ulna. To investigate this damping effect, mechanical vibration was applied to a mouse ulna by using our mechanical stimulator described above, and, simultaneously, mechanical strains were measured at the medial surface of the ulna midshaft. [Figures 4a](#) and [b](#) show the waveforms of mechanical force applied to the ulna and bone strain measured on the ulna, respectively. Fast fourier transform (FFT) analysis was performed to show the load and strain signals in frequency domain. [Figures 4c](#) and [d](#) represent mean power per decade Hz through 0 to 50 Hz in waveforms of the applied load and the measured bone strain, respectively. Frequency components were observed up to 50 Hz for both applied load and the measured bone strain. The mean power was reduced slightly in 30–40 Hz and 40–50 Hz ranges for the measured bone strain. This result suggests that the vibration applied to ends of a forearm of a mouse by our mechanical stimulator transmits high-frequency strains into the ulna, although there was some damping of mechanical signals over 30 Hz.

## Bone histomorphometry

All mice were given a calcein injection (0.1 ml/each) 2 and 6 days after and were killed 13 days after the last loading session. The right and left ulnae were removed for bone histomorphometry, fixed in 10% formalin for 48 h, dehydrated by sequential changes of ascending concentrations of ethanol and acetone, and embedded in methyl methacrylate (MMA; K-Plast; Delaware Diamond Knives, Wilmington, DE). Transverse sections 50  $\mu\text{m}$  in thickness were cut 1 mm distal from the ulnar midshaft by using a diamond wire saw (Histo-saw; Delaware Diamond Knives). These sections were examined by using a fluorescence microscope (Nikon Melville, NY). The following bone formation parameters were measured on the periosteal and endosteal perimeter by using the Bioquant semiautomatic digitizing system (R&M Biometrics, Nashville, TN): 1) mineralizing surface (MS/BS, %) =  $100 \times (\text{sum of the length of double-labeled perimeter and half of single-labeled perimeter}) / (\text{total length of perimeter})$ ; 2) mineral apposition rate (MAR,  $\mu\text{m}/\text{day}$ ) =  $(\text{average radial distance between the two labels}) / (\text{time interval between calcein injections, 4 days})$ ; 3) bone formation rate (BFR/BS,  $\mu\text{m}^3/\mu\text{m}^2/\text{year}$ ) =  $\text{MS/BS} \times \text{MAR} \times 3.65$ , which represents the volumetric rate of new bone formation per year. Relative bone formation parameters, rMS/BS, rMAR, and rBFR/BS, were determined by subtracting the parameters in the left ulna from those in the right ulna.

## Statistical methods

Analysis of variance (ANOVA, Statview, SAS Institute, Cary, NC) was used to examine statistically significant differences in the bone-formation parameters. Statistical significance was examined if the *P*-value was 0.05 or lower. Paired *t*-tests were performed for each loading group to determine significant differences in bone formation parameters between right and left ulnae. Fisher's Protected Least Significant Difference tests were conducted for comparisons among loading and control groups. Sections with missing calcein labels were excluded from the analyses. Two mice in the S+V group and one mouse from the sine group expressed woven bone on their right ulna. These mice were excluded from the analyses.

## RESULTS

New bone formation on the ulnar periosteal surface was enhanced by sine and S+V loading, but no detectable increase of new bone formation was observed in the vibration-stimulated ulnae (Fig. 5). S+V stimulation increased bone formation parameters to a greater degree than sine loading (Fig. 6). Bone formation parameters, that is, rMS/BS, rMAR, and rBFR/BS, caused by S+V stimulation were 1.6-fold ( $P<0.05$ ), 3.3-fold ( $P<0.0001$ ), and 3.9-fold ( $P<0.0001$ ) greater than those caused by sine stimulation.

The effects of mechanical loading regimens on the ulnar endosteal surface were far less dramatic than those observed on the periosteal surface. Sine and S+V loading each had a mild anabolic effect on endosteal bone formation rate (rBFR/BS;  $P<0.05$  for each group compared with control). S+V stimulation did not enhance endosteal rBFR/BS significantly more than sine stimulation alone. Vibration loading by itself had no significant effect on any bone formation parameter measured at either the periosteal or endosteal surface.

