Vascular endothelium as a target tissue for short-term exposure to low-frequency noise that increases cutaneous blood flow

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Abstract

Harmful health effects of exposure to low-frequency noise (LFN) defined as noise with frequencies at ≤100 Hz on the circulatory system have been a concern. However, there has been no study on the effects of exposure to LFN on the circulatory system with consideration of its frequencies and decibels. In this study, the effects of short-term exposure to broad-band LFNs and their pure-tone components (pure-tone LFNs) on cutaneous blood flow in the extremities including the hands were investigated. In our fieldwork study, we first sampled some kinds of common broad-band LFNs. Our human study then showed that broad-band LFN with a narrower frequency range more strongly increased cutaneous blood flow than did broad-band LFN with a wider frequency range. Pure-tone LFNs of 70-100 Hz at ≤85 dB(Z), but not pure-tone LFNs exceeding 100 Hz, further increased levels of cutaneous blood flow. Our wavelet-transform spectrum analysis of cutaneous blood flow next revealed that the nitric oxide (NO)-dependent and -independent vascular activities of the vascular endothelium were specifically increased by exposure to pure-tone LFN. Our animal study again indicated that exposure to pure-tone LFN increased cutaneous blood flow in mice with impairments of bilateral inner ears as well as cutaneous blood flow in control mice, suggesting a limited effect of inner ear function on the LFN-mediated increase in cutaneous blood flow. The NO-dependent suppressive effect of pure-tone LFN on cutaneous blood flow was confirmed by inhibition of vascular endothelial activity through intravenous injection of an NO inhibitor in wild-type mice. Taken together, the results of this study demonstrated that the vascular endothelium is a target tissue of LFN and that NO is an effector of the LFN-mediated increase in cutaneous blood flow. Since improvement of peripheral

circulation could generally promote human health, short-term exposure to LFN may be beneficial for health.

Keywords: Low-frequency noise, target tissue, cutaneous blood flow, endothelium, nitric oxide

1. Introduction

We are exposed to various kinds of noise generated from household electric appliances, air conditioners, ventilation systems and transportation systems in our daily life (Berglund et al., 1999). The physical characteristics of noise are defined by the intensity (decibels: dB) and the frequency (hertz: Hz). Noise has sound components including broad-band low-frequency noise (broad-band LFN) that consists of frequencies at ≤100 Hz (Baliatsas et al., 2016; Berglund et al., 1999; Tamura et al., 2012; Luecke et al., 2020). It has been suggested that there are various harmful health effects of long-term exposure to noise including broad-band LFN (Baliatsas et al., 2016; Kempen et al., 2018; Ising and Ising, 2002). However, it is ethically difficult to determine the harmful effect of long-term exposure to broad-band LFN on human health in an intervention trial, since it is important to avoid an intervention causing irreversible harmful effects in humans. In fact, there have been very limited interventional studies in which the human health effects of broad-band LFN with scientifically defined physical characteristics of broad-band LFN were investigated.

Previous studies showed that hearing levels were comparable in control mice and exposure mice after excessive exposure to LFN at 100 Hz, 95 dB(Z) for 1 hour (Negishi-Oshino et al., 2019a) and for 60 hours (Ninomiya et al., 2018), suggesting very limited influence of LFN on hearing. In contrast, excessive exposure to a pure-tone component of LFN (pure-tone LFN) at 100 Hz, ≥95 dB(Z) for 1 hour caused imbalance in mice (Negishi-Oshino et al., 2019a). The vestibule but not the organ of Corti in inner ears was morphologically identified as a target tissue for LFN that causes the imbalance (Ohgami et al. 2020; Negishi-Oshino et al., 2019a; Ninomiya et al., 2018; Ohgami et al., 2017). On the other hand, information about target tissues for LFN other than the vestibule is very limited.

A previous animal study showed that exposure for 4 days to environmental noise recorded around an airport increased the level of blood pressure with increased sensitivity of vasoconstricting factors in the aorta of mice (Münzel et al., 2017). Previous cross-sectional studies epidemiologically suggested that exposure to noise including broad-band LFN caused hypertension in humans (Berglund et al., 1996; Chang et al., 2014). Those studies suggested that noise affects the circulatory system. Furthermore, a correlation between blood pressure and cutaneous blood flow was shown in humans (Lossius et al., 1993; Tsuchida et al., 1991) and in experimental animals (Haddy, 1960). Thus, measurement of cutaneous blood flow could be a useful tool for evaluating the effects of noise on the circulatory system. However, it remains unclear whether LFN affects the circulatory system including

cutaneous blood flow because there was no information about frequencies of the exposed noise in previous reports.

A laser speckle blood flow imager was used in a previous study to non-invasively evaluate the level of cutaneous blood flow with two-dimensional images (Vaz et al., 2016). Spectrum analysis of cutaneous blood flow data obtained by laser doppler flowmetry was shown to provide factors contributing to the change of cutaneous blood flow in humans (Tankanag et al., 2014), and it could be a strong tool for identifying a target tissue of environmental stimulation that affects cutaneous blood flow.

In this study, we first sampled some kinds of broad-band LFN generated in our daily life in our fieldwork study. We then investigated the short-term effects of exposure of hands to local broad-band LFN and pure-tone LFNs on cutaneous blood flow with consideration of their frequencies and decibels in the exposed site. We next tried to identify a target tissue of pure-tone LFNs in humans. We finally tried to clarify the mechanism of the LFN-mediated effect on cutaneous blood flow in mice after confirming the same effects of LFN in humans and mice.

2. Methods

2.1. Subjects

106 The study was carried out with healthy participants. The basic characteristics of
107 the subjects in each experiment are shown in table S1. All of the subjects were non-
108 smokers. All experiments were performed in a quiet room [background noise level =
109 66 dB(Z)] with a temperature of 22 ± 1˚C. The present study was approved by the
110 Ethics Committee of Nagoya University, and all subjects gave informed written
111 consent for participation in the study (approval number: 21016-0036).

