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ORIGINAL PAPER

Inaudible components of the human infant cry influence haemodynamic responses in the breast region of mothers

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Abstract

Distress vocalizations are fundamental for survival, and both sonic and ultrasonic components of such vocalizations are preserved phylogenetically among many mammals. On this basis, we hypothesized that ultrasonic inaudible components of the acoustic signal might play a heretofore hidden role in humans as well. By investigating the human distress vocalization (infant cry), here we show that, similar to other species, the human infant cry contains ultrasonic components that modulate haemodynamic responses in mothers, without the mother being consciously aware of those modulations. In two studies, we measured the haemodynamic activity in the breasts of mothers while they were exposed to the ultrasonic components of infant cries. Although mothers were not aware of ultrasounds, the presence of the ultrasounds in combination with the audible components increased oxygenated haemoglobin concentration in the mothers' breast region. This modulation was observed only when the body surface was exposed to the ultrasonic components. These findings provide the first evidence indicating that the ultrasonic components of the acoustic signal play a role in human mother–infant interaction.

Keywords Parenting · Cry · Mother · Infant · Ultrasonic

Introduction

The cry qua distress vocalization is fundamental for survival and is preserved phylogenetically among many mammals [1, 7]. The vocalizations emitted by infants are acoustically similar across a wide array of taxonomic families [26]. Moreover, parental behaviour is governed by many phylogenetically preserved principles that are conserved from rodents to humans [35]. Determining the acoustic constituents of the cry and their functions are at the core of understanding human mother–infant interaction because of the signal role of the cry in mammalian caregiving.

In mammals other than humans, such as rodents, cats, and primates [5, 9, 36, 37], high-frequency components in cry sounds (> 20 kHz) are emitted by young offspring to

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signal distress [47] due to hunger, physical discomfort, isolation, or capture by predators. These vocalizations elicit strong physiological and behavioural responses in caregivers. Considering that humans share similar neural circuits for processing infant cries with other mammalian species [3, 23], it seems plausible to hypothesize that humans also possess the neural machinery to process the ultrasonic cry sounds of infants [29].

To date, the cry sounds of human infants have been thought to contain only audible frequencies, with an average fundamental frequency of 300–600 Hz [17]. Here we ascertained that human infant cries contain ultrasonic components with frequencies (in some cases) exceeding 80 kHz (see Fig. 1) by using a purpose-made apparatus that allowed us to record and reproduce sounds with audible (< 20 kHz) and ultrasonic (> 20 kHz) components. Inspired by this initial observation, we then investigated the functional value of ultrasonic sounds in infant cry sounds.

Breastfeeding is a defining mammalian maternal behaviour [18]. It has been demonstrated that infants in a state of hunger emit cry sounds with particular acoustic characteristics that prompt breastfeeding [26]. Of particular relevance to the present study, Vuorenkoski et al. [43] reported that exposure to the cry sounds of an infant induces an increase in the temperature of the mother's breast region. Skin temperature rise in the breast region related to breastfeeding has been observed in other studies [20, 42] and is generally attributed to increased blood influx induced by oxytocin secretion [42], partly because there is a close linkage between thermal regulation and blood circulation [15, 39]. Further, exposure to infant cry sound is reported to induce increases in heart rate [16, 32]. On the basis of these, we decided to assay the potency of the ultrasonic components of cry sounds to modulate haemodynamic responses in the breast region.

Experiment 1 was designed to elucidate the nature of the ultrasonic effect of the infant cry by, first, determining whether ultrasonic components of a typical infant cry influence the haemodynamic response in mothers and, second, by determining whether ultrasonic components of the cry alone would be sufficient to induce a haemodynamic response in mothers. We measured haemodynamic responses in the breast region of mothers in response to three types of cry sounds: natural cries, scrambled cries, and ultrasonic only cries. Both natural cries and scrambled cries contained audible and inaudible components, but the frequency structure of the inaudible components was disrupted in the scrambled cries. Because the audible components were left intact in the scrambled cries as well as in natural cries, these two types of cries sounded the same. Ultrasonic only cries contained only the inaudible components of the cry sound.

Haemodynamic activity in the mothers' breasts was recorded through dual-channel near-infrared spectroscopy (NIRS), with two sensors attached directly to the skin surface of the right and left breasts. Analyses focused on the concentration of oxygenated and deoxygenated haemoglobin (oxyHb/deoxyHb) during the presentation of the cries. OxyHb/deoxyHb measurement is a sensitive indicator of a

