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RESEARCH ARTICLE

Prenatal daily musical exposure is associated with enhanced neural representation of speech fundamental frequency: Evidence from neonatal frequency-following responses

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Abstract

Fetal hearing experiences shape the linguistic and musical preferences of neonates. From the very first moment after birth, newborns prefer their native language, recognize their mother's voice, and show a greater responsiveness to lullabies presented during pregnancy. Yet, the neural underpinnings of this experience inducing plasticity have remained elusive. Here we recorded the frequency-following response (FFR), an auditory evoked potential elicited to periodic complex sounds, to show that prenatal music exposure is associated to enhanced neural encoding of speech stimuli periodicity, which relates to the perceptual experience of pitch. FFRs were recorded in a sample of 60 healthy neonates born at term and aged 12–72 hours. The sample was divided into two groups according to their prenatal musical exposure (29 daily musically exposed; 31 not-daily musically exposed). Prenatal exposure was assessed retrospectively by a questionnaire in which mothers reported how often they sang or listened to music through loudspeakers during the last trimester of pregnancy. The FFR was recorded to either a /da/ or an /oa/ speech-syllable stimulus. Analyses were centered on stimuli sections of identical duration (113 ms) and fundamental frequency ($F_0 = 113$ Hz). Neural encoding of stimuli periodicity was quantified as the FFR spectral amplitude at the stimulus F_0. Data revealed that newborns exposed daily to music exhibit larger spectral amplitudes at F_0 as compared to not-daily musically-exposed newborns, regardless of the eliciting stimulus. Our results suggest that prenatal music exposure facilitates the tuning to human speech fundamental frequency, which may support early language processing and acquisition.

KEYWORDS
infants, language, newborns, plasticity, prenatal, speech auditory brainstem response

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Research Highlights

- Frequency-following responses to speech were collected from a sample of neonates prenatally exposed to music daily and compared to neonates not-daily exposed to music.
- Neonates who experienced daily prenatal music exposure exhibit enhanced frequency-following responses to the periodicity of speech sounds.
- Prenatal music exposure is associated with a fine-tuned encoding of human speech fundamental frequency, which may facilitate early language processing and acquisition.

1 | INTRODUCTION

Fetal hearing experiences shape the linguistic and musical preferences of newborns (Chorna et al., 2019; Gervain, 2018; May et al., 2011; Partanen et al., 2013). Behavioral studies have shown that newborns prefer their mother's voice (DeCasper & Fifer, 1980) and their native language (Moon et al., 1993), and even recognize stories only heard during pregnancy (DeCasper & Spence, 1986), proving that babies respond differently to native and non-native sounds just a few hours after birth (Moon et al., 2012). Likewise, recent studies using a range of neuroimaging techniques such as cranial ultrasonography, functional magnetic resonance imaging (fMRI), and functional near-infrared spectroscopy (fNIRS) demonstrated the influence of hearing experiences on the neonate's brain through several findings, such as distinct hemisphere specialization (Vannasing et al., 2016), differential brain activation in newborns for native and non-native languages (May et al., 2011), and bilateral volume increase of auditory cortices (Webb et al., 2015). Furthermore, evidence from neurophysiological studies in newborns using event-related potentials (ERP) revealed greater neural activation to lullabies (Partanen et al., 2013) and to changes in speech sounds (Partanen et al., 2013) heard during pregnancy. Research thus suggests a paramount influence of exposure to sound during prenatal neural plasticity windows (Gilmore et al., 2018), molding auditory processing and perception since gestation.

This growing body of evidence points to a very early form of auditory learning that takes place in utero, shaping the infant's future neurodevelopment and processing of language. This is hardly surprising, considering that most of hearing development occurs between the 26th and the 28th weeks of pregnancy (Anbuhl et al., 2016; Granier-Deferre et al., 2011; May et al., 2011; Moore & Linthicum, 2007; Ruben, 1995), and that by the third trimester of gestation, the sense of hearing is already functional in some aspects similar to that of adults (Ullal-Gupta et al., 2013).