112 **2.2. Short-term exposure to broad-band LFN and pure-tone LFN**

113 The actual environmental noises generated from a ventilation system and rotary
114 pump were recorded with a digital recorder (DM-750, OLYMPUS, Japan). The
115 frequency components of environmental noise were analyzed using Sonic Visualiser
116 4.4 version, and it was shown that the noise components generated by the devices
117 had frequencies mainly distributed in the frequency range of broad-band LFN below
118 100 Hz (Fig. 1A). Scales of noise levels included A-weighted noise level [dB(A)],
119 which reflects the sensitivity of hearing in humans, and Z-weighted noise level
120 [dB(Z)], which is the actual noise level at each frequency without weighting. In this
121 study, the scale of dB(Z) was used for assessment of LFN since previous studies
122 suggested that the scale of dB(A) underestimates the influence of LFN below 100 Hz
123 due to A-weighted correction (reduction) of actual noise levels at frequencies below
124 100 Hz (Berglund et al., 1999; Leventhall, 2004), and the effects of LFN in humans
125 and mice were assessed in this study with the same scales of noise levels, although
126 the scale of dB(A) is generally used for assessments of the health risks of noise

exposure. Short-term exposure of hands to broad-band LFN at 85 dB(Z) sound pressure level was performed for 2 min with repetitive playback of a 10-sec-long recorded broad-band LFN shown in Fig. 1B. A sound level meter (TYPE 6236 equipped with an FFT card, ACO CO., LTD, Japan) was used to monitor the noise levels at the site of exposure in humans. Pure-tone LFN contained in broad-band LFN was produced by a multifunction generator (WF1947, NF Corporation, Japan) as a continuous sine wave at frequencies of 60, 70, 80, 90, 100, 120 and 150 Hz and noise intensities of 75, 80 and 85 dB(Z). The recorded broad-band LFN and pure-tone LFN were output by a speaker (KSC-SW11, KENWOOD, Japan) attached to a flexible stand fixed to a separate bench used for the LFN exposure. In our experimental conditions, exposure to broad-band LFN with 85 dB(Z) and pure-tone LFN at 70-100 Hz with 85 dB(Z) did not increase the levels of vibration at the site of exposure (Fig. S1).

2.3. Isolation of pure-tone LFN from environmental LFN

An active noise filter system consisting of a microcontroller board (NUCLEO-F401RE, STMicroelectronics) and a DAC (MCP4821, Microchip Technology) was used to isolate frequency components from broad-band LFN (Fig. S2). The speaker output environmental noise was set up in an LFN-proof box (Shizuka Co. Japan) to prevent sound leakage. Inside the box, the noise was captured by an electric microphone with an amplifier (MAX4466, Analog Devices) that was connected to the

active noise filter system. LFN before and after filtration was used for monitoring cutaneous blood flow.

2.4. Measurement of cutaneous blood flow using a laser speckle blood flow imager

Each subject sat in a comfortable chair with his/her back supported for 10 min to acclimate to the environment before starting the measurement. The right hand of the subject was placed on a vibration-proof sponge while the speaker was 5 cm over the dorsum of the hand. The edges of the speaker were 1 cm from the wrist and 10 cm from the tips of fingers (Fig. 1B). A laser speckle blood flow imager system (Omegazone OZ-2 STD, Omegawave, Inc., Tokyo, Japan) was used to measure cutaneous blood flow in two dimensions. Cutaneous blood flow was recorded continuously with the HS-AVG mode, which provided an average of 24 high-speed images per second using LSI software (Omegawave, Inc., Tokyo, Japan). The averaged BF enclosed by the region of interest (ROI) was analyzed using LIA software (Omegawave, Inc., Tokyo, Japan) shown in Figs. 1C and 5B. Cutaneous blood flow was measured 30 sec before LFN exposure and for 2 min during LFN exposure.

2.5. Cutaneous temperature measurement using an infrared camera

An infrared camera (FLIR ONE PRO, FLIR Systems Inc., Sweden) was used to measure cutaneous temperature. The infrared camera was set at a distance of 50 cm

from the hand. Changes of cutaneous temperature in the dorsa of the hands were measured every 10 sec before (baseline for 30 sec) and during no exposure (control) for 2 min and LFN exposure for 2 min.

2.6. Wavelet transform analysis of blood flow signals measured by laser doppler flowmetry

For spectral analysis, cutaneous blood flow was recorded using laser doppler flowmetry (wavelength of 780 nm, ATBF-LN1, Unique Medical) with a contact probe (LP-C2) attached to the dorsum of the hand avoiding the venous network. Cutaneous blood flow signals with a sampling rate of 100 Hz were digitized using an analog-to-digital converter (USB-6211) (Jarm et al., 2010). Cutaneous blood flow data were recorded as a baseline of 30 sec and 2 min with exposure to pure-tone LFN [70 Hz, 85 dB(Z)]. The continuous wavelet transform of a cutaneous blood flow signal g(u) is defined as $\tilde{g}(s,t) = \int_{-\infty}^{+\infty} \bar{\psi}_{s,t}(u) g(u)\ du$ as shown in a previous study (Söderström et al. 2003). The time-averaged wavelet transform value [arbitrary unit (a.u.)] within each frequency was determined to assess vascular regulatory mechanisms. The characteristic frequencies divided into five different intervals (Söderström et al., 2003) are described in the Results section. The endothelial activity was further divided into nitric oxide (NO)-dependent (0.0095-0.020 Hz) and NO-independent (0.005-0.0095 Hz) endothelial factors (Grinevich et al., 2019). Amplitude changes within each frequency interval were calculated by subtracting the baseline average amplitude from the amplitude during exposure.

2.7. Animal experiments

Male C57BL/6J and ICR mice (purchased from Japan SLC, Inc.) were maintained in a specific pathogen-free (SPF) environment with room temperature at 23 ± 2˚C, humidity of 55 ± 10% and a 12-h light/dark cycle. The mice had free access to standard mouse chow and water. All experiments were approved by the Institutional Animal Care and Use Committee in Nagoya University (approval number: M220179-001) and followed the Japanese Government Regulations for Animal Experiments.

After 30 min of anesthesia injection, baseline values of cutaneous blood flow and temperature were measured for 10 sec and 30 sec before LFN exposure, respectively. Immediately after the baseline measurements, LFN exposure was performed during measurements of blood flow and temperature with an LFN exposure setting similar to the setting in human trials. The detailed conditions of each experiment are shown in Fig. 5.

For the anesthesia, a mixture of anesthetic agents including 0.75 ml of medetomidine (1 mg/ml) (Kyoritsu Seiyaku Corporation), 5.0 ml of midazolam (2 mg/ml) (Wako Pure Chemical Industries, Ltd) and 2.5 ml of butorphanol (5 mg/ml) (Meiji Seika Pharma Co., Ltd.) was intraperitoneally injected into the mice (10 ml/kg body weight) as in a previous study (Kawai et al., 2011). In order to have a stable baseline of blood flow, we waited for 30 min after the anesthesia injection (Fig. S3),

since the baseline of cutaneous blood flow was shown to be stable after 30 min of the anesthesia injection in a previous study (Gargiulo et al. 2013).

2.8. Preparation of vestibular lesion mice

Bilateral intratympanic injection of 25 µl of gentamicin sulfate (Wako, Japan) dissolved in saline at 200 µg/µl under a stereomicroscope (Olympus, SZ61) was performed in ICR mice with the same anesthesia as that described above. The administrations were performed on 3 consecutive days (Ishibashi et al., 2009; Negishi-Oshino et al., 2019b). One week after the final administration, before assessments of the effect of pure-tone LFN on cutaneous blood flow in vestibular lesion mice, typical phenotypes of vestibular impairment were confirmed by rotarod and beam tests (Fig. S4), results of which have been shown to be correlated with vestibular functions in mice (Isgrig et al., 2022; Negishi-Oshino et al., 2019a).