## DISCUSSION

Both sine and S+V loading promoted new bone formation in the mouse ulna. In particular, S+V stimulation induced as much as 3.9-fold more bone formation on the periosteal surface compared with sine stimulation. These results demonstrate that new bone formation in response to simulated exercise (sine stimulation) can be enhanced by low-amplitude, broad-frequency vibration, suggesting an effect of stochastic resonance. Stochastic resonance might affect mechanosensitivity and/or signal transduction mechanisms in bone cells. Calcium channels and other ion channels play fundamental roles in osteoblastic responses to external mechanical forces (10), and it has been proposed that certain ion channels exhibit stochastic resonance (11).

Our study failed to demonstrate an osteogenic effect at either the periosteal or endosteal ulnar surfaces for low-amplitude, broad-frequency vibration by itself. Previously, Rubin et al. (12, 13) reported that low-amplitude, high-frequency (30 Hz) loading is anabolic for bone; however, the anabolic response was observed only in trabecular bone at the ends of long bones. They failed to demonstrate an osteogenic response in cortical bone tissue. Because our analysis was restricted to cortical bone, our results appear to be consistent with those of Rubin et al., that is, low-amplitude vibration by itself is incapable of promoting of cortical bone formation. It is unclear why low-amplitude vibration might have anabolic effects on trabecular but not cortical bone. One might speculate that vibration loading across joints creates low-amplitude pressure waves in the bone marrow, which in turn stimulate osteoblasts lining trabecular bone surfaces. These marrow pressure waves may be damped as they propagate toward the midshaft of the bone, thus explaining the lack of effect of vibration loading on bone formation at the ulnar midshaft. We did not measure trabecular bone formation in at the ends of the ulna, so we were unable to confirm the results of Rubin et al.

Our results imply the important role of low-amplitude, broad-frequency bone strain observed in vivo (14–16) in maintenance or enhancement of the bone adaptive response to mechanical stimuli caused by daily activities like walking, running, or jumping. It is possible that muscle

vibration is responsible for some of the high-frequency bone strains measured in vivo. The amplitude of this muscle-induced vibration over 20 Hz has been reported to decline with increasing age (17). Rubin (13) hypothesized that loss of muscle-induced vibration in elderly contributes to progressive osteoporosis. Stochastic resonance might be one mechanism by which broad-frequency bone strains derived from muscle vibration sensitize osteoblasts to mechanical stimuli caused by physical activities to maintain bone mass.

Our results suggest a possibility that stochastic resonance can be exploited to enhance the osteogenic effects of exercise. Exercise can improve both bone mass and bone strength in growing children and adolescents, but the osteogenic potential of exercise diminishes greatly after puberty (18). The adult skeleton is only moderately responsive to mechanical loading, and this responsiveness decreases with age (19). Vibration exercise is one promising new technique for stimulating bone formation in the aging skeleton. Low-amplitude, high-frequency vibration by itself is reported to enhance new trabecular bone formation in sheep (12, 13, 20) and callus formation in a rabbit osteotomy model (21), yet vibration alone has no measurable effect on new cortical bone formation. Our results demonstrate a potent anabolic effect on cortical bone when low-amplitude vibration is delivered concurrent with simulated exercise. Cortical bone provides the majority of the biomechanical support in long bones and many clinically important sites like the proximal femur. The application of stochastic resonance offers a new way to enhance bone formation where it is biomechanically most important.