Thus, previous research has revealed that the newborn's brain, albeit its limited language and auditory experience, is already able to encode and perceive different components of speech, such as pitch, in an adult-like manner (Arenillas-Alcón et al., 2021a; Cabrera & Gervain, 2020; Jeng et al., 2011). Pitch is defined as the perceptual attribute of the periodicity rate of a sound waveform that allows sounds to be ordered in a musical scale (Plack et al., 2014). Thus, pitch relates to the perception of sound periodicity, and hence mainly depends on the lowest frequency of a periodic waveform, that is, the so-called fundamental frequency (F_0), (Krizman & Kraus, 2019; Plack et al., 2014). The accurate encoding and tracking of F_0 play an essential role in the future acquisition of language and sound processing, including the perception of melodies, harmony in music or prosody in speech, as well as language comprehension in noisy environments, perception of the emotional content of a conversation, phoneme acquisition in tonal languages, recognition of speakers or speech segmentation, among others (Arenillas-Alcón et al., 2021a; Benavides-Varela et al., 2012; Cabrera & Gervain, 2020; Gervain, 2018; Musacchia et al., 2007; Partanen et al., 2013; Plack et al., 2014; Ribas-Prats et al., 2022). Because the mother's womb acts as a low-pass filter, only allowing the transmission of sound frequencies below 500 Hz (Gerhardt & Abrams, 2000; Jeng, 2017; McCarthy et al., 2019; Parga et al., 2018), sounds available to the fetus are mainly dominated by these frequency ranges. The consequences of this prenatal exposure to low-frequencies, which are typical of human speech (from 100–255 Hz (Traunmüller & Eriksson, 1995)), support the idea of an increased sensitivity of the auditory system regarding low-frequency ranges and may offer an explanation to the remarkable adult-like status observed already at birth in the encoding of F_0 (Arenillas-Alcón et al., 2021a).

After birth, F_0 processing abilities are enhanced by a wealth of auditory experiences, entailing auditory training periods and language or music exposure (Carcagno & Plack, 2011). For instance, a greater exposure to enriched linguistic contexts, as occurs in bilingual environments or with tonal languages, which employ pitch to convey word meaning, has been found to yield a more robust neural encoding of F_0 (Bidelman et al., 2011; Jeng et al., 2011; Krishnan et al., 2005). Likewise, musicians from different ages show enhanced neural encoding of F_0 (Musacchia et al., 2007; Wong et al., 2007), exhibit superior detection of linguistic pitch manipulations (Bidelman et al., 2011; Deguchi et al., 2012; Magne et al., 2006; Schön et al., 2004), as well as finer perception of prosody (Thompson et al., 2003). Importantly, musical exposure is pervasive in infancy (Mendoza & Fausey, 2021). Regardless of socioeconomic status, ethnicity and technical developments such as recorded music availability, most parents direct singing to their offspring on a daily basis (Yan et al., 2021), with arousing, pleasing and soothing effects

(Cirelli & Trehub, 2020). Lullabies, characterized by an overall lower pitch, reduced accentuation and slower tempos than, for example, play songs (Tsang & Conrad, 2010), exert an especially relaxing effect on the infant, even if unknown and stemming from different cultures (Bainbridge et al., 2021). Interestingly, infant-directed speech (i.e., 'parentese') is characterized, among other features, by an overall higher pitch and pitch variability, as well by purer and less harsh vocal timbres (Hilton et al., 2022), which emphasize the periodicity of the sound waveform over noise. These characteristics have led some authors to conclude that 'the constellation of acoustic features that characterize infant-directedness in speech, across cultures, are rather musical' (Hilton et al., 2022). Hence, while lullabies use lower pitches to exert relaxing effects, infant-directed speech, which supports language acquisition (Ma et al., 2011; Trainor & Desjardins, 2002) and coordinates communicative interactions with infants (Mehr et al., 2021), resembles other song types in its use of a higher pitch and pitch variation.

Thus, according to previous findings showing an enhanced F_0 encoding due to a higher language and music exposure, and considering the pervasive presence of music and infant-directed speech in early life periods and their characteristic pitch modulation patterns, it seems likely to consider that a musically enriched prenatal experience could also have enhancing effects on F_0 encoding skills (Chorna et al., 2019; Gervain, 2018).

