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Preterm birth is associated with an increased fundamental frequency of spontaneous crying in human infants at term-equivalent age

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Human infant crying has been researched as a non-invasive tool for assessing neurophysiological states at an early developmental stage. Little is known about the acoustic features of spontaneous cries in preterm infants, although their pain-induced cries are at a higher fundamental frequency ($F_0$) before term-equivalent age. In this study, we investigated the effects of gestational age, body size at recording and intrauterine growth retardation (IUGR) on the $F_0$ of spontaneous cries in healthy preterm and full-term infants at term-equivalent age. We found that shorter gestational age was significantly associated with higher $F_0$, although neither smaller body size at recording nor IUGR was related to increased $F_0$ in preterm infants. These findings suggest that the increased $F_0$ of spontaneous cries is not caused by their smaller body size, but instead might be caused by more complicated neurophysiological states owing to their different intrauterine and extrauterine experiences.

1. Introduction
For several decades, acoustic features of infant crying have been studied as a possible non-invasive tool for assessing neurophysiological states [1–5]. Previous studies have indicated that an abnormally high frequency ($F_0$) (e.g. mean $F_0 > 600$ Hz) of infant cries is associated with medical conditions, including chromosomal, endocrine, metabolic and neurological disturbances at an early developmental stage [1]. Preterm birth is also a factor in higher $F_0$ cries during early infancy [1–3]. Pain-induced cries in preterm infants have been reported to be higher in $F_0$ before term-equivalent age compared with those of full-term newborns [2,3], although such differences disappeared around term-equivalent age [2,4]. A higher vocal $F_0$ is generally related to smaller body size, especially shorter vocal folds [6]; therefore, it is possible that the higher $F_0$ of preterm infants simply reflects premature body development.

Vocal $F_0$ also depends on a complex interaction between laryngeal and respiratory controls [1,6]. Specifically, vagal inputs from the right nucleus ambiguus of the medulla are assumed to have inhibitory effects on laryngeal muscle contraction and tightening of the vocal folds [5]. Therefore, diminished vagal activity might cause laryngeal muscle contraction and tightening of the vocal folds, resulting in a higher $F_0$ [5]. As preterm infants exhibit reduced vagal activity even at term-equivalent age [7], the $F_0$ of their cries might be affected by the altered vagal activity as well as smaller body size.
However, the effects of body size and other factors related to neurophysiological states (e.g. intrauterine growth retardation (IUGR)) on the $F_0$ of cries in preterm infants have not been investigated [2–4]. In addition, although spontaneous cries (those unaffected by external acute stress) have a higher internal consistency than pain-induced cries in full-term neonates [8], to our knowledge, no studies have assessed the linearity of predictors due to strong correlations among the variables related to preterm birth or body size at recording. Post hoc testing (Bonferroni) revealed that the minimum ($p < 0.0001$) and mean ($p = 0.04$) were significantly different between the VP and MLP groups. In addition, the minimum ($p = 0.03$), mean ($p < 0.001$) and maximum $F_0$ ($p < 0.0001$) were significantly different between the VP and FT groups, whereas the maximum $F_0$ ($p = 0.01$) was significantly different between the MLP and FT groups. We also examined the differences between the SGAP and AGAP groups using the two-tailed Student’s $t$-test; however, there were no differences between the groups for any of the $F_0$ variables (table 2).

Pearson’s and Spearman’s correlation analyses for all participants ($n = 64$) revealed that gestational age was significantly correlated with the minimum ($r_S = -0.32, p < 0.01$; figure 1a), mean ($r_S = -0.38, p < 0.01$; figure 1b) and maximum $F_0$ ($r_S = -0.48, p < 0.0001$; figure 1c). However, infant weight at recording was marginally correlated with minimum $F_0$ ($r_S = 0.24, p = 0.06$), but not mean ($r_S = 0.11, p = 0.39$) or maximum $F_0$ ($r_S = -0.01, p = 0.96$). Other body size variables (height, head and chest circumference) at recording were not significantly correlated with any $F_0$ variable (see the electronic supplementary material).

For multiple regression analyses, we used gestational age, postmenstrual age and weight at recording to avoid the collinearity of predictors due to strong correlations among the variables related to preterm birth or body size at recording. All $F_0$ variables could be predicted by gestational age rather than postmenstrual age or weight at recording (table 3).