2.9. Intravenous injection of an NO inhibitor

Thirty µg/µl of L-NAME (WAKO), an NO inhibitor, was intravenously injected into tail veins in C57BL/6J mice at the dose of L-NAME (250 mg/kg) used in a previous study (Morita et al. 1996). After obtaining preliminary results to decide the appropriate treatment time for L-NAME and to validate its effect by the change of blood flow in soles as shown in previous studies (Ghafouri et al., 2011; Gohin et al. 2016), we performed the experiments with 20-min treatment time after L-NAME injection. Anesthesia was performed in mice prior to the intravenous injection of L-

NAME, since the intravenous injection requires mice to be kept under an immobile condition. At 10 min after the anesthesia injection that allowed us to have immobile mice, we performed intravenous injection of L-NAME and then waited for 20 min as mentioned above, followed by short-term exposure to pure-tone LFN during blood flow measurement to evaluate the suppressive effect of L-NAME on LFN-mediated blood flow.

2.10. Statistical analysis

Non-parametric statistical tests were performed using data analysis software (SPSS version 23, IBM). Comparisons between two groups were performed by using the Wilcoxon signed-rank test for matched samples and the Mann–Whitney *U* test for independent samples. The Friedman test was performed for comparing more than two related groups. Differences with $P < 0.05$ were considered to be statistically significant.

3. Results

3.1. Influence of broad-band LFN on cutaneous blood flow in humans

In this study, sampling of LFN sources generated in our daily life was performed. Since we found that a ventilation system and a rotary pump generated typical patterns of LFN (Fig. 1A), a hand of each subject was exposed to broad-band LFN by repeating output of noise recorded from the ventilation system and the rotary pump

through a speaker as shown in Fig. 1B. The Z-weighted 10-second equivalent sound pressure level at the dorsum of the hand was 85 dB(Z). Blood flow images of the hands during broad-band LFN exposure are shown in Fig. 1C. Cutaneous blood flow in the dorsa of hands was significantly increased by local exposure to 85 dB(Z) of broad-band LFN generated from the rotary pump but not to that generated from the ventilation system (Fig. 1D). Since the exposure to broad-band LFN affected cutaneous blood flow, we further determined the influence of LFN with a narrow frequency range on cutaneous blood flow in humans. We determined the influence of LFN at 70 Hz isolated from broad-band LFN with an active noise filter system (Fig. 1E), since broad-band LFN from the rotary pump that showed an effect on cutaneous blood flow included the majority of noise components around 70 Hz (Fig. 1A). Using the active noise filter system with an LFN-proof box enabled us to isolate LFN from broad-band LFN (Fig. S2). Cutaneous blood flow at the dorsa of right hands was significantly increased by local exposure to 85 dB(Z) of isolated LFN at 70 Hz but not by local exposure to LFN before isolation (Fig. 1F). Semi-quantitative analysis of the spectra of broad-band LFN showed that the broad-band LFN from the rotary pump had a narrow frequency range of 41% less noise components than that of broad-band LFN from the ventilation system (Fig. S5A). The isolated LFN had a narrow frequency range of 80% less noise components than that of broad-band LFN before isolation (Fig. S5B).

3.2. Influence of pure-tone LFN on cutaneous blood flow in humans

We next determined the influence of pure-tone LFN with a further narrow frequency range on cutaneous blood flow in humans (Fig. 2). Typical pure-tone LFN (70 and 150 Hz) spectrograms produced from the digital sound generator are shown in Fig. 2A. Cutaneous blood flow at the dorsa of right hands was significantly increased by local exposure to 85 dB(Z) of pure-tone LFN at 60, 70, 80, 90 and 100 Hz (Fig. 2B, left graph). Since pure-tone LFN at 70 Hz showed the highest median of cutaneous blood flow among the frequencies, we then focused on determining the effects of different intensities [75, 80 and 85 dB(Z)] of pure-tone LFN at 70 Hz. Cutaneous blood flow in the dorsa of hands was significantly increased by pure-tone LFN at 80 and 85 dB(Z) but not at 75 dB(Z) (Fig. 2B, right graph). We further verified the influence of pure-tone LFN on cutaneous blood flow by determining cutaneous temperature, since cutaneous blood flow is known to be positively correlated with cutaneous temperature (Itokawa et al., 2020). Cutaneous temperature at the dorsa of right hands was significantly increased by local exposure to 85 dB(Z) of pure-tone LFN at 70 Hz (Fig. 2C), which showed the highest median of cutaneous blood flow as can be seen in Fig. 2B. Semi-quantitative analysis of the spectra of broad-band LFN and pure-tone LFN at 70 Hz showed that the pure-tone LFN had a further narrower range of frequencies of 99% less noise components than that of broad-band LFN (Fig. S5B).

3.3. Spectrum analysis of a target tissue contributing to the influence of pure-tone LFN on cutaneous blood flow in humans

We investigated a target tissue contributing to the pure-tone LFN-mediated increase of cutaneous blood flow. Blood flow in the hands was not increased during the bilateral auditory stimulation for 2 min by 85 dB(Z) of broad-band LFN and pure-tone LFN at 70 Hz with headphones (Fig. 3A, B). The sound level at the ears was 56 dB(Z) at 70 Hz, which was comparable to the background level, when pure-LFN at 70 Hz, 85 dB(Z) was output targeting the hands in humans (Fig. 3C). We then used wavelet-transform spectrum analysis of cutaneous blood flow measured by laser doppler flowmetry (Fig. 4). In previous studies, wavelet-transform spectrum analysis was used to identify target factors [vascular endothelial activity (0.005-0.020 Hz), neurogenic activity representing the sympathetic nervous system (0.02-0.06 Hz), myogenic activity (0.06-0.2 Hz), respiratory activity (0.2-0.6 Hz) and cardiac activity (0.6-1.6 Hz)] contributing to the change of cutaneous blood flow in humans (Grinevich et al., 2019; Söderström et al., 2003; Stefanovska et al., 1999). Vascular endothelial activity represents the release of vasodilator factors in peripheral tissues, which can be further divided into nitric oxide (NO)-dependent and NO-independent endothelial factors (Grinevich et al., 2019; Hodges and Cheung, 2020). The wavelet-transform spectrum analysis showed that the amplitude of endothelial activity including NO-dependent and NO-independent factors significantly increased compared to that in the control group during exposure to the pure-tone LFN. In contrast, in sympathetic nervous, respiratory and cardiac systems, the amplitude changes were not increased during exposure to pure-tone LFN (Fig. 4).