Studies investigating F_0 encoding have gained interest in using the frequency-following response (FFR) as a precise neural activity correlate of early processing stages in the auditory pathway. The FFR is an auditory evoked potential originating from combined cortical and subcortical sources that mimics with high fidelity the acoustic features of the eliciting auditory stimulus (Coffey et al., 2019; Gorina-Careta et al., 2019, 2021; Skoe & Kraus, 2010), providing a non-invasive lens into sound processing in the brain. The growing attention it has obtained stems from its potential to predict the future development of language (Schochat et al., 2017), considering that abnormal FFR patterns in children have been related to reading impairments, learning problems, deficits in phonological awareness, dyslexia and even to clinical conditions such as autism (Banai et al., 2009; Basu et al., 2010; Chandrasekaran et al., 2009; Font-Alaminos et al., 2020; Hornickel et al., 2012; King et al., 2002; Lam et al., 2017; Otto-Meyer et al., 2018; Rosenthal, 2020). Indeed, the FFR is sensitive not only to several clinical conditions, but also to many different auditory contexts, such as training or musical experience (Carcagno & Plack, 2017; Gorina-Careta et al., 2019; Kraus & Chandrasekaran, 2010; Russo et al., 2005; Song et al., 2008). As a result of the abovementioned evidence, together with the feasibility to record the FFR in newborns (Arenillas-Alcón et al., 2021a; Gardi et al., 1979; Gorina-Careta et al., 2022; Jeng et al., 2011, 2016; Ribas-Prats et al., 2019, 2022; Richard et al., 2020), the idea that the FFR could become a potential biomarker for identifying auditory and speech processing impairments has recently emerged (Arenillas-Alcón et al., 2021a, 2021b; Coffey et al., 2016; Font-Alaminos et al., 2020; Ribas-Prats et al., 2019; Richard et al., 2020; Schochat et al., 2017).

Considering the enhancement of pitch processing by musical training and exposure and its potential to foster language development (Bidelman et al., 2011; Musacchia et al., 2007; Wong et al., 2007), together with the importance of prenatal musical exposure on fetal and neonatal well-being (Brillo et al., 2021; Çatalgöl & Ceber Turfan, 2021; He et al., 2021; Poćwierz-Marciniak & Harciarek, 2021), it appears reasonable to expect that prenatal music exposure could be related to language and speech encoding abilities at birth. However, previous findings relating prenatal music exposure and language in newborns have been mostly based on behavioral measures such as the register of the number of movements, heart rate accelerations or decelerations, respiratory rate or feeding volume; or through the analysis of ERP components related to the brain's automatic detection of changes, rather than neurophysiological responses that accurately reflect the neural encoding of speech sounds.

The present study was hence set to investigate the encoding of the fundamental frequency of speech stimuli at birth through recording the FFR in newborns with different degrees of exposure to music during the prenatal period. We hypothesized better F_0 encoding in the group of neonates with daily exposure to music during pregnancy, that is, a significant increase in the magnitude of the neural signal at the stimulus F_0. Should this hypothesis be confirmed, our findings would support early neural plasticity in audition, and critically, would point out to the relevance of prenatal music exposure to facilitate the tuning of the fetus' auditory system to human speech F_0, which is crucial for a successful future language acquisition.

2 | METHODS

2.1 | Participants

A sample of 29 newborns daily-exposed to music during pregnancy (DE; 11 females; mean gestational age = 39.85 ± 0.79 weeks; mean birth weight = 3329 ± 256 g) and 31 not-daily exposed (NDE; 16 females; mean gestational age = 39.92 ± 1.06 weeks; mean birth weight = 3367 ± 305 g) was recruited from *SJD Barcelona Children's Hospital* in Barcelona (Spain), based on a successful completion of a musical exposure questionnaire filled out by the babies' mothers (see below). No significant differences are reported across groups in gestational age ($t_{(58)} = -0.307$, $p = 0.760$) or birth weight ($t_{(58)} = -0.525$, $p = 0.602$). All newborns passed positively the universal hearing screening as part of the hospital routine, based on the detection of the auditory brainstem responses (ALGO 3i, Natus Medical Incorporated, San Carlos, SA), and obtained Apgar scores higher than 8 at 1 and 5 min of life. High-risk gestations as well as newborns with obstetric pathologies or other risk factors related to hearing impairment according to the Joint Committee of Infant Hearing (Joint Committee on Infant Hearing, 2019), were excluded from the recruitment.

Additionally, to double-check the integrity of the auditory pathway as well as the neural transmission time—as performed in previous studies from our laboratory—both groups of newborns received a standard click-evoked auditory brainstem response (ABR) test. The click stimulus had a duration of 100 μs and was presented at a rate of 19.30 Hz, at an intensity of 60 dB sound pressure level (SPL) until a total of 4000 artifact-free sweeps, divided in two runs of 2000, were collected. Identification of a reliable wave V peak was a requirement for all newborns to proceed to the experiment. The study was approved by the Ethical Committee of Clinical Research (CEIC) of the Sant Joan de Déu Foundation (Approval ID: PIC-53-17), and required the mothers to fill out a sociodemographic questionnaire and to sign an informed consent prior to the participation, in line with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Because FFRs are modulated by socioeconomic status (Krizman et al., 2021; Skoe et al., 2013), we checked for group differences in the mothers' educational level and employment status, considering these variables as proxies available in the collected sociodemographic questionnaire. Summary statistics and analysis, as well as a graphical representation of the distribution, can be found in Figure S1 and Table S1, respectively. In short, we did not find any differences across groups (Educational level of the mother (DE vs. NDE): $\chi^2{}_{(2)} = 0.229$, $p = 0.892$; Employment status of the mother (DE vs. NDE): $\chi^2{}_{(2)} = 2.092$, $p = 0.351$), suggesting that the socioeconomic status is not a confounding factor in our data analyses.