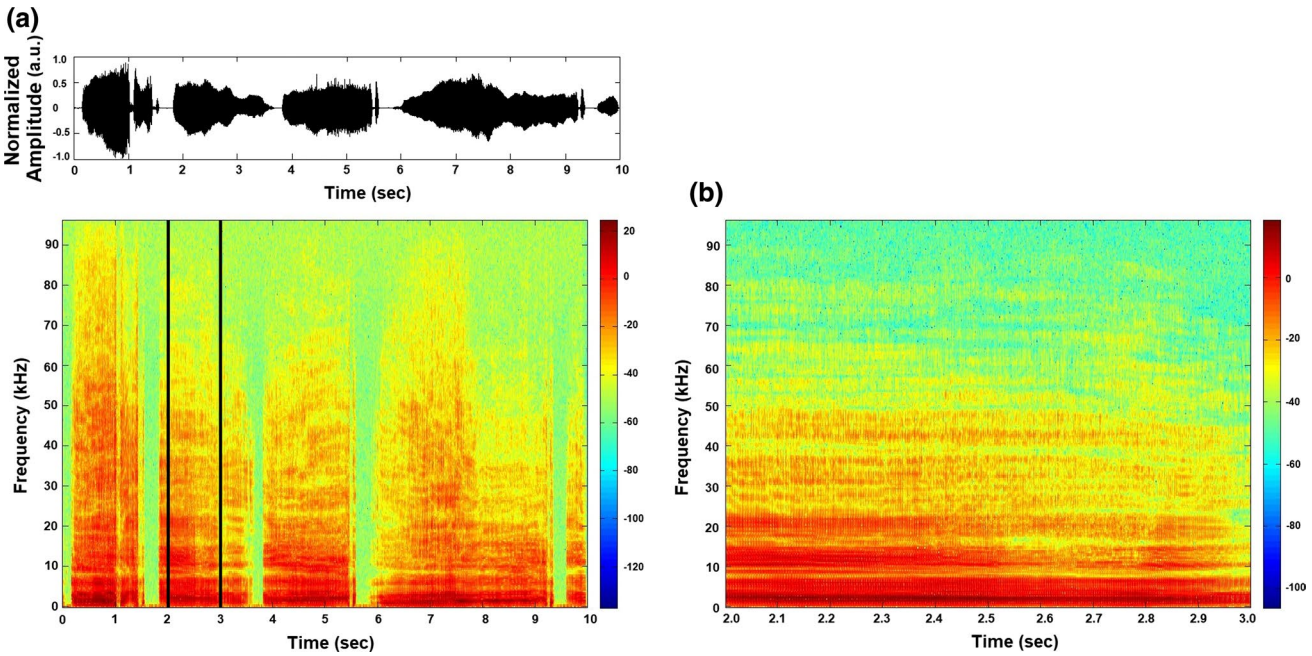

Fig. 1 **a** Spectrogram and normalized amplitude of one natural infant cry. **b** Magnified spectrogram within the time-window (2.0–3.0 s) indicated by two vertical black lines in (**a**). Colour bars represent magnitude in dB

change in breast blood flow [40]. The comparison between haemodynamic responses to natural and scrambled cries supposedly reveals effects, if any, of the ultrasonic components in infant cry sounds. We included ultrasonic only cries as sound stimuli to ascertain whether the ultrasonic cry sounds alone would induce haemodynamic responses in mothers.

In their seminal study on the effects of ultrasonic sounds on humans, Oohashi et al. [30] claimed that the effects of ultrasonic components of sounds on neural and behavioural responses ("hypersonic effect") are observed only when the listener's entire body is exposed to the ultrasonic sounds, indicating a reliance of the "hypersonic effect" on systems other than, or in addition to, the auditory system. Thus, it is possible that, if there are any modulatory influences of ultrasonic components of the infant cry on the haemodynamics of the mother's breast, they may be mediated by a mechanism similar to that proposed by Oohashi et al. [30].

To investigate this possibility, we conducted a second experiment, in which mothers were exposed to the same set of cry sounds used in experiment 1, but through headphones that conveyed ultrasonic as well as audible components of the sounds. If the perceptual system outside the inner ear plays a pivotal role in the induction of the ultrasonic effects of the infant cry, an effect of ultrasonic cry sounds similar to that observed in experiment 1 should not be observed in experiment 2, because the mothers' bodily surface is not exposed to the cry sounds.

Experiment 1

Methods

Participants

Seventeen healthy mothers (*M* age = 32. 3 years, SD = 4.5) took part (babies' *M* age = 5.3 months; SD = 2.1) after giving written informed consent.

Materials and stimuli

The original cry sounds used for the creation of the experimental stimuli in experiment 1 and experiment 2 were chosen from a database of infant cries. We used spontaneous infant cries recorded from four different infants (aged 4–10 months). All infants were born at term and showed no signs of clinical conditions at birth or at the time of recording. Cries were recorded at least 2 h after the most recent breastfeeding to collect recordings of one bout of hunger cry from each infant. Recordings were performed using a free-field microphone (40BE; G.R.A.S Sound & Vibration, Vedbaek, Denmark), a microphone preamplifier (26CB; G.R.A.S. Sound & Vibration, Vedbaek, Denmark), and a dual-channel sensor amplifier (SR-2200; Ono Sokki, Tokyo, Japan). The signals were digitized by a signal processor (0202 USB 2.0 Audio Interface; E-MU Systems, Scotts Valley, California, USA), with an A/D sampling frequency of 192 kHz, and stored on a PC. The microphone was situated at a constant distance of 15 cm from the infants' mouth, and the total duration of the infants' crying was recorded.

Recorded sounds of cries originally differed in length, with two cries having short recording lengths (1.35 and 2.07 s) and two having longer recording lengths (21.97 and 20.5 s). To create cry segments of equal duration and of a reasonable length to elicit an ultrasonic effect [31], four sound files of cries lasting for 45 s were made by duplicating and concatenating the original cry recordings.

In experiment 1, four different natural cries (original recordings of cry sounds, containing both audible and intact ultrasonic components, produced by four different babies) were used. Two further versions of each cry were created: one with a scrambled ultrasonic component (scrambled cries) and one containing only the ultrasonic cry components (ultrasonic only cries). To create the scrambled cries, we first isolated the ultrasonic components of each cry by applying a high-pass filter to the sound using a 22-kHz cut-off frequency. The waveforms above the cut-off frequency were divided into 20 ms segments. Each ultrasonic waveform segment was Fourier-transformed, its phase values within frequency domain being scrambled, and then inverse Fourier-transformed to yield scrambled waveform segments. Then, scrambled ultrasonic components were created by concatenating these scrambled waveform segments in the original order [2]. Finally, after adjusting the RMS of the sound pressure of scrambled ultrasonic components with that of corresponding natural cry, we spliced the scrambled ultrasonic components onto the audible components of the cry to synthesize the scrambled cries.

Ultrasonic only cries were created using high-pass filtering of each of the natural cries with a cut-off frequency of 22 kHz. In contrast to the natural cries and scrambled cries, the ultrasonic only cries did not contain audible components and were inaudible to participants. Spectrograms of example sounds in each condition are shown in Fig. 2. The averaged sound pressure levels of each type of sound against background noise were 56.9 ± 4.47 dB for natural cry, 57.0 ± 4.43 dB for scrambled cry, and 30.3 ± 2.24 dB for ultrasonic only cry.