### 2. Material and methods

**(a) Participants**

Forty-four healthy preterm infants (gestational age less than 37 weeks, weight at birth less than 2500 g) and 20 full-term (FT) newborn infants (gestational age more than or equal to 37 weeks, weight at birth more than or equal to 2500 g) were enrolled in this study. Preterm infants were assigned to two subgroups according to gestational age: very preterm (VP) infants (gestational age less than 32 weeks, $n = 22$) and moderate-to-late preterm (MLP) infants (gestational age between 32 and less than 37 weeks, $n = 22$). They were also grouped according to intrauterine growth: small for gestational age preterm (SGAP) infants ($n = 19$), and adequate for gestational age preterm (AGAP) infants ($n = 25$) (see the electronic supplementary material).

**(b) Cry recording and acoustic analysis**

Cry recordings were performed at term-equivalent age (postmenstrual age between 37 and less than 42 weeks) at Kyoto University Hospital. The spontaneous cries of each infant in an open crib less than 30 min before feeding were recorded for 60 s using a wave recorder (ED100 R-09; Roland Corp., Los Angeles, CA, USA) at a 44.1 kHz sampling rate and 16 bit quantization. Cry utterances containing broad regions of environmental noise were excluded from acoustic analyses to avoid artefacts when determining $F_0$. $F_0$ measurements were performed using PRAAT v. 5.2.35 [9]. A total of 2321 cry utterances were extracted manually from the PRAAT spectrogram of all cry recordings for the acoustic analysis. The cry utterances were down-sampled to 22.05 kHz and low-pass filtered at 10 kHz to eliminate outliers and artefacts; then the minimum, mean and maximum $F_0$ were determined using the PRAAT autocorrelation algorithm. The $F_0$ values were averaged for each infant (see the electronic supplementary material for more information).

### 3. Results

One-way ANOVA of the gestational group revealed significant group differences in the minimum ($F_{2,61} = 7.87, p < 0.001$, $\eta^2 = 0.10$), mean ($F_{2,61} = 9.17, p < 0.001$, $\eta^2 = 0.23$) and maximum $F_0$ ($F_{2,61} = 13.32, p < 0.0001$, $\eta^2 = 0.30$) (table 1). Post hoc testing (Bonferroni) revealed that the minimum ($p < 0.0001$) and mean $F_0$ ($p = 0.04$) were significantly different between the VP and MLP groups. In addition, the minimum ($p = 0.03$), mean ($p < 0.001$) and maximum $F_0$ ($p < 0.0001$) were significantly different between the VP and FT groups, whereas the maximum $F_0$ ($p = 0.01$) was significantly different between the MLP and FT groups. We also examined the differences between the SGAP and AGAP groups using the two-tailed Student’s $t$-test; however, there were no differences between the groups for any of the $F_0$ variables (table 2).

Pearson’s and Spearman’s correlation analyses for all participants ($n = 64$) revealed that gestational age was significantly correlated with the minimum ($r_S = -0.32, p < 0.01$; figure 1a), mean ($r_S = -0.38, p < 0.01$; figure 1b) and maximum $F_0$ ($r_S = -0.48, p < 0.0001$; figure 1c). However, infant weight at recording was marginally correlated with minimum $F_0$ ($r_S = 0.24, p = 0.06$), but not mean ($r_S = 0.11, p = 0.39$) or maximum $F_0$ ($r_S = -0.01, p = 0.96$). Other body size variables (height, head and chest circumference) at recording were not significantly correlated with any $F_0$ variable (see the electronic supplementary material).

For multiple regression analyses, we used gestational age, postmenstrual age and weight at recording to avoid the collinearity of predictors due to strong correlations among the variables related to preterm birth or body size at recording. All $F_0$ variables could be predicted by gestational age rather than postmenstrual age or weight at recording (table 3).

### 4. Discussion

This is the first study to determine the relationships between preterm birth and the $F_0$ of spontaneous cries in human infants at term-equivalent age. We found that shorter gestational age was significantly associated with higher $F_0$.
of spontaneous cries; however, neither smaller body size at recording nor IUGR was related to increased $F_0$ of spontaneous cries in preterm infants. Preterm infants are usually smaller in body size than full-term infants at term-equivalent age. Therefore, the higher $F_0$ of spontaneous cries may reflect the smaller body size at recording in preterm infants. However, we did not find any negative effects of body size at recording on any $F_0$. As the relationship between body size and vocal folds is not strong, especially in the same age and sex class within species [10], it is possible that the body size measurements used in this study did not reflect individual differences in vocal fold size. By contrast, we found a weak positive effect of weight at recording on minimum $F_0$. Vocal $F_0$ is related to subglottic pressure from the lungs as well as the size of the vocal folds [6]. Therefore, higher subglottic pressure might increase the minimum $F_0$ in infants with increased body weight at recording. As the MLP group had lower body weight at recording than the FT and VP groups (see the electronic supplementary material), the relatively low minimum $F_0$ in the MLP group might have been due to their lower body weight at recording. However, our data at least suggest that the