2.2 | Musical exposure

The musical exposure that newborns underwent during pregnancy was assessed by a short retrospective questionnaire delivered to the babies' mothers. Based on previous research in newborns that conceptualizes musical exposure in terms of hours of exposure per week (Coffey et al., 2017; Musacchia et al., 2007; Strait et al., 2012; Zuk et al., 2013), mothers were asked the frequency with which they used to sing or listen to music through loudspeakers during the last 3 months before delivery (an English version of the musical questionnaire employed can be found in the Appendix). They were instructed to spurn situations in which music exposure was not intentional, such as ambient music in shops, elevators or restaurants. Instead, they were told to consider as an "exposed day" those days with periods in which their exposure to music through loudspeakers and/or singing was a minimum of around 30 min, regardless whether they were solely listening to it and/or singing, or while carrying out other activities (e.g., cleaning, cooking, exercising). Newborns were then classified into two groups attending to their musical exposure. The DE group included 29 neonates whose mothers were listening to music through loudspeakers and singing on a daily basis. The NDE group included 31 neonates whose mothers did not sing or listen to music through loudspeakers on a daily basis. Summary statistics of the mother's reports can be found in Table 1.

We acknowledge that our study would have benefited from allowing answers in a continuous manner (i.e., number of days per month), rather than in incremental steps (daily, weekly, once every 2 weeks, monthly, and never), enabling a correlation approach in data analysis. With the collected data, though, such an analysis would be subject to statistical issues given the distribution of counts per answer. Counts per answer to days in a month with music listening or singing exhibited a left skewed distribution, revealing that about half the sample (29/60) listened to music through loudspeakers and/or sang daily, while the rest (31/60) did so on a lesser degree. Interestingly, the number of participants who listened to music weekly (N = 24) was similar to that of participants who listened to music daily (N = 29). Taking into account that group size was well balanced between DE (N = 29) and NDE (N = 31), and that NDE contained a 77.4% of participants (24/31) with weekly musical exposure, any differences between groups we may found would be even more conclusive.

TABLE 1 **Summary statistics of mother's reports**: Frequency of responses for listening to music and singing as reported by the newborns' mothers through the musical questionnaire

	DE		NDE	
	Frequency	% of the sample	Frequency	% of the sample
Listen to music				
Daily	29	100	–	–
Weekly	–	–	24	77.4
Once every 2 weeks	–	–	3	9.7
Monthly	–	–	0	0
NA/Never	–	–	4	12.9
Sing				
Daily	29	100	–	–
Weekly	–	–	12	38.7
Once every 2 weeks	–	–	4	12.9
Monthly	–	–	2	6.5
NA/Never	–	–	13	41.9
Total	29	100	31	100

2.3 | Stimuli

Neonatal FFRs were recorded to two different speech stimuli: a consonant-vowel syllable /da/ (Ribas-Prats et al., 2019, 2022) and a two-vowel syllable /oa/ (Arenillas-Alcón et al., 2021a) in a group design (i.e., a single newborn was stimulated either with the /da/ or the /oa/ stimulus). The stimulus /da/ was chosen since it is the most commonly employed in FFR research with newborns (Lemos et al., 2021; Ribas-Prats et al., 2019, 2022; Richard et al., 2020). This stimulus, created by Klatt-based synthesizer (Klatt, 1980) and modified by *Praat* (Boersma & Weenink, 2020), has a duration of 170 ms, divided in 10 ms of onset period, 47 ms of consonant transition, and 113 ms for the /a/ vowel section (consonant transition: 10–57 ms, $F_0 = 113$ Hz, $F_1 = 553–688$ Hz; /a/ vowel section: 57–170 ms, $F_0 = 113$ Hz, $F_1 = 688$ Hz; Figure 1a), and was presented at a rate of 3.7 Hz. The F_0 was kept steady at 113 Hz during the whole duration of the stimulus. Of the total number of newborns whose FFR were recorded with the /da/ stimulus, eight of them were considered to belong to the DE group, and 11 belonged to the NDE group.