3.4. Validation of a target tissue for pure-tone LFN affecting cutaneous blood flow in mice

We further verified the results obtained by the human study by research on the biosphere (Fig. 5A). Typical blood flow images of ear auricles during pure-tone LFN exposure are shown in Fig. 5B. Cutaneous blood flow in ear auricles of mice was significantly increased by 85 dB(Z) of pure-tone LFN at 60, 70, 90 and 100 Hz (Fig. 5C, left graph). Cutaneous blood flow in ear auricles of mice was significantly increased by 70 Hz of pure-tone LFN at 85 dB(Z) (Fig. 5C, right graph). In addition, cutaneous temperature in ear auricles was significantly increased by exposure to 85 dB(Z) of pure-tone LFN at 70 Hz (Fig. 5D), which showed the highest median of blood flow in mice as can be seen in the right graph of Fig. 5C. We next used vestibular lesion mice with impairment of inner ears caused by administration of the ototoxic chemical gentamicin to verify the possibility that inner ears are not involved in the change of cutaneous blood flow in mice exposed to pure-tone LFN. Vestibular lesion mice showed typical imbalance behavior (Fig. S4), which has been shown to correlate with vestibular function (Negishi-Oshino et al., 2019b). Cutaneous blood flow in ear auricles of vestibular lesion mice was significantly increased by exposure to pure-tone LFN at 70 Hz, 85 dB(Z) (Fig. 5E). The degrees of increased cutaneous blood flow stimulated by pure-tone LFN at 70 Hz, 85 dB(Z) in control mice and VL mice were comparable (Fig. 5E). The increase of cutaneous blood flow in ear

auricles was significantly suppressed in mice with intravenous injection of the NO inhibitor L-NAME (Fig. 5F).

4. Discussion

4.1. Finding of LFN affecting cutaneous blood flow

We demonstrated for the first time the existence of broad-band LFN with a narrow frequency range below 100 Hz that increased cutaneous blood flow in humans. Pure-tone LFN at 70-100 Hz with a further narrower frequency range than that of broad-band LFN further increased cutaneous blood flow by a median value of 20%, while pure-tone exposures to 120 and 150 Hz, which are not included in the definition of LFN, did not significantly increase cutaneous blood flow. Our results demonstrated that specific frequencies and intensities of pure-tone LFN (sound spice®) as well as a narrow frequency range of broad-band LFN are required for increasing cutaneous blood flow in humans and mice.

4.2. Limited effect of auditory and vestibular-sympathetic nervous systems on cutaneous blood flow by local exposure to pure-tone LFN.

Previous studies showed that stimulation of the vestibule by gravity affects the sympathetic nervous system, leading to an increase in blood pressure (Yates, 2004) and that exposure to noise was associated with cardiovascular risk via the sympathetic nervous system (Ising and Kruppa, 2004; Lusk et al., 2004). Those

studies indicate the possibility that LFN affects cutaneous blood flow via the vestibular-sympathetic nervous system. However, wavelet-transform spectrum analysis of cutaneous blood flow revealed that vascular endothelial activation, but not the sympathetic nervous system, is correlated with the increased cutaneous blood flow by pure-tone LFN exposure in our human study. The noise level detected at ears was the background level under the condition of local exposure of hands to pure-tone LFN at 70 Hz with 85 dB(Z) in our human study. No significant increase of cutaneous blood flow in a hand was observed by bilateral auditory exposure to pure-tone LFN at 70 Hz with 85 dB(Z) by the headphones in our human study. These results suggest that the auditory system has a limited effect on cutaneous blood flow by local exposure to pure-tone LFN in humans. Moreover, our animal study showed that there were comparable levels of increased cutaneous blood flow in wild-type mice and vestibular lesion mice with bilateral impairments of inner ears by exposure to pure-tone LFN at 100 Hz (Fig. S6) as well as at 70 Hz (Fig. 5E). Our results obtained in humans and mice suggest that the vestibular-sympathetic nervous system has limited effects on the increase of cutaneous blood flow caused by exposure to pure-tone LFN.

4.3. Vascular endothelium could be a target tissue for pure-tone LFN affecting cutaneous blood flow

In this study, wavelet-transform spectrum analysis of cutaneous blood flow showed that both NO-dependent and NO-independent factors, which regulate

vascular endothelium activities, were increased by the pure-tone LFN exposure in humans. An NO inhibitor partially suppressed the pure-tone LFN-mediated increase of cutaneous blood flow in mice. Our results indicated that the vascular endothelium could be a target tissue for pure-tone LFN that affects cutaneous blood flow through regulation of NO. In previous studies in which wavelet transform spectrum analysis of cutaneous blood flow was performed, the NO-independent factor was shown to correspond to the release of prostaglandins and endothelial-derived hyperpolarizing factors in the vascular endothelium (Grinevich et al., 2019; Hodges and Cheung, 2020). Since the pure-tone LFN-mediated increase of the NO-independent factor could not be suppressed by an NO inhibitor in our animal study, its suppressive effect may be partial. Further study is needed to elucidate the NO-independent factor in mice.

4.4. Scientific contributions

In this study, short-term exposure to pure-tone LFN increased cutaneous blood flow in various parts of the extremities including the hand, forearm and toes (Fig. S7). Since increased cutaneous blood flow in the extremities has been shown to contribute to the prevention of cold constitution, pressure ulcers, and diabetic circulatory disorders (Claeys, 1997; Cracowski and Roustit, 2020; Liao et al., 2013; Petrofsky, 2012), short-term exposure to pure-tone components in broad-band LFN may be beneficial for the prevention of disorders in humans. Meanwhile, this study showed that long-term exposure to broad-band LFN at 100 dB(Z) 3 times for 12 hours each time significantly decreased cutaneous blood flow in mice (Fig. S8),

suggesting that long-term exposure to broad-band LFN has a harmful effect on cutaneous blood flow. A previous study showed that long-term exposure for 4 days to environmental noise recorded around an airport caused damage of the vascular endothelium in mice (Münzel et al., 2017). Therefore, it is possible that short-term exposure to LFN is linked to long-term exposure to LFN via the same target tissue, although the effects are different (i.e., endothelium stimulation by short-term exposure and endothelium damage by long-term exposure). Our hypothesis partially corresponds to the results of previous studies showing that short-term exposure to ultraviolet (UV) light increased cutaneous blood flow via stimulation of the vascular endothelium (Liu et al., 2014), while long-term exposure to UV light caused damage of the vascular endothelium (Schuch et al., 2017). Thus, our results scientifically contribute to a new understanding of LFN having a beneficial effect as well as a hazardous effect on our health. Further studies are needed to verify whether a target tissue for long-term exposure is the same as that for short-term exposure.