## ACKNOWLEDGMENTS

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Fig. 1

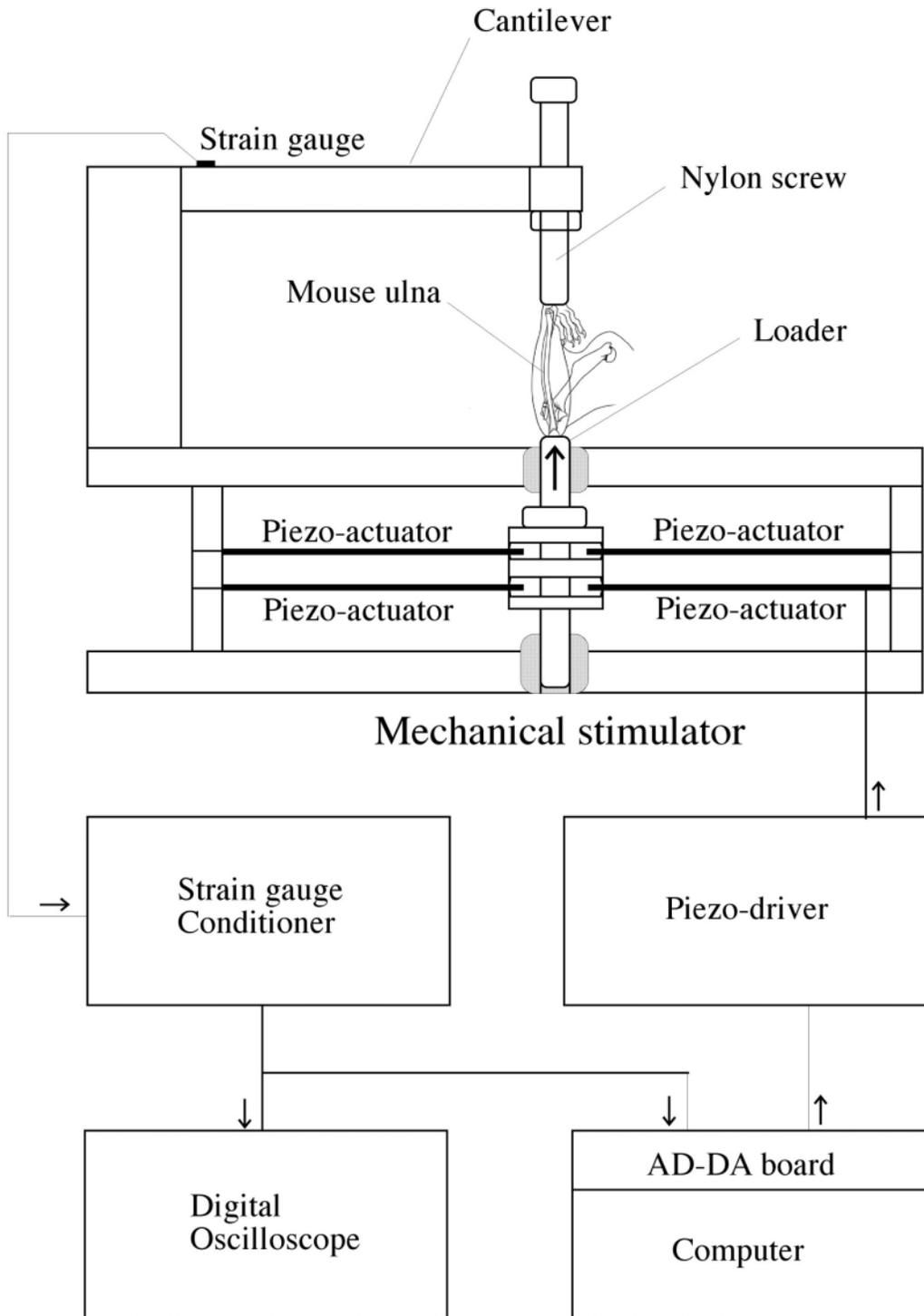


Figure 1. Schematic diagram of mechanical stimulator for mouse ulna and control system.