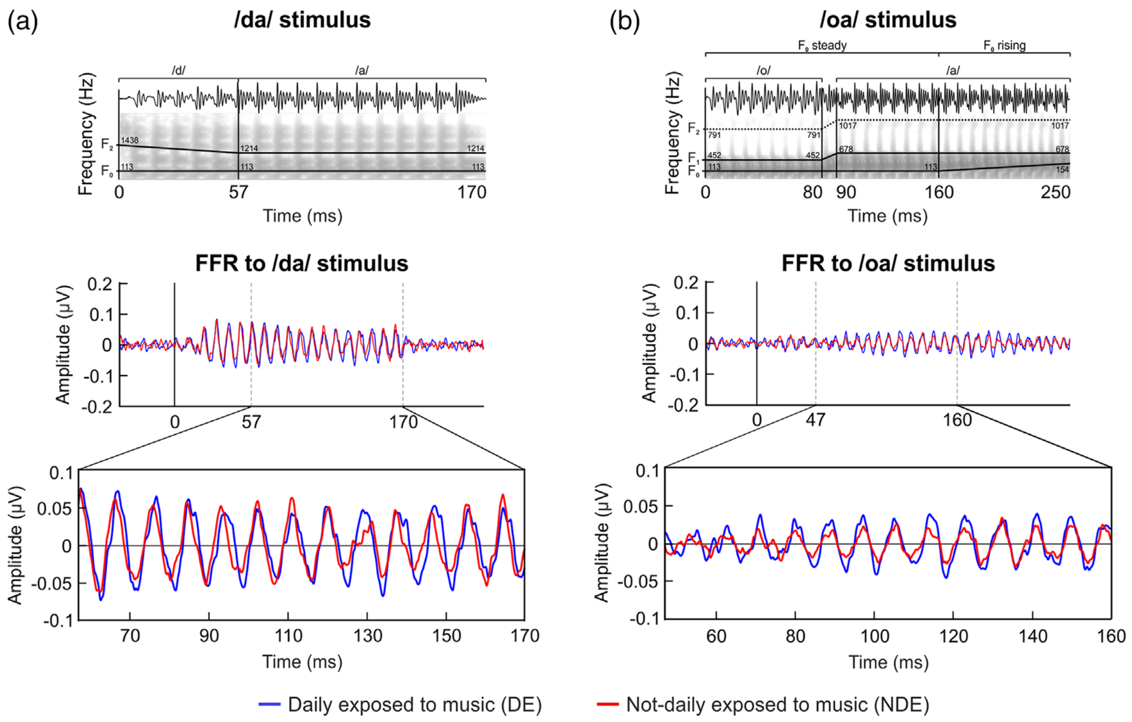

FIGURE 1 Temporal representations of the FFR elicited in the DE (blue) and the NDE (red) groups separately. **(a) (top)**: Acoustic waveform and spectrogram of the /da/ stimulus with a schematic overlay of the formant structure trajectory; **(a) (center)**: Grand averaged time-domain waveform of the FFR elicited by the /da/ stimulus. **(a) (bottom)**: Zoom of the equivalent analyzed section (57-170 ms) of the waveform neural response. **(b) (top)**: Acoustic waveform and spectrogram of the /oa/ stimulus with schematic overlay of the formant structure trajectory; **(b) (center)**: Grand averaged time-domain waveform of the FFR elicited by the /oa/ stimulus. **(b) (bottom)**: Zoom of the equivalent analyzed section (47-160 ms) of the waveform neural response.

In turn, the stimulus /oa/ (Arenillas-Alcón et al., 2021a), was created in *Praat* (Boersma & Weenink, 2020) with a total duration of 250 ms divided in two vowel sections as well as two different F_0 sections (/o/ section: 10–80 ms, $F_0 = 113$ Hz, $F_1 = 452$ Hz; /a/ steady section = 90–160 ms, $F_0 = 113$ Hz, $F_1 = 678$ Hz; /a/ rising section = 160–250 ms, $F_0 = 113$–154 ms, $F_1 = 678$ Hz; Figure 1b), and was presented at a rate of 3.39 Hz. Attending to its pitch, F_0 was kept steady at 113 Hz during the first part of the stimulus (0-160 ms) and increased linearly until 154 Hz (160-250 ms). Of the total number of newborns whose FFR were recorded with the /oa/ stimulus, 21 of them were considered to belong to the DE group, and 20 belonged to the NDE group.

