Supplementary Information for "**Enhancing the regulatory function of the autonomic nervous system using sounds with inaudible high-frequency components**"

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Supplementary Figure

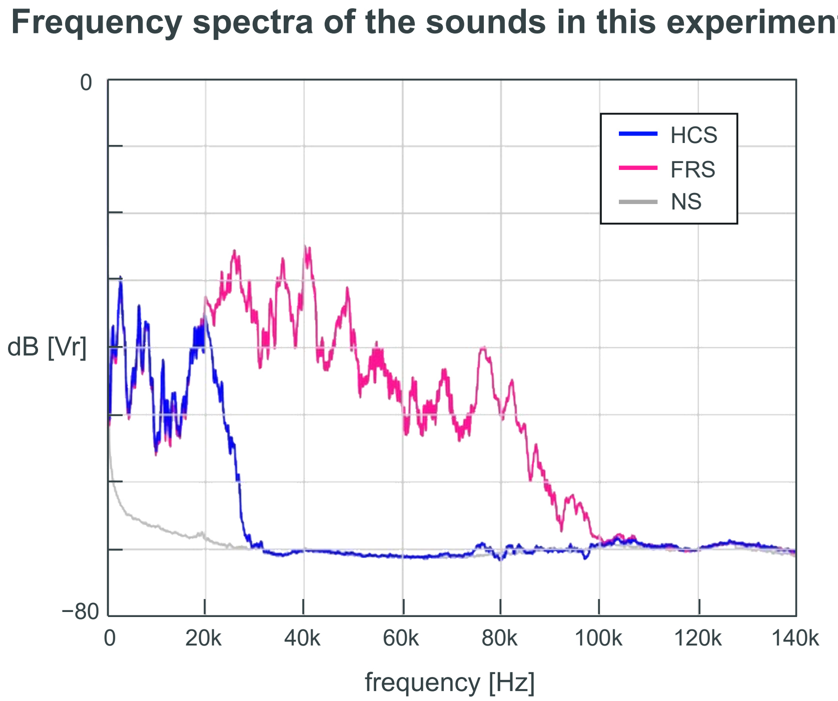

Figure S1.
Frequency spectra of the sounds presented by the sound presentation system used in this experiment. These frequency spectra were obtained by spectrum analyzer (SoundCheck, Listen., Inc., USA) based on the signals measured with a microphone (Type 4939, Brüel & Kjær, Denmark) placed at the center of the participant's head. The blue and pink lines represent the power spectra of high-cut sound (HCS) and full-range sound (FRS) conditions, respectively. The gray line represents the power spectrum of background noise in no-sound (NS) condition. FRS contains high-frequency components above the upper limit of the human audible range (20 kHz), whereas HCS contains only components within the audible range, below approximately 20 kHz. Notably, the power spectra below 20 kHz are nearly identical between FRS and HCS.

Supplementary Methods

Recording and analysis of indices for the ANS Assessment

Heart Rate Variability: Heart rate variability (HRV) was used as an index of ANS activity, with particular emphasis on the high-frequency (HF; 0.15–0.4 Hz) component as a marker of PNS function [1,2]. HRV was measured using a wearable heart rate sensor (myBeat WHS-1; Union Tool, Japan) attached to the left side of the chest. During the 5-min N-back task and relaxation conditions, the R-R interval (RRI) data were recorded. All artifacts in the RRI data were removed. Next, spline interpolation was applied. The participants exhibiting frequent arrhythmias were excluded from the analysis. The RRI time-series data, sampled at 1,000 Hz, were analyzed using MATLAB (The MathWorks, the USA). The HF components were obtained by calculating the power spectral density within a range of 0.15–0.4-Hz using the Fast Fourier Transform of the RRI time-series data.

Skin Conductance: Skin conductance (SC) is a physiological signal that reflects SNS activity. Increased SNS activity induces sweat secretion, particularly from the palms and soles, resulting in an elevated SC. The SC time-series data comprises two distinct components: tonic skin conductance level (SCL) and phasic skin conductance response (SCR) [3]. SCL changes gradually over time and reflects baseline SNS activity. Meanwhile, SCR is characterized by rapid, transient fluctuations in response to unexpected stimuli and is associated with somatic nervous system activity [3]. In the current study, SCL, which represents general SNS activity, was adopted as the index of SNS activation. During the measurements, an SC sensor (ProComp Infiniti System SC-Flex/Pro; Thought Technology, Canada) was used, with electrodes affixed to the index and ring fingers of the nondominant hand. For data analysis, the SC signals were recorded over a 5-min period at a sampling frequency of 8 Hz. The recorded data were processed via continuous deconvolution analysis [4] implemented using Ledalab V3.4.9 [4], an open-source SC analysis software operating on the MATLAB platform. The mean SCL over the 5-min period was calculated and utilized as the quantitative index of SNS activity.

Skin Temperature: Skin temperature (ST) is a physiological signal that reflects ANS activity. Vasodilation and vasoconstriction at the body surface alter blood flow, thereby increasing or decreasing ST. Activating the SNS induces vasoconstriction around the nasal area, resulting in a decrease in the ST. Further, unlike other body regions where the blood vessels typically run beneath a layer of subcutaneous fat, the vessels around the nasal region are located in a narrow space between the skin and the nasal bone. Hence, blood flow changes are readily detectable with ST alterations [5]. Facial thermal images were captured at 10-s intervals using an infrared thermography camera (InfRec R550, Nippon Avionics, Japan) equipped with a 2× telephoto lens (IRL-TX02D, Nippon Avionics, Japan). The camera was positioned such that the height from the floor to the lens was 80 cm and the distance from the lens to the participant's face (height: approximately 110 cm) was 150 cm. The differential skin temperature (DST) between the nasal tip and forehead was analyzed. Considering the difference between the nasal tip (more susceptible to sympathetic modulation) and the forehead (less susceptible) helps to cancel out environmental factors such as ambient temperature [6]. During the N-back and relaxation conditions, for each 5-min analysis period (30 thermal images per period), thermal images were extracted and then analyzed using a thermal imaging analysis software (InfRec Analyzer NS9500 Standard, Nippon Avionics, Japan). The regions of interest (ROIs) were established on the nasal tip and forehead in each image, and their mean temperatures were calculated. The nasal tip ROI was defined as an 11 × 11-pixel square centered at the midpoint between the lateral edges of the alae nasi (nostrils). Similarly, the forehead ROI was defined as an 11 × 11-pixel square centered on a point that is symmetric (relative to the nasal tip center) across the line connecting the inner corners of the eyes. The DST was calculated by subtracting the mean temperature of the forehead ROI from that of the nasal tip ROI for each thermal image. The 5-min average of these values was used as an index of SNS activity.

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