Fig. 2

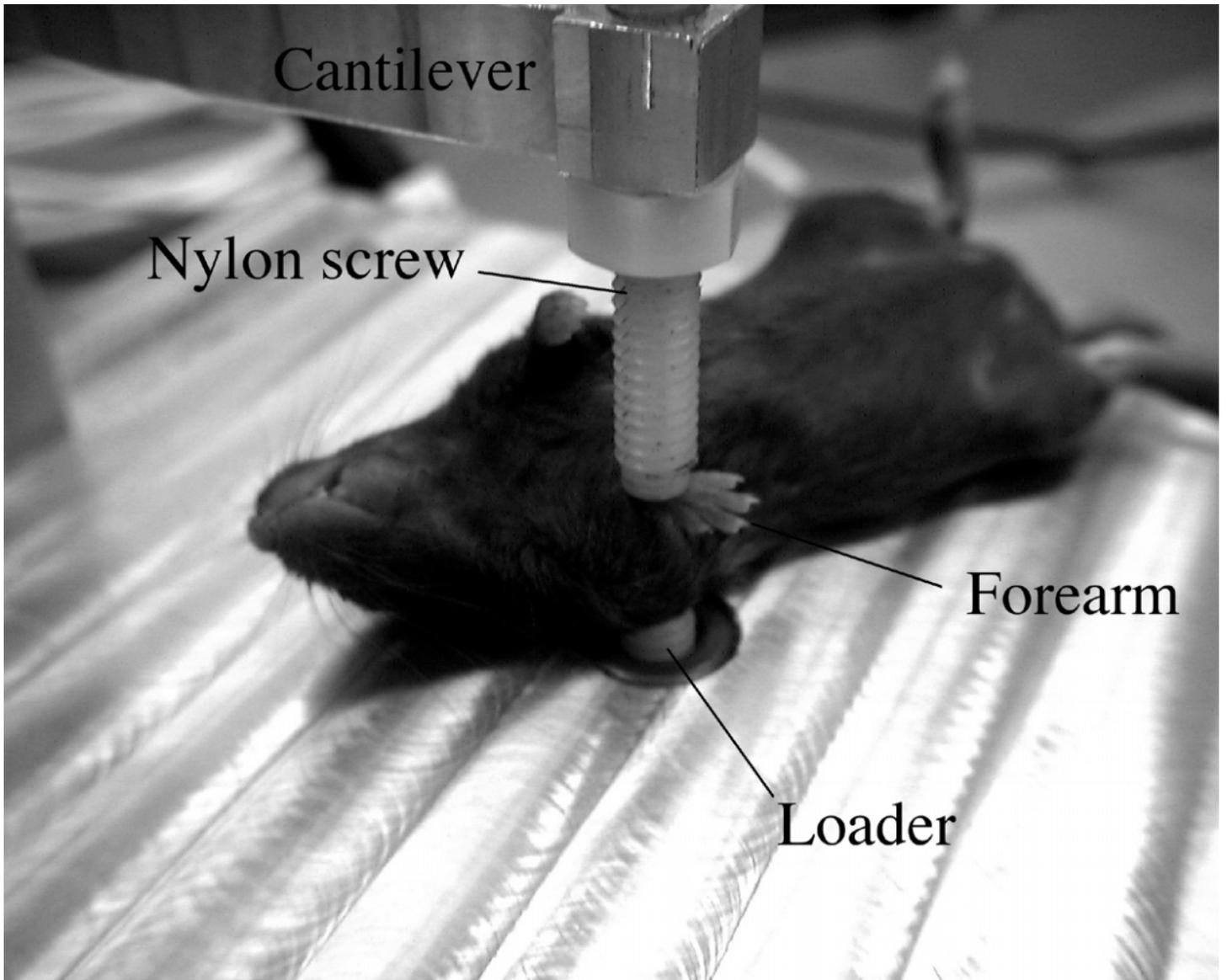