4.5. Innovative points

The general purpose of noise control is simply to reduce exposure levels in order to reduce health risks since noise has been regarded as one of the hazardous air pollutants for human health (Berglund et al., 1999). In this study, the usefulness of the active noise control system for LFN (sound recycle®) was shown, because the system could isolate the pure-tone LFN, which further increased cutaneous blood flow, from broad-band LFN. It is known that broad-band LFN is usually generated from eco-friendly devices (e.g., heat pump systems) that enable us to save energy (Gadalla et al., 2005). Our results suggest one possible technology as innovative

417 solutions providing not only beneficial effects on health (i.e., increase of cutaneous
418 blood flow) by positive utilization of pure-tone LFN isolated from noise pollutants but
419 also reduction of unnecessary exposure to noise.

420 **4.6. Study limitations**

421 There are several limitations in this study. For the human study, the number of
422 subjects and the number of environmental noise sources evaluated in this study were
423 limited. Further studies are needed to evaluate the effects of broad-band LFN
424 generated from other noise sources using a larger number of subjects. In addition,
425 short-term exposure to LFN was used in this study in order to safely perform the
426 interventional trial, since previous studies showed that long-term exposure to LFN
427 had harmful effects in humans (Baliatsas et al., 2016) and mice (Negishi-Oshino et
428 al., 2019a; Ninomiya et al., 2018). The health risk of long-term exposure to LFN in
429 humans should be investigated in the future in a safe interventional study. For the
430 mouse study, the effect of LFN on cutaneous blood flow in mice was not evaluated
431 under the same awake condition as that in the human study, since the laser speckle
432 blood flow imager requires the mice to be kept under an immobile condition.
433 Therefore, anesthesia was required for assessments in mice. In a future study, the
434 effect of LFN on cutaneous blood flow in mice should be investigated by using a
435 wearable sensor to measure cutaneous blood flow under an awake condition.

436 **5. Conclusion**

437 In conclusion, our combined fieldwork (atmosphere), human (anthroposphere)
438 and animal (biosphere) studies demonstrated for the first time that short-term
439 exposure to broad-band LFN with a narrower frequency range than that in our
440 environment and to pure-tone LFN promoted cutaneous blood flow. Our results

obtained from multiple spheres then clarified a target tissue as well as the mechanism of NO-mediated increase in cutaneous blood flow by short-term exposure to pure-tone LFN.

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601 **Figure legends**
602 **Fig.1 Effects of broad-band LFN and isolated LFN on cutaneous blood flow in**

hands. (A) Physical characteristics of broad-band LFN recorded from different devices (ventilation system and rotary pump) were analyzed using frequency spectrograms shown with frequency on the y axis and time on the x axis. The color scale bar is a measure of the intensity (Decibels Full Scale, dB FS) of the signal, ranging from a high intensity (-20 dB) to a low intensity (-80 dB). (B) Test setting. The subject was in the sitting position during the measurement. A hand was put on a vibration-proof sponge and was exposed to LFN output by a speaker (all pass= 85 dB). The distances between the speaker and the hand are shown in the inset. A laser speckle blood flow imager was set at a distance of 50 cm from the hand. (C, D) Typical images of blood flow in hands captured by the laser speckle blood flow imager (C) and change of blood flow in the dorsa of hands (n=9) (D) during no exposure for 2 min (ctrl) and exposure for 2 min to broad-band LFN at 85 dB(Z) (all pass) recorded from different sources (venti: ventilation, R Pump: rotary pump). Regions of interest (ROIs) shown by squares indicate the areas of blood flow analysis. (E) The physical characteristics of broad-band LFN before and after isolation from broad-band LFN (ventilation) with the active filter are shown by frequency spectrograms with frequency on the y axis and time on the x axis. (F) Changes of blood flow in the dorsa of hands (n=8) during no exposure for 2 min (ctrl) and exposure for 2 min to LFN at 70 Hz, 85 dB(Z) (all pass) before and after isolation from broad-band LFN. Significant difference (*$p<0.05$) and no significant difference compared to the no exposure group were analyzed by the Friedman test (D, F).

Fig.2 Effects of pure-tone LFN on cutaneous blood flow and temperature in hands. (A) Spectrograms of 85 dB(Z) of pure-tone LFN at 70 Hz (left) and 150 Hz (right) generated by a digital sound generator are shown with frequency (Hz) on the y axis and time (seconds) on the x axis. The color scale bar is the intensity (Decibels Full Scale, dB FS), ranging from a high intensity (-20 dB) to a low intensity (-80 dB). An arrow and an arrowhead indicate pure-tone LFN at 70 Hz and 150 Hz, respectively. (B)

Changes of cutaneous blood flow (%, relative to baseline) in the dorsa of hands during no exposure for 2 min (gray box plot, ctrl) and exposure to 85 dB(Z) of pure-tone LFN at different frequencies and different intensities (dB) of pure-tone LFN at 70 Hz for 2 min (open box plots) are shown (n=16). Cutaneous blood flow was measured by a laser speckle blood flow imager with the same ROIs as those shown in Fig. 1C. (C) Changes of cutaneous temperature (˚C, subtracted by baseline) in the dorsa of hands during no exposure for 2 min (gray box plot, ctrl) and exposure to 85 dB(Z) of pure-tone LFN at different frequencies for 2 min (open box plots) are shown (n=17). Significant difference (*$p < 0.05$, **$p<0.01$) and no significant difference (N.S.) compared to no exposure (ctrl) were analyzed by the Friedman test.

Fig.3 Influence of auditory exposure to LFN on cutaneous blood flow in hands of humans. (A) Boxplot of increased blood flow measured in hands during 2-min exposure of ears to broad-band LFN (from a rotary pump), all pass = 85 dB (n=4). (B) Blood flow measured in hands during exposure to pure-tone LFN output by a headphone (n=13). (C) Sound pressure level measured at ears when pure-tone LFN output targeted hands. N.S.: no significant difference compared to the control group by the Wilcoxon signed-rank test.

Fig.4 Wavelet-transform spectrum analysis of cutaneous blood flow in human hands exposed to pure-tone LFN. The time-averaged wavelet transform was applied in spectral analysis of cutaneous blood flow signals measured by laser doppler flowmetry to determine factors contributing to change of blood flow in response to exposure to 85 dB(Z) of pure-tone LFN at 70 Hz (n=13). The relative amplitude shifts from baseline of the frequency spectrum of the blood flow signals (a.u.) during no exposure for 2 min (gray box plot, control) and exposure to pure-tone LFN for 2 min (open box plot) are shown. The frequency ranges for each factor are shown in boxplots. Significant differences (**$p<0.01$, *$p<0.05$) and no significant difference (N.S.) between

exposure to no exposure (control) were analyzed by the Wilcoxon signed-rank test.