Apparatus and procedures

Each participant engaged in fNIRS measurement and a detection task that aimed to verify the validity of experimental manipulation. The detection task was conducted after the completion of the fNIRS measurement.

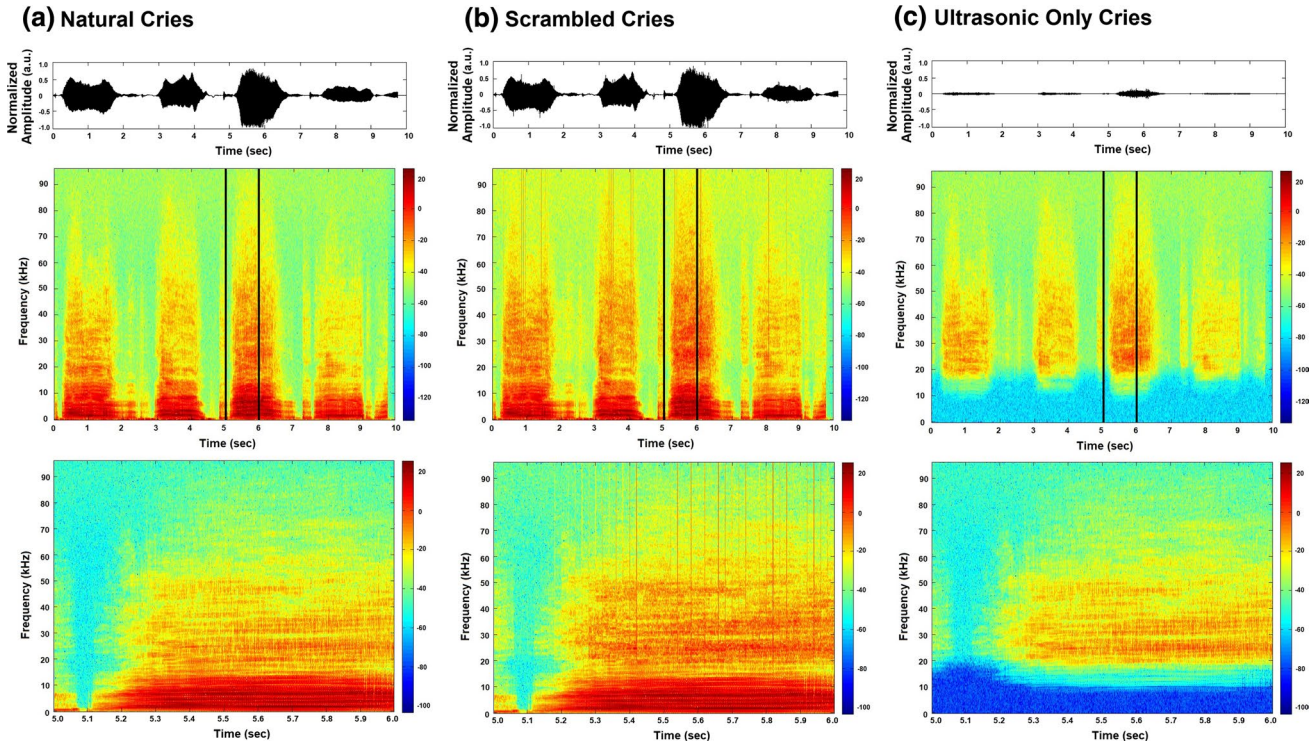

Fig. 2 Examples of normalized amplitudes (uppermost panel) and spectrograms (middle panel) of stimulus sounds of the three cry types (natural cries, scrambled cries, and ultrasonic only cries) used in experiment 1 and experiment 2. The spectrograms within time-window (5.0–6.0 s) flanked by two black vertical lines are magnified and described in finer temporal resolution in the lowermost panels. The amplitudes were normalized by the maximum signal value of the natural cry waveform. Colour bars represent magnitude in dB

fNIRS measurement Stimuli were presented through a 192-kHz high-resolution audio system, which allowed us to control stimulus presentation and play the ultrasonic components and audible components of cries through a speaker and a super tweeter. Specifically, we used a system designed with a 2-way monitor speaker (RL906; musikelectronic githain gmbh, Germany) for the presentation of audible range components and a custom-made super tweeter (Trb-001-ngs; Katou Acoustics Consultant Office, Japan) with frequency response 20–96 kHz for the presentation of inaudible high-frequency range components. The two speakers were positioned in front of the participant at a distance of approximately 50 cm, as shown in Fig. 3. We presented the cry sounds through the simultaneous presentation of low and high frequencies. Sounds within audible and ultrasonic frequency ranges were presented through speaker and super tweeter, respectively.

For fNIRS measurement, we measured the oxyHb and deoxyHb in participants' breast region using a dual-channel NIRS (NIRO-220, Shimadzu. Co.) during the presentation of the three types of cries. fNIRS emitters and probes were attached to the upper inner quadrant of both breasts [40], as shown in Fig. 3. To attach the emitters and probes, a rubber probe holder (approximately 60 × 30 mm) was affixed to the breast. The modified Lambert–Beer law was used to calculate the oxyHb and deoxyHb. The sampling rate was 1 Hz.