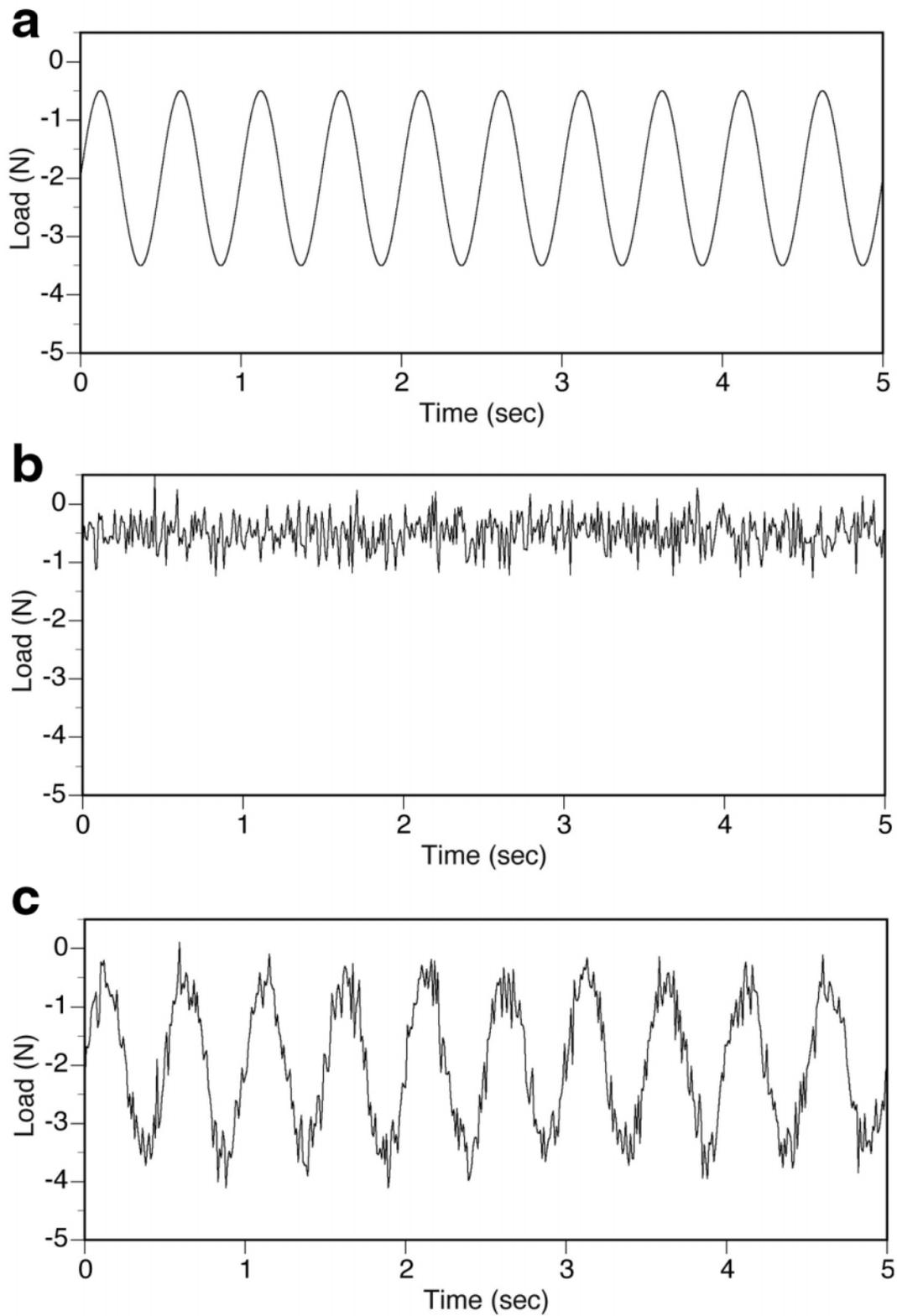