Fig.5 Influence of pure-tone LFN on cutaneous blood flow in vestibular lesion mice with impairments of bilateral inner ears and mice with intravenous injection of an NO inhibitor. (A) Experimental setting. A mouse was put on the vibration-proof sponge and was exposed to pure-tone LFN output by a speaker (all pass = 85 dB). The distance between the speaker and the mouse was 5 cm. The laser speckle blood flow imager was set at a distance of 20 cm to the mouse. (B) Typical images of cutaneous blood flow in auricles of mice (C57BL/6J, male, 2 months of age) during no sound (control) and exposure to 85 dB of pure-tone LFN at 70, 100 and 150 Hz are shown. The region of interest (ROI) shown by a dotted line indicates the area of blood flow analysis. (C) Changes of cutaneous blood flow (%, relative to baseline) in auricles of mice (n=6) during no exposure for 10 sec (no sound) and exposure to 85 dB(Z) of pure-tone LFN at different frequencies (left graph) and different intensities (dB) of pure-tone LFN at 70 Hz (right graph) for 10 sec are shown. (D) Cutaneous temperatures (˚C, shift from baseline) in auricles of mice (C57BL/6J, male, 4 months of age) during no exposure for 1 min (no sound) and exposure to 85 dB(Z) of pure-tone LFN at 70 Hz for 1 min (pure-tone LFN) are shown (n=6 in each group). (E, F) Changes of cutaneous blood flow (%, relative to baseline) in auricles of (E) vestibular lesion mice (ICR, male, 6-7 weeks of age) with impairments of bilateral inner ears caused by intratympanic injections of gentamicin (VL+, n=7) and control mice (VL-, n=5) and (F) mice (C57BL/6J, male, 4-6 months of age) with intravenous injection of L-NAME (+) (n=8 ear auricles) and control (-) (n=7 ear auricles) during no exposure for 10 sec (no sound) and exposure to 85 dB(Z) of pure-tone LFN at 70 Hz for 10 sec (pure-tone LFN) are shown. VL stands for vestibular lesion mice. Significant differences (*$p< 0.05$, **$p < 0.01$, N.S: no significant difference) compared to the control group were analyzed by the Friedman test in (C), Wilcoxon signed-rank test (D) and Mann–Whitney *U* test (E, F).