Participants sat in front of a 19-inch computer screen and speakers and passively listened to the cries. The temporal sequence of stimulus presentation was as follows. A white fixation cross subtending approximately 1.8° in height and 1.8° in width was displayed against black background at the centre of the screen for 15 s to serve as the baseline. The cry stimulus was then presented for 45 s. Simultaneously with the onset of cry stimulus, the colour of the fixation cross changed from white to red. The colour change of fixation cross was incorporated into the experimental design so that participants noticed the start of stimulus presentation even when only inaudible sounds were being played in the ultrasonic only condition. At the end of cry stimulus, the fixation colour changed back to white, and there was a 20-s post-stimulation period during which a white fixation cross was presented at the centre of the screen. Trials were separated by 5-s inter-trial intervals during which the screen was blank (only black background was presented). Before starting the experiment, participants received verbal instructions from the experimenter and were asked to minimize their bodily movements. Three types of experimental blocks were created: one for the presentation of the natural cries, one for the presentation of scrambled cries, and one for the presentation

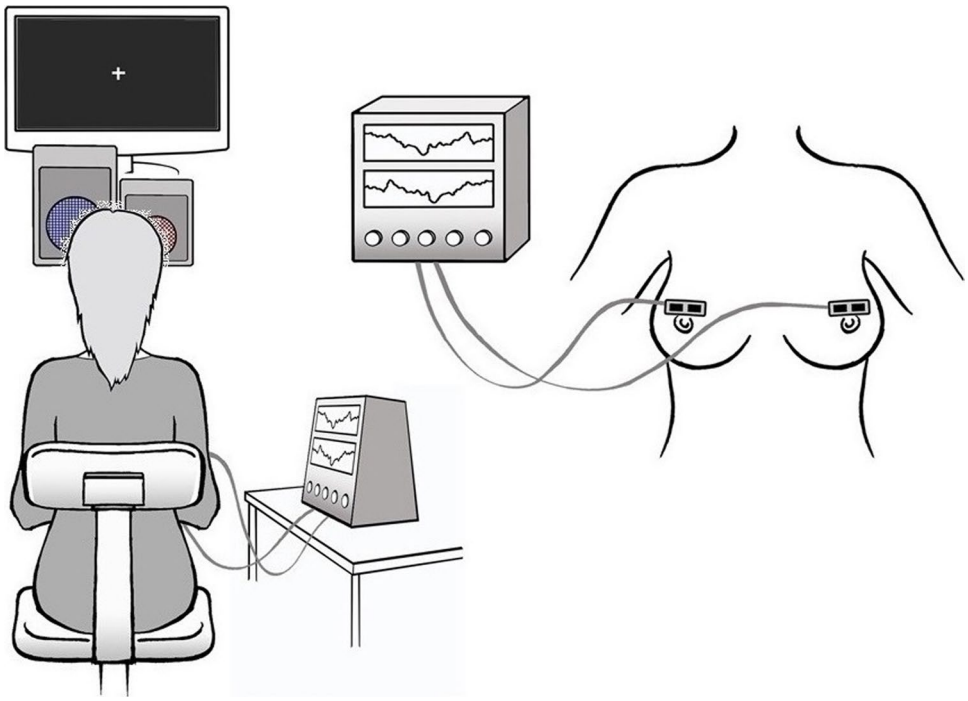

Fig. 3 Schematic diagrams of experimental setup. Illustration of the apparatus and sensor positions on participants' breasts. Participants sit in front of the two speakers that play cry stimuli. The blue and red speaker grills represent the speakers used to play audible and ultrasonic components of the stimuli, respectively

of ultrasonic only cries. Each type of experimental block was presented twice, resulting in a total of six blocks. The order of the presentation of the four sound files of each cry type was randomized within each block, and the block order was pseudo-randomly determined across participants. The entire session lasted for approximately 45 min.

Detection task At the start of each trial, a white fixation cross subtending approximately 1.8° in height and 1.8° in width appeared on the screen. 1 s after the appearance of the fixation cross, a short (3 s) excerpt of a cry sound was presented. The participant's task was to press the "l" key with her right index finger as soon as she heard a sound. When the participant pressed a key, the sound presentation was terminated and the experiment proceeded to the next trial. If the participant did not press the key, the sound file was played for 3 s and the experiment automatically proceeded to the next trial. The white fixation cross remained on the screen while the sound was played, and there was no inter-trial interval. Thus, the fixation cross was presented throughout the task. The short excerpts of the four sound files that were used in each condition (natural cries, scrambled cries, ultrasonic only cries) of the fNIRS measurement were each presented twice in a pseudo-random order.

Data analysis

In the analysis, oxyHb waveforms were smoothed with a five-point moving average procedure and linearly detrended, and the oxyHb value in each temporal point was transformed into standardized oxyHb. The standardized oxyHb was computed as follows: First, the mean of the oxyHb values during the 15-s baseline period was subtracted from the oxyHb. Then, the oxyHb value was divided by the standard deviation of the oxyHb values obtained during the baseline period. Thereafter, the waveforms of the standardized oxyHb in all the trials of the same condition were averaged to generate the waveforms of standardized oxyHb for each participant in each condition. Standardized deoxyHb waveforms were computed for each participant in the same manner. Due to the high peak sound pressure in the original recordings, there were segments with signal overflow in some of the sound files, which introduces the possibility of clipping in some segments of stimulus sounds. However, we used data of all the eligible trials in the final analysis to increase the signal-to-noise ratio.

In the first set of statistical analyses, the average of the standardized oxyHb/deoxyHb during the whole 45-s stimulation period was used as the dependent variable. OxyHb/deoxyHb were then analysed by a two-way analysis of variance (ANOVA) with the type of cry (natural cries vs scrambled cries vs ultrasonic only cries) and the channel side (left vs. right) as within-participant factors.

The measured waveforms of oxyHb/deoxyHb in each condition showed clear temporal fluctuation. Thus, in the second set of analyses, we examined the temporal course of the influences of cry type on haemodynamic response. To achieve this, baseline period, stimulation period and post-stimulation period were segmented into 5-s time-windows. Then, oxyHb/deoxyHb in each condition was

averaged within each time-window. This resulted in total of 3 cry types × 2 channel sides × 16 time-windows (3 time-windows during 15-s baseline, 9 time-windows during 45-s cry stimulus presentation and 4 time-windows during 20-s post-stimulation period) = 96 values for oxyHb and deoxyHb each. We decided to include the post-stimulation period in this analysis because several fNIRS studies have reported lasting influence of sensory stimulation on cortical haemodynamic responses after the end of stimulus presentation ([8, 24] for a review). OxyHb/deoxyHb were then analysed by a three-way ANOVA with the channel side (2), time-window (16), and the type of cry (3) as within-participant factors.