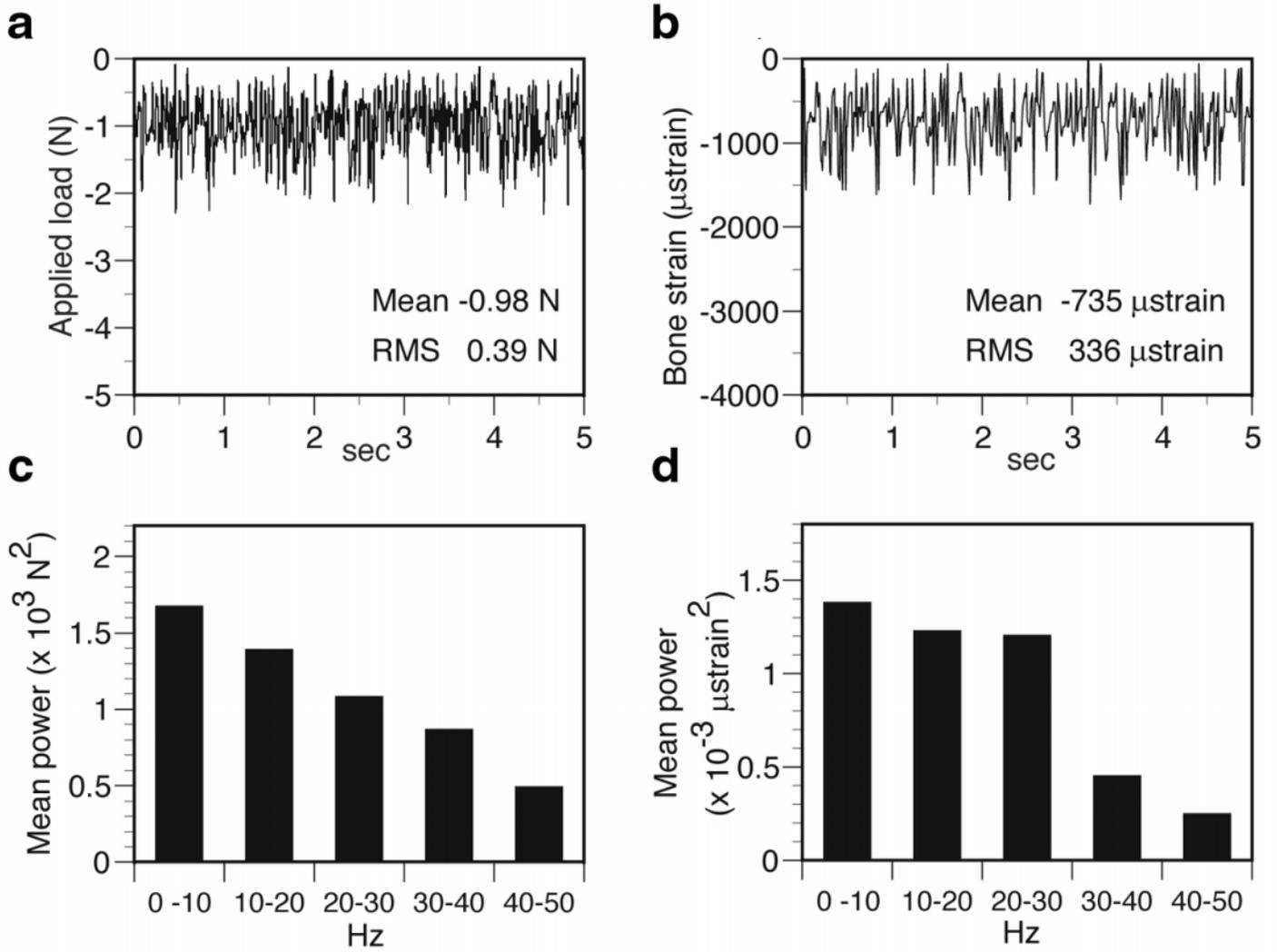
Figure 2. Right forearm of a mouse held in the loading device.