![Figure 1. Scatter plots showing the relationships between gestational age and (a) minimum ($r_s = -0.32, p < 0.01$), (b) mean ($r_s = -0.38, p < 0.01$) and (c) maximum ($r_s = -0.48, p < 0.0001$) fundamental frequency ($F_0$) of spontaneous cries at term-equivalent age for all participants ($n = 64$). The groups of infants were VP (white circles), MLP (grey circles) and FT (black circles).](rsbl.royalsocietypublishing.org_Biol_Lett_10_20140350_f1.png)

![Table 2. Difference in fundamental frequency ($F_0$) of spontaneous crying for SGAP infants and AGAP infants.](rsbl.royalsocietypublishing.org_Biol_Lett_10_20140350_t2.png)
increased $F_0$ of spontaneous cries in preterm infants is not due to their smaller body size at recording. Nevertheless, it remains unclear why preterm birth is associated with an increased $F_0$ of spontaneous cries at term-equivalent age. Our data did not provide a direct answer to this question. However, one possibility is that the increased $F_0$ might reflect the reduced vagal activity in preterm infants. Vagal input has an inhibitory effect on laryngeal contraction and vocal fold tightening; therefore, diminished vagal activity is assumed to cause increased vocal fold tension and higher $F_0$ [5]. As preterm infants at term-equivalent age exhibit lower resting vagal activity than full-term newborns [7], their reduced vagal activity might cause higher tension in the vocal folds, resulting in an increased $F_0$ of spontaneous cries. To clarify this relationship, further studies involving direct investigation of vagal activity by measuring respiratory sinus arrhythmia are required.

An additional important point is that we included preterm infants who exhibited IUGR because no studies have assessed $F_0$ of cries in SGA infants. As some preterm infants could exhibit IUGR, which can negatively affect neurobehavioural maturation [11], it is possible that this negative maturation might be related to the increased $F_0$ of spontaneous cries in preterm infants. However, we were unable to identify the effects of IUGR on the $F_0$ in preterm infants, suggesting that the increased $F_0$ in preterm infants does not reflect a neurophysiological vulnerability specific to IUGR. Moreover, it is important to note that our reported $F_0$ value in preterm infants did not necessarily deviate from the normal $F_0$ range (i.e. 200–600 Hz) reported previously [1]. This might have been because we included low-risk preterm infants without severe complications. Nevertheless, we should investigate the effects of preterm birth in a study with stronger homogenization including full-term SGA infants.

It is also possible that the increased $F_0$ of spontaneous cries in preterm infants is due to the longer postnatal period. This study did not include postnatal age in the major analyses because of a strong negative correlation between gestational and postnatal age. Nevertheless, each postnatal and postmenstrual age was positively correlated with every $F_0$, even after controlling for gestational age (see the electronic supplementary material). In mammals, the production of a cry involves cortical regions including the anterior cingulate gyrus, as well as reflexive central pattern generators in the periaqueductal grey and nucleus retroambigus [12,13]. Recent study of full-term infants shows that cry melodies (variation in $F_0$ over time) become increasingly complex during early interaction with the environment in the first months of life [14], suggesting the early contribution of cortical control to the $F_0$. However, it remains unclear in preterm infants at term-equivalent age; therefore, additional longitudinal studies will help to assess the

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**Table 3.** The results of the multiple regression analysis predicting fundamental frequency ($F_0$) of spontaneous crying. $\beta$, standardized regression coefficient; s.e., standard error.

<table>
<thead>
<tr>
<th>predictor</th>
<th>$F_0$</th>
<th>s.e.</th>
<th>$t$-value</th>
<th>$p$-value</th>
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<tr>
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<td>2.70</td>
<td>0.01</td>
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<td>1.70</td>
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<td>weight at cry recording</td>
<td>0.26</td>
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<td>2.23</td>
<td>0.03</td>
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<td>$F_{3,60} = 6.19$</td>
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<th>$t$-value</th>
<th>$p$-value</th>
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<td>0.02</td>
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<th>$p$-value</th>
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<td>2.87</td>
<td>0.01</td>
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<td>0.07</td>
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<td>$F_{3,60} = 8.95$</td>
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effects of the postnatal and maturational period on the $F_0$ in preterm infants.

In conclusion, this study revealed that preterm birth was associated with an increased $F_0$ of spontaneous cries at term-equivalent age, regardless of the smaller body size at recording or IUGR. Hence, the increased $F_0$ in preterm infants may have been caused not by smaller body size at recording, but rather by more complicated neurophysiological states due to different intrauterine and extrauterine experiences.

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