Results

The temporal course of oxyHb in each condition is shown in Fig. 4a. A 2 × 3 ANOVA with oxyHb as the dependent variable showed a main effect of the type of cry [F (2, 32) = 6.47, $p = 0.004$, $\eta_p^2 = 0.29$]. The ANOVA table is presented in Table 1.

Multiple comparisons by Holm's sequentially rejective Bonferroni's method revealed a higher level of oxyHb on presentation of natural cries than on presentation of scrambled cries [t (16) = 3.06, adjusted $p = 0.022$] and ultrasonic only cries [t (16) = 2.70, adjusted $p = 0.031$]; responses to the scrambled cries and ultrasonic only cries did not differ from each other [t (16) = 0.27, adjusted $p = 0.78$]. No effect of channel side was observed, and no interaction between the channel side and the type of cry emerged (Fs < 2, ps > 0.20).

A 2 × 16 × 3 ANOVA with oxyHb as the dependent variable revealed a significant main effect of the type of cry [F (2, 32) = 6.39, $p = 0.0046$, $\eta_p^2 = 0.29$]. This main effect was qualified by a significant two-way interaction between time-window and the type of cry [F (30, 480) = 2.22, $p = 0.0003$, $\eta_p^2 = 0.12$]. No other effect reached significance (Fs < 1.3, ps > 0.14).

Simple main effect analysis revealed a significant simple main effect of the type of cry in seventh to eleventh time-windows that roughly correspond to the latter half of stimulus presentation period as summarized in Table 2. Pairwise comparisons by Holm's sequentially rejective Bonferroni's

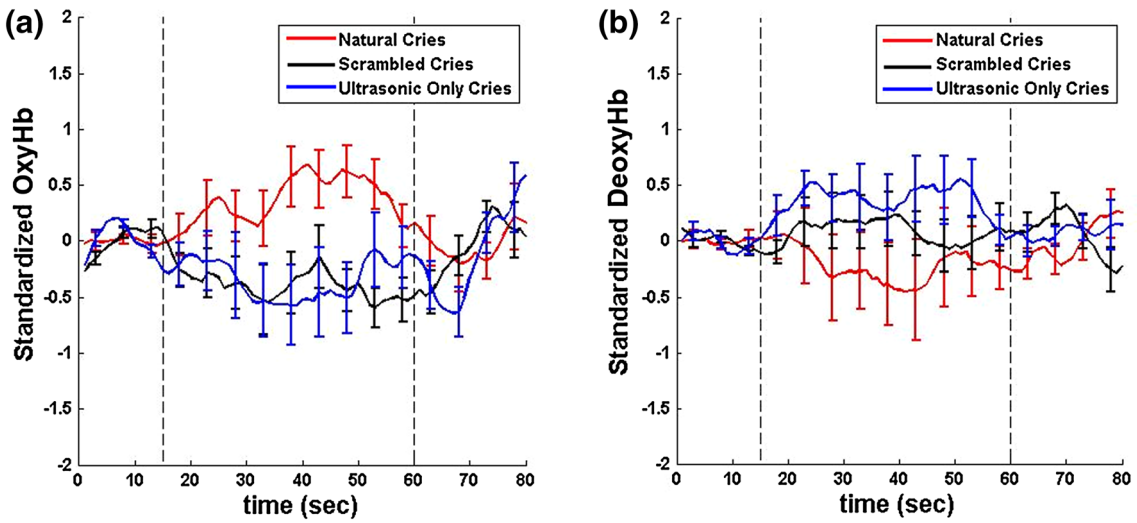

Fig. 4 Temporal course of **a** oxyHb change and **b** deoxyHb change in mothers' breasts (two-channels average) in the three conditions (Red: natural cries, Black: scrambled cries, and Blue: ultrasonic only cries) in experiment 1. The two vertical dashed lines indicate the beginning and the end of the cry stimulus. The error bars represent standard errors of standardized oxyHb values within 5-s time windows

Table 1 Table of ANOVA results on oxyHb in experiment 1

Source	SS	*df*	MS	*F*	*p*	η_p^2
Channel side	0.69	1	0.69	0.99	0.335	0.06
Error	11.14	16	0.7			
Cry type	12.02	2	6.01	6.47	0.004**	0.29
Error	29.7	32	0.93			
Channel side × cry type	1.42	2	0.71	1.64	0.21	0.09
Error	13.81	32	0.43			

**$p < 0.01$

Table 2 ANOVA table of simple main effect of the type of cry on oxyHb in each time-window in experiment 1

Period	Time window	Source	SS	*df*	MS	*F*	*p*	η_p^2
Baseline	1	Cry type	0.16	2	0.08	1.55	0.229	0.09
		Error	1.65	32	0.05			
	2	Cry type	0.01	2	0.01	0.33	0.718	0.02
		Error	0.51	32	0.02			
	3	Cry type	0.11	2	0.05	0.86	0.432	0.05
		Error	1.99	32	0.06			
Stimulation	4	Cry type	1.61	2	0.8	1.61	0.215	0.09
		Error	15.92	32	0.5			
	5	Cry type	7.1	2	3.55	2.85	0.073#	0.15
		Error	39.93	32	1.25			
	6	Cry type	8.22	2	4.11	2.21	0.126	0.12
		Error	59.51	32	1.86			
	7	Cry type	12.81	2	6.4	3.35	0.048*	0.17
		Error	61.17	32	1.91			
	8	Cry type	24.78	2	12.39	6.03	0.006**	0.27
		Error	65.76	32	2.06			
	9	Cry type	21.03	2	10.52	4.4	0.021*	0.22
		Error	76.51	32	2.39			
	10	Cry type	24.22	2	12.11	5.14	0.012*	0.24
		Error	75.34	32	2.35			
	11	Cry type	18.54	2	9.27	4.26	0.023*	0.21
		Error	69.7	32	2.18			
	12	Cry type	8.57	2	4.28	2.7	0.083#	0.14
		Error	50.83	32	1.59			
Post stimulation	13	Cry type	3.59	2	1.8	1.58	0.222	0.09
		Error	36.38	32	1.14			
	14	Cry type	3.47	2	1.73	1.19	0.317	0.07
		Error	46.53	32	1.45			
	15	Cry type	1.76	2	0.88	1.59	0.219	0.09
		Error	17.67	32	0.55			
	16	Cry type	1.53	2	0.77	0.43	0.657	0.03
		Error	57.72	32	1.8			