**Fig. 3**



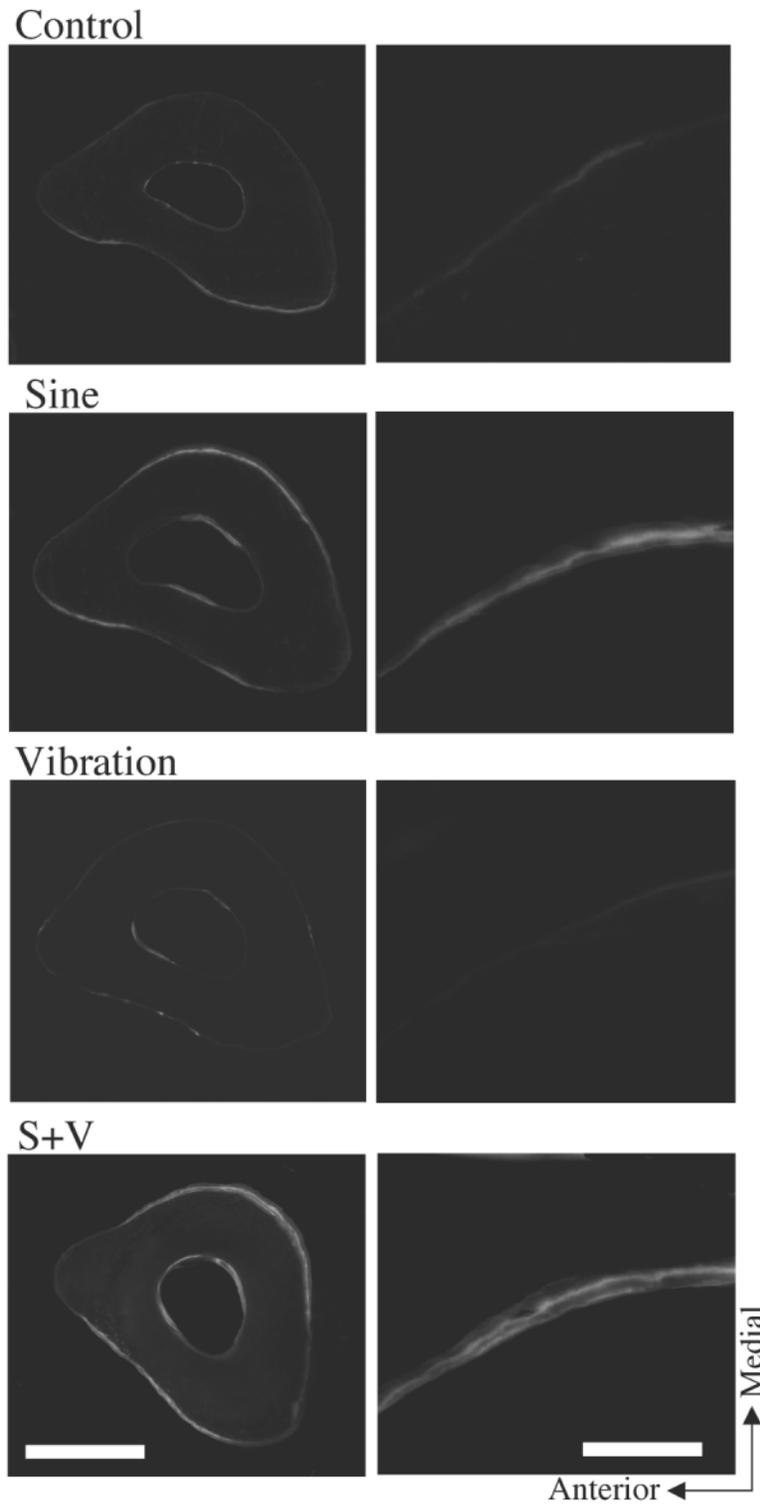
**Figure 3. Applied load waveforms. a)** High-amplitude, low-frequency sinusoidal wave at 2 Hz with an amplitude of 3 N to simulate exercise; **b)** low-amplitude, broad frequency vibration with frequency components 0–50 Hz and 0.3 N mean amplitude; **c)** the sinusoidal wave combined with vibration (S+V) to invoke stochastic resonance.