Figure 1

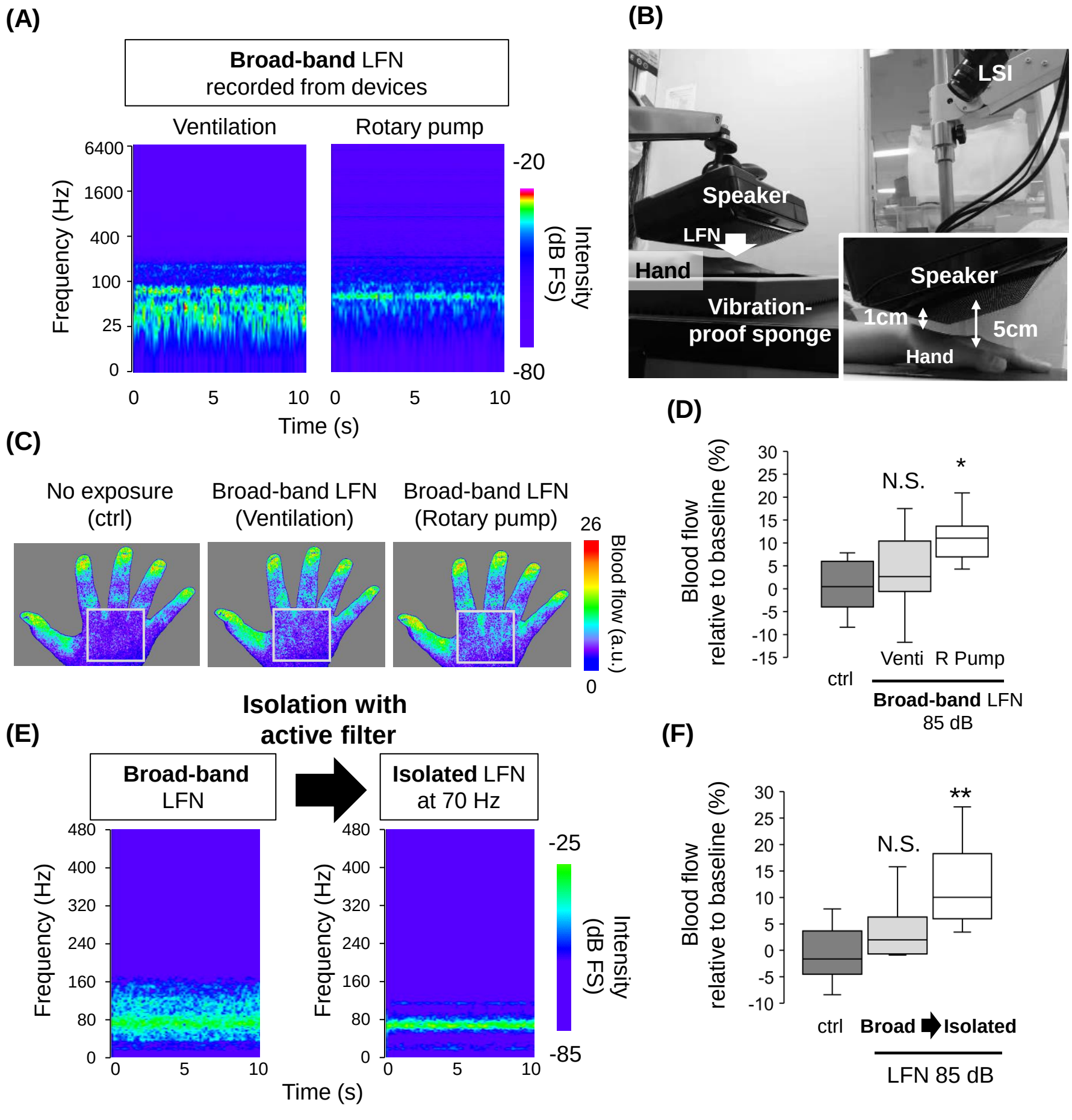

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Figure 2

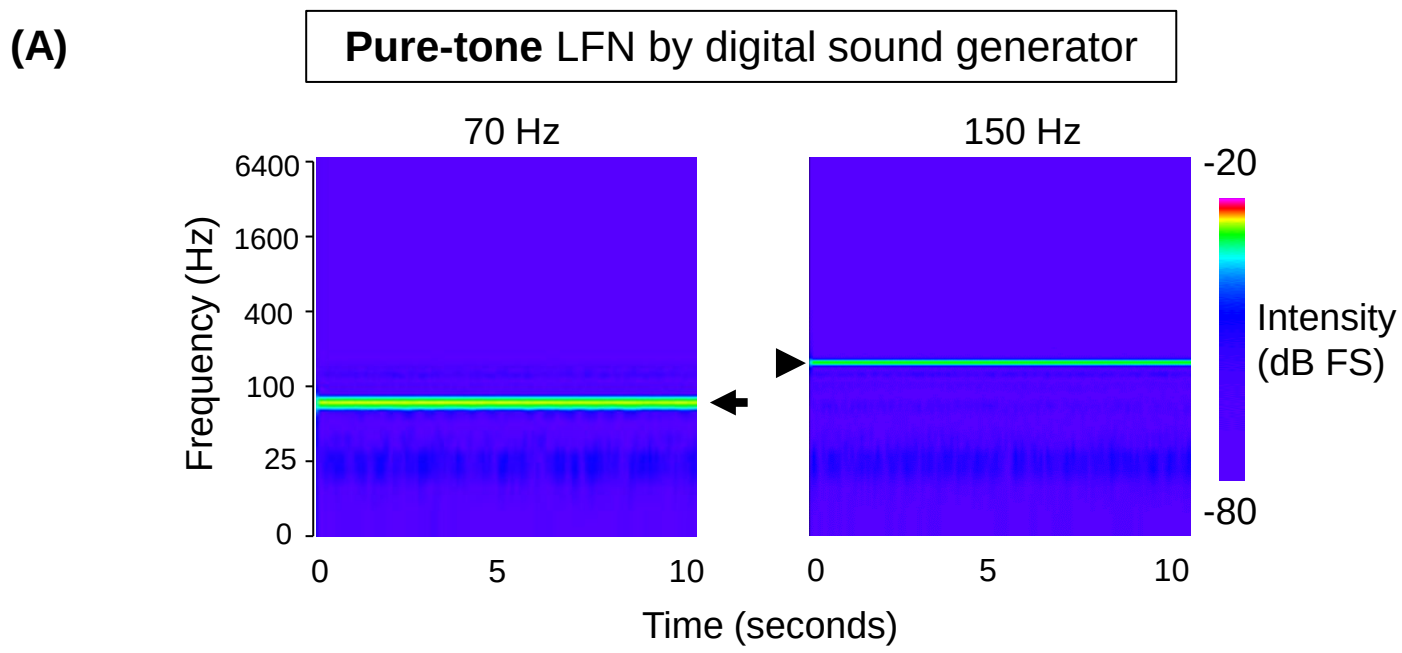

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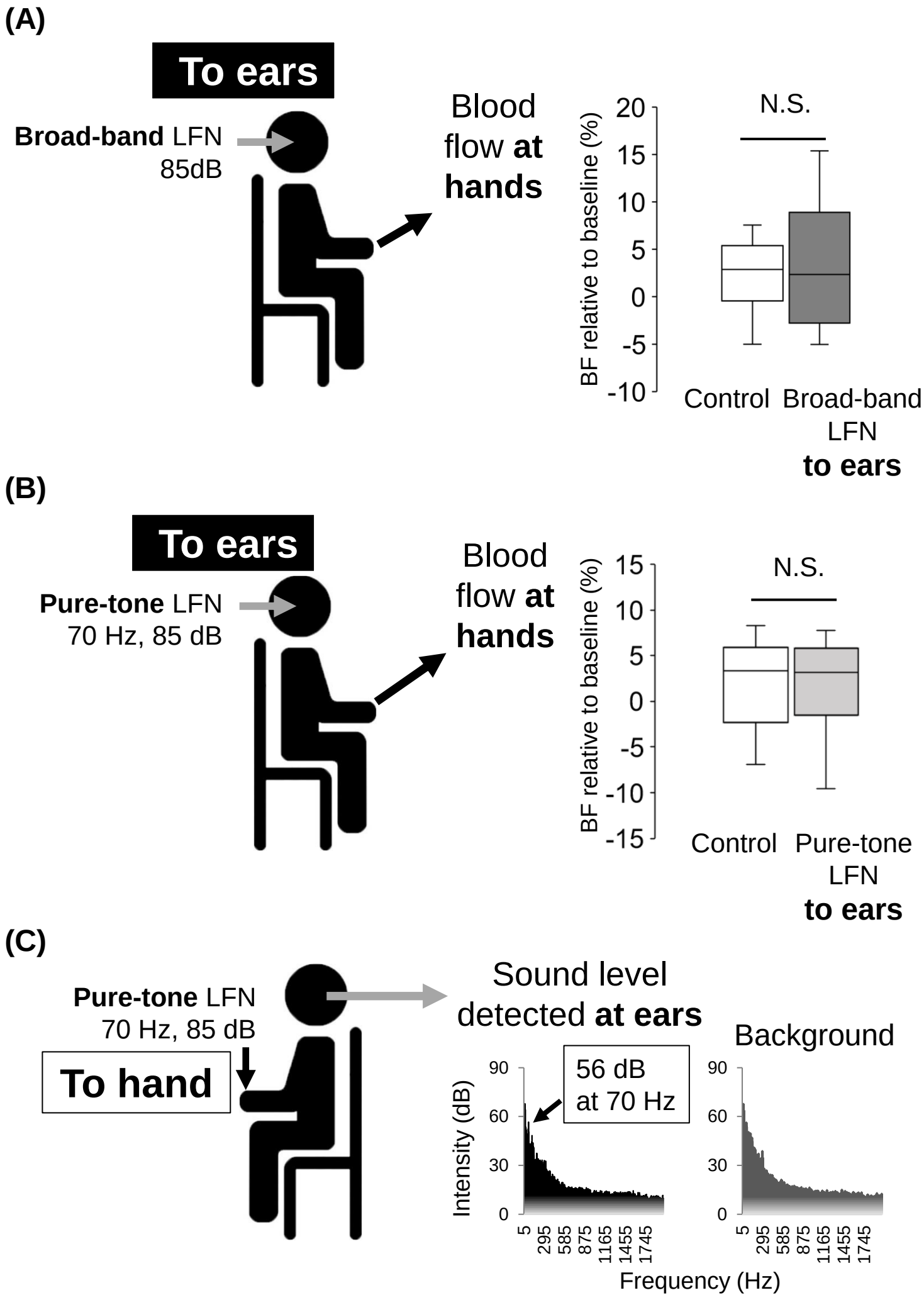
Figure 3