#$p < 0.10$, *$p < 0.05$, **$p < 0.01$

method were carried out in each time-window. The results of pairwise comparisons are summarized in Table 3. As can be seen, oxyHb in response to natural cry sounds was higher than both scrambled and ultrasonic only cries in the eighth time-window around the apex of oxyHb fluctuation, but the conditional difference was less clear in the other time-windows.

The temporal course of deoxyHb in each condition is shown in Fig. 4b. A 3 × 2 ANOVA with deoxyHb as the dependent variable revealed no significant effects (Fs < 2.4, ps > 0.10). The ANOVA results are summarized in Table 4.

A 2 × 16 × 3 ANOVA with deoxyHb as the dependent variable revealed a marginally significant main effect of the type of cry [F (2, 32) = 2.70, $p = 0.082$, $\eta_p^2 = 0.14$]; deoxyHb tended to decrease most prominently in the natural cry condition. This main effect was qualified by a significant two-way interaction between time-window and the type of cry [F (30, 480) = 2.03, $p = 0.012$, $\eta_p^2 = 0.11$]. No other effect reached or approached significance (Fs < 1.5, ps > 0.25). Simple main effect analysis revealed a significant simple main effect of the type of cry in the fourteenth time-window during the post-stimulation period [F (2, 32) = 5.14, $p = 0.012$, $\eta_p^2 = 0.24$]. Pairwise-comparisons revealed significantly higher deoxyHb to the scrambled than natural cry [t (16) = 2.98, adjusted $p = 0.03$]. No other pairwise comparisons reached significance after adjustment (ts < 1.75, adjusted ps > 0.20). Simple main effect of the type of cry failed to reach significance in the other time-windows (Fs < 2.8, ps > 0.10).

In the detection task, participants pressed the key every time they were exposed to sound excerpts of natural cries or scrambled cries (100%). Participants almost never pressed

Table 3 Results of pairwise comparisons in time-windows in which simple main effect of the type of cry reached significance

Time window	Comparison	Difference	*t*	Adjusted *p* value
7	NC > SC	0.74	2.35	0.097#
	NC > UOC	0.77	2.06	0.112
	SC > UOC	0.03	0.1	0.921
8	NC > UOC	1.12	2.7	0.047*
	NC > SC	0.94	2.64	0.047*
	SC > UOC	0.18	0.72	0.479
9	NC > UOC	1.06	2.56	0.063#
	NC > SC	0.82	2.01	0.123
	SC > UOC	0.25	0.84	0.411
10	NC > UOC	1.06	2.81	0.038*
	NC > SC	1.00	2.43	0.055#
	SC > UOC	0.06	0.19	0.855
11	NC > SC	1.04	2.8	0.038*
	NC > UOC	0.63	1.74	0.204
	SC < UOC	-0.4	1.19	0.251

NC natural cry, *SC* scrambled cry, *UOC* ultrasonic only cry
#$p < 0.10$, *$p < 0.05$

the key on the presentation of the ultrasonic only cries (< 1.5%).

Experiment 2

Methods

Participants

Seventeen healthy mothers (*M* age = 32.7 years, SD = 3.0) took part in experiment 2 (babies' *M* age = 5.1 months; SD = 1.1). All participants in the present study provided written informed consent.

Stimuli and procedure

The same set of cries that were used in experiment 1 (natural cries, scrambled cries, and ultrasonic only cries) were played through headphones (EAH-T700, Panasonic Co, Japan) with response frequency 3 Hz–100 kHz using a custom-made headphone amplifier. To equate the frequency responses of the sounds in experiment 1 and 2, we manipulated the sound files using an equalizer function in Audacity version 2.1.3 (audacity team). Except for the use of headphones and modification of frequency responses of cry sounds, the apparatus, stimulus, and procedures of both the fNIRS measurement and the detection task were exactly the same as in experiment 1.

Results

The temporal course of the standardized oxyHb in each condition is shown in Fig. 5a. A 2 × 3 ANOVA with oxyHb as the dependent variable revealed no significant effects (Fs < 0.88, ps > 0.4). The detailed results of the ANOVA are summarized in Table 5. A 2 × 16 × 3 ANOVA revealed no significant effects either (Fs < 1.4, ps > 0.10).

The temporal course of deoxyHb in each condition is shown in Fig. 5b. A 3 × 2 ANOVA, with deoxyHb as the dependent variable, using the same factorial design as described above revealed no significant effects (all Fs < 0.8, ps > 0.4). The ANOVA table is presented in Table 6. A 2 × 16 × 3 ANOVA revealed a significant main effect of time-window [F (15, 240) = 3.72, $p < 0.001$, $\eta_p^2 = 0.19$]. No other effect reached significance (Fs < 1.3, ps > 0.13).