**Fig. 4**



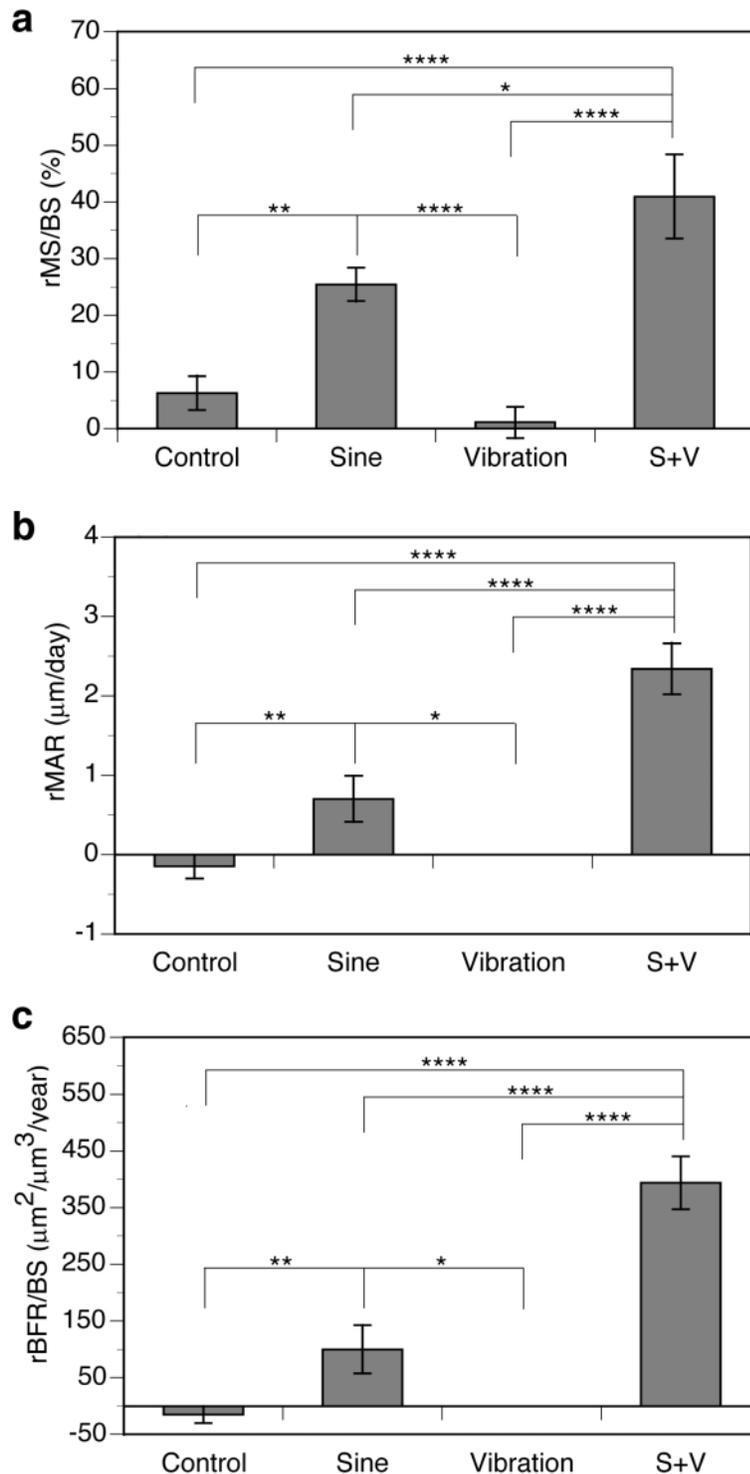
**Figure 4. Vibration load applied to a mouse forearm (a) and bone strain measured by a strain gauge placed on the medial surface of the ulnar midshaft (b). Mean power spectrum per decade Hz for the applied load (c) and for the measured bone strain (d). The vibration applied to the bone tissue caused bone strains ranging up to 50 Hz.**

**Fig. 5**



**Figure 5. New bone formation on cross sections through right (loaded) ulnae.** Photos to the right are a larger magnification of the medial periosteal surface shown in the left photos. White outlines represent fluorescent labels indicating the locations of the mineralization front at 2 and 6 days after loading. More labeled surface or greater distance between labels demonstrates greater bone formation. Bars on the left and right photos represent 280  $\mu\text{m}$  and 140  $\mu\text{m}$ , respectively. Sine and sine plus vibration (S+V) loading induced significant new bone formation, whereas vibration by itself had no effect on bone formation.

Fig. 6



**Figure 6. Comparison between loading groups for relative bone formation parameters on the periosteal surface in ulnar sections.** Histomorphometric measurements included mineralizing surface (rMS/BS), mineral apposition rate (rMAR), and bone formation rate (rBFR/BS). These parameters represent relative values, which were determined by subtracting the parameters in the left ulna (no loading) from those in the right ulna (loaded). The S+V-stimulated group had significantly higher values for all bone formation parameters compared with the other loading groups. No significant differences were observed between vibration-stimulated and control groups. Data represent the mean  $\pm$  standard error. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*\*  $P < 0.0001$ .