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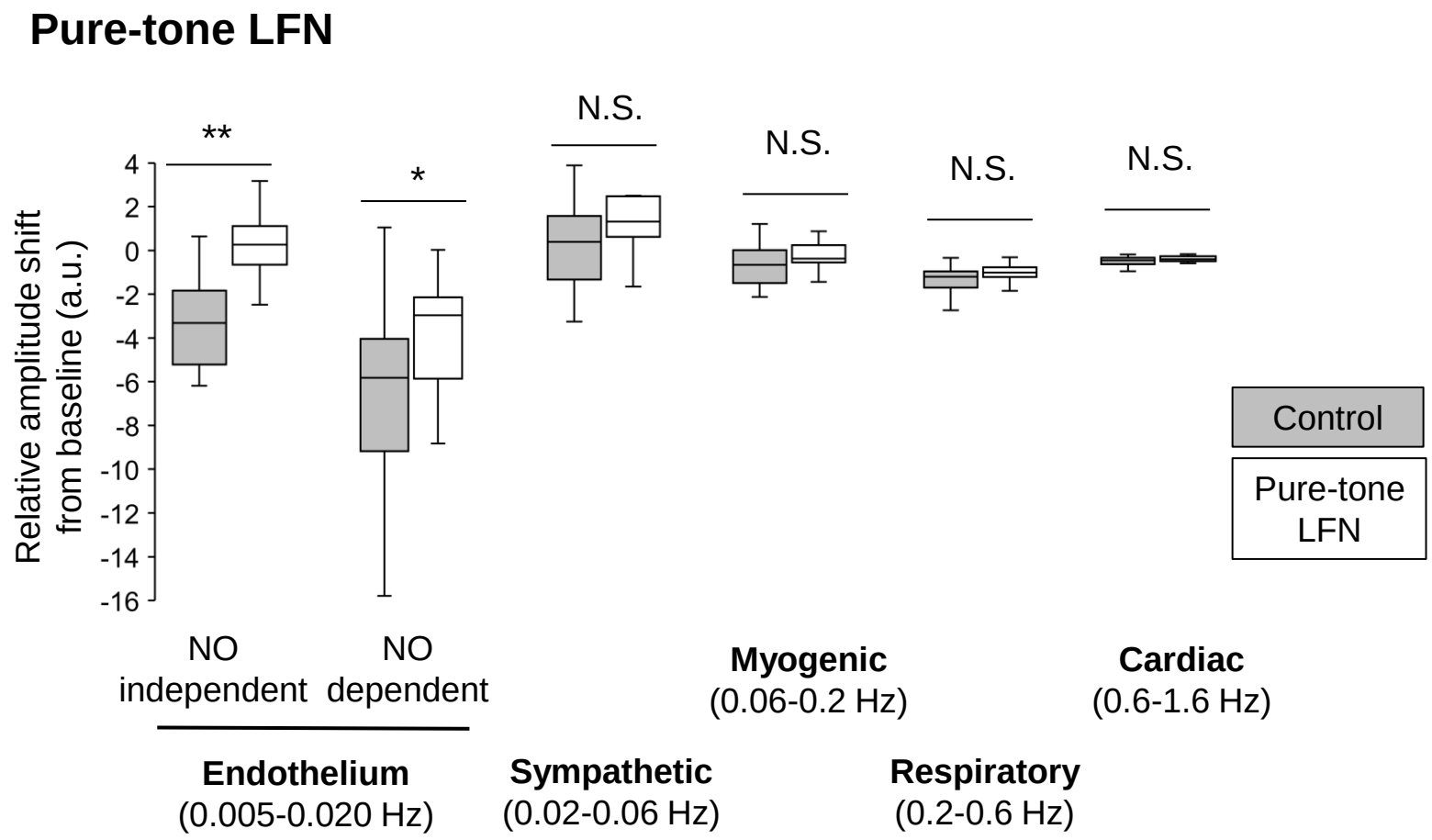

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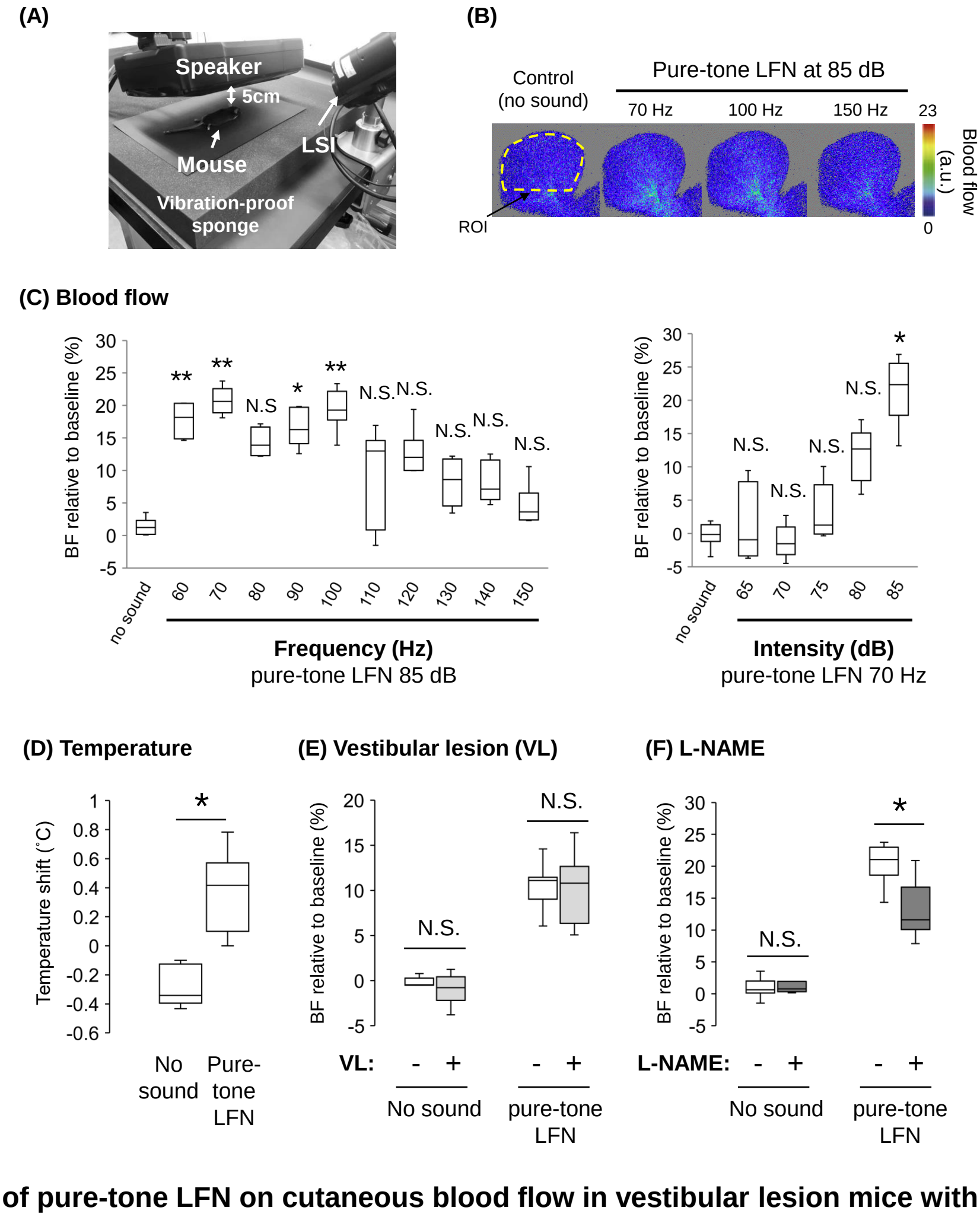
Figure 5



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