As in experiment 1, the detection task demonstrated that the participants did not consciously perceive the ultrasonic only sound (no participants pressed the button during the presentation of the ultrasonic only cries). Experiment 2 did not show an effect of the ultrasonic sounds on maternal haemodynamic responses at the breast. The small effect size, described in Tables 5 and 6, indicates that the inner ear does not play a major role in the induction of the ultrasonic effects of haemodynamic responses in the mothers' breasts.

Discussion

The present study revealed that human infant cries contain ultrasonic components, and, together, the results of two experiments demonstrate that the ultrasonic components of the infant cry influence haemodynamic activity in the breasts

Table 4 Table of ANOVA results on deoxyHb in experiment 1

Source	SS	*df*	MS	*F*	*p*	η_p^2
Channel side	0.05	1	0.05	1.19	0.291	0.07
Error	0.66	16	0.04			
Cry type	1.28	2	0.64	2.32	0.115	0.13
Error	8.84	32	0.28			
Channel side × cry type	0.35	2	0.18	1.33	0.278	0.08
Error	4.2	32	0.13			

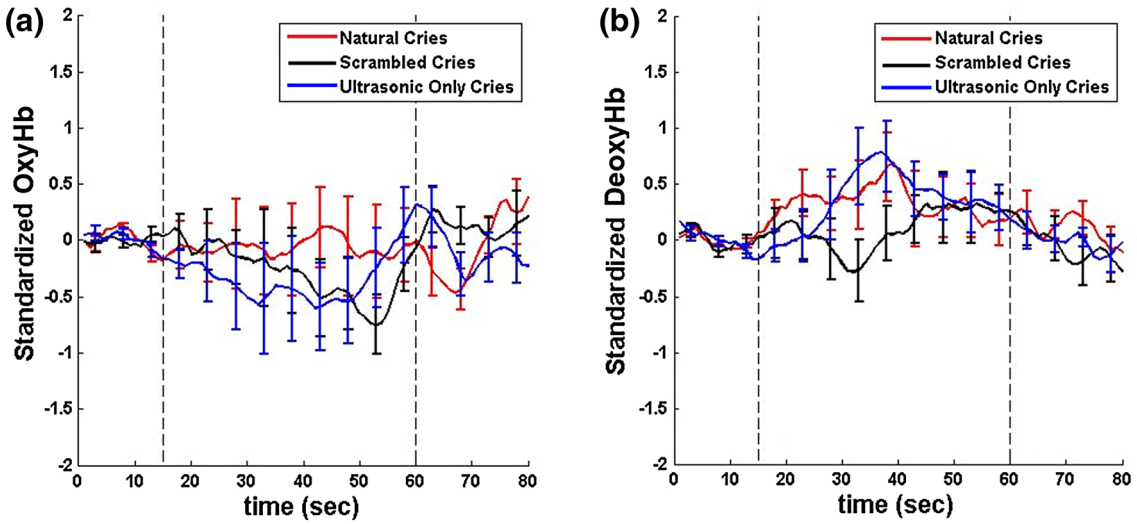

Fig. 5 Temporal course of **a** oxyHb change and **b** deoxyHb change in mothers' breasts (two-channels average) in the three conditions (Red: natural cries, Black: scrambled cries, and Blue: ultrasonic only cries) in experiment 2. The two vertical dashed lines indicate the beginning and the end of the cry stimulus. The error bars represent standard errors of standardized oxyHb values within 5-s time windows

Table 5 Table of ANOVA results on oxyHb in experiment 2

Source	SS	*df*	MS	*F*	*p*	η_p^2
Channel side	0.36	1	0.36	0.58	0.459	0.03
Error	9.88	16	0.62			
Cry type	1.45	2	0.72	0.73	0.488	0.04
Error	31.62	32	0.99			
Channel side × cry type	1.15	2	0.58	0.88	0.426	0.05
Error	21.04	32	0.66			

Table 6 Table of ANOVA results on deoxyHb in experiment 2

Source	SS	*df*	MS	*F*	*p*	η_p^2
Channel side	0.32	1	0.32	0.39	0.542	0.02
Error	13.29	16	0.83			
Cry type	1.3	2	0.65	0.79	0.463	0.05
Error	26.38	32	0.82			
Channel side × cry type	0.33	2	0.16	0.49	0.615	0.03
Error	10.7	32	0.33			

of mothers. Specifically, the concentration of oxyHb in the breast region increased in response to infant cry sounds with intact ultrasonic components. Concomitantly, deoxyHb showed trend-level fluctuation in the direction opposite to oxyHb, which is considered to be a reliable sign of oxygen-rich arterial blood influx [8, 28]: inflow of oxygenated blood into blood vessels replaces deoxyHb and consequently decreases deoxyHb concentration in blood. Oohashi et al. [31] reported brain responses in human listeners to ultrasonic components contained in Gamelan music (traditional music of Java and Bali in Indonesia), suggesting that "inaudible" high-frequency components (> 20 kHz) are processed by human listeners in fully appreciating instrumental music. Our findings agree with their results that the inaudible ultrasound components of the human infant cry can modulate haemodynamic responses in the breast region of mothers. The present study, therefore, constitutes the first demonstrations that ultrasonic components are present in the human infant cry and that inaudible components of infant vocalizations induce physiological responses in mothers.

The observed effects of the ultrasonic components in the typical human infant cry share many characteristics with the "hypersonic effect" observed by Oohashi et al. [30, 31]. First, for this effect to emerge, listeners need to be exposed

to audible carrier sounds simultaneously with ultrasonic sounds; in other words, no modulatory influence on maternal haemodynamic responses was observed when only ultrasonic components were present. Second, the inner ear does not play a primary role in the induction of this effect. These observations suggest that the ultrasonic effects of the typical infant cry rely on a similar perceptual mechanism as the "hypersonic effect" [30, 31, 45].

Ultrasonic communication is common in the mother–infant interaction in a wide variety of mammalian species. The emission of ultrasounds by young offspring is usually prompted by distress of various sorts [47], and in turn it often elicits prompt maternal responses [27, 44]. Distress vocalizations, as well as non-distress vocal communications and calls, are used by mammals and sensitivity to them is attributable to shared neural structures that arose from a common ancestor [1]. On the basis of this line of reasoning, the present finding may indicate that some parental behaviours are governed by phylogenetically preserved principles conserved from rodents to humans [35]. At the same time, there seems to be an important difference in the mechanism to process conspecific's high-frequency vocalizations between humans and the other mammalian species. Researchers generally agree that rodents process conspecific ultrasonic vocalizations in auditory cortex and presumably "hear" them as sounds [4, 33]. By contrast, the present results indicate that human mothers perceive ultrasonic components of infant cries using receptors other than inner ear as discussed above. These results cast doubt on the contention that sensitivity to ultrasonic components in humans is phylogenetically linked to ultrasonic communication in other mammalian species.

Several aspects of the current findings require further explanation. First, auditory components of cry sounds without intact ultrasonic component (scrambled cry) did not exert modulatory effects on haemodynamic responses in breast region in the present study despite the fact that scrambled and natural cries were consciously indistinguishable. This pattern contradicts previous studies that showed strong effects of infant cry sounds on physiological responses in mothers [6, 12, 16, 32] even when mothers were exposed to cry sounds only within audible range. One explanation for the lack of any effects of audible components in cry sounds in the present study might be the contrast effect [19]; when one is exposed to two sensory stimuli successively, the perceived quality of the second stimulus is influenced by the preceding one. In the present study, every participant was exposed to both natural cry sounds with intact ultrasonic components and scrambled cry sounds whose ultrasonic components were destroyed in its frequency structure. Considering the previous studies indicating the unconscious effect of inaudible components on behaviour [45, 46] and neural activation [21, 22, 31], the neural system may have detected subtle "unnaturalness" in the scrambled cries due to a contrast effect induced by the presentation of high-fidelity natural cry sounds. This design might have attenuated physiological responses to scrambled cries in the present study. Though we did not present audible cry sound without ultrasonic components, it seems likely that cry sounds with only audible components do not have notable effects on haemodynamic responses in mothers due to contrast effect similarly to scrambled sounds in the present study.

At the same time, if the inner ear does not contribute to the perception of ultrasonic vibration as discussed above, a contrast effect alone would not explain the results of experiment 2. The neural system had no clue to discriminate natural and scrambled cries when the two were played through headphones. Thus, no contrast effect should have emerged in experiment 2. Exposure to infant cry sounds through headphones severely degrades the ecological validity of experimental settings. Such lack of ecological validity might be one cause of our failure to observe any effects of cry sounds, irrespective of the existence of ultrasonic components, on haemodynamic responses in experiment 2. However, this is mere speculation, and further investigation would be required to resolve this issue.

The second unexpected result was the statistically significant conditional difference in deoxyHb in the post-stimulation period in experiment 1; natural cries induced larger decrease in deoxyHb than scrambled cry. As mentioned above, decreases of deoxyHb often accompany oxyHb increases [8, 28]. However, we found no conditional difference in oxyHb fluctuation in post-stimulation period in the present study. At this point, we have no definitive explanation for this unexpected result. Concentration changes of deoxyHb could be influenced by multiple factors, such as cardiac responses and the degree of vasodilation. Furthermore, milk duct expansion as observed in milk ejection is supposed to mechanically compress microvasculature, which sometimes leads to apparent reduction of blood volume and oxyHb/deoxyHb change in the breasts [13, 40, 42]. Thus, mechanical compression of tissue in breast region induced by prolonged exposure to infant cry sounds might have partly contributed to this unexpected pattern of haemodynamic response after the end of stimulus presentation.

The average frequencies of linguistic formants are distributed below 10 kHz, which indicates that normal human adult vocal conversation does not rely mainly on the ultrasound components. Why do human infants utilize the ultrasonic channel to signal their distress? One reason may derive from the particular structure of the young human infant's body. Vocal sounds with higher frequencies are usually produced by smaller animals due to the short length of the vocal tract [25]. This unique anatomical characteristic, i.e. a short vocal tract, likely gives rise to infant ultrasounds. The functional significance of ultrasonic component of cry sounds remains

unclear at this point. One possibility is that ultrasonic components of the infant cry might prompt oxytocin secretion [34]. Oxytocin is known to have vasodilatory effect [14], which conceivably leads to increased blood perfusion [10, 42] and temperature rise [43] in the breasts. Thus, further study is warranted to elucidate the nature of the modulatory effects of the ultrasonic cry on maternal behaviour through the inclusion of endocrinological measurements.

Another interesting future venue of future research would be to clinical settings. Takahashi et al. [38] have demonstrated atypical patterns of ultrasonic vocalizations in mice with a rare copy number variant that was identified as risk factor of autism spectrum disorder (ASD). Esposito and Venuti [11] previously identified atypicalities in the cry sounds in human infants who were later diagnosed with ASD. Taking these findings into consideration, our results suggest the possibility that infants with risk factors of ASD might show atypicality in the ultrasonic components of their cry, leading to less optimal maternal responsiveness. Thus, investigation into the functional significance of ultrasonic components in infant cry might play an important role in social cognition research and may be clinically relevant.

Conclusions

We present the first evidence of ultrasounds in the human infant cry and demonstrate effects of those ultrasonic components. Even when mothers are unaware of their presence, ultrasonic components of the human infant cry modulate haemodynamic responses in breast region in mothers. Similarly to the observation that some blind individuals utilize mouth-click sounds for echolocation [41], the present findings represent a novel demonstration of the remarkable ability in humans to transmit and recognize abundant information through air vibrations.

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Compliance with ethical standards

Conflict of interest We declare no conflict of interest.

Ethical approval The experimental protocol was approved by the ethical committee of Nagasaki University (No. 08102894-5). The participants were given information about the research and gave written informed consent.

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