For the present study, only a section with equal duration (113 ms) and steady fundamental frequency (113 Hz) was chosen for analysis in both stimuli (/da/ from 57–170 ms, $F_0 = 113$ Hz; /oa/ from 47–160 ms, $F_0 = 113$ Hz). Both speech sounds were delivered monaurally to the right ear, in alternating polarities, at 60 dB SPL of intensity with an earphone connected to a Flexicoupler disposable adaptor (Natus Medical Incorporated, San Carlos, CA).

2.4 | Procedure and data acquisition

After passing the universal neonatal hearing screening, newborns were tested in their crib at the hospital room while they were sleeping. Four disposable Ag/AgCl electrodes were placed in a vertical montage (active at Fpz, ground at forehead, one reference at each mastoid; Figure S2a) with impedances below 7 kΩ, though in the current study only data referenced to the electrode located at the right mastoid (ipsilateral to the auditory stimulation) was considered for analyses. Click and speech stimuli were presented by using a *SmartEP* platform connected to a *Duet* amplifier, that includes the *cABR* and the *Advanced Hearing Research* modules (Intelligent Hearing Systems, Miami, Fl, USA).

Following procedures from previous newborn studies carried out in our laboratory, four blocks of 1000 artifact-free responses to the /da/ or /oa/ stimulus, respectively, were recorded after the ABR blocks described above. Any electrical activity exceeding $\pm$ 30 μV was automatically rejected until a total of 4000 presentations to each stimulus were collected. The total mean duration of the recording session was approximately 25 min (sessions with /da/: two click blocks $\times$ 2000 repetitions $\times$ 51.81 ms of stimulus-onset asynchrony (SOA) + four /da/ blocks $\times$ 1000 repetitions $\times$ 270 ms SOA + duration of rejected repetitions; sessions with /oa/: two click blocks $\times$ 2000 repetitions $\times$ 51.81 ms SOA + four /oa/ blocks $\times$ 1000 repetitions $\times$ 295 ms SOA + duration of rejected repetitions). The continuous electroencephalography signal was acquired at a sampling rate of 13,333 Hz, bandpass filtered online from 30–1500 Hz and epoched and averaged online from −40.95 ms (pre-stimulus period, for both stimuli) to 229.32 ms (/da/ stimulus) or to 249.975 ms (/oa/ stimulus).

2.5 | Data processing and analysis

Data were bandpass filtered offline from 80 to 1500 Hz. To emphasize the encoding of the stimulus (F_0) and to minimize the contribution of cochlear microphonics, neural responses elicited to the two opposite stimulus polarities were averaged [(Condensation + Rarefaction)/2] (Aiken & Picton, 2008; Krizman & Kraus, 2019), to obtain the envelope-following FFR. All parameters were computed with custom scripts from Matlab R2019b (The Mathworks, 2019) developed in our laboratory and used in similar analyses performed in previous studies.

2.5.1 | Parameters extracted from ABR

Wave V. The latency of wave V peak in the ABR was determined by automatically identifying the major positive peak from 8–9.90 ms and its corresponding amplitude, and was taken as an estimation of the brainstem conduction time from the stimulus reception in the cochlea to the inferior colliculus in the midbrain (Ribas-Prats et al., 2019; Stuart et al., 1994). Additionally, the automatic detection was visually reviewed to identify peaks slightly outside the established time range.

2.5.2 | Parameters extracted from FFR

Neural lag. Neural lag was considered as an inference of the neural transmission delay of the auditory system, since this value provides evidence of the amount of time passed from the reception of the stimulus at the cochlea until the start of the neural phase-locking (Arenillas-Alcón et al., 2021a; Jeng et al., 2010; Liu et al., 2015; Ribas-Prats et al., 2019, 2022). It was calculated by computing a cross-correlation between the auditory stimulus and the neural response within a 3–13 ms time window, obtaining a correlation value at each time lag. The neural lag was obtained by selecting the time lag corresponding to the maximum cross-correlation value.

Pre-stimulus root mean square (RMS) amplitude. The RMS of the pre-stimulus period was taken as an indicator of the overall magnitude of neural activity along time, and used to discard electrophysiological differences in the pre-stimulus region (Liu et al., 2015; Ribas-Prats et al., 2019, 2022; White-Schwoch et al., 2015). It was calculated by squaring each point of the pre-stimulus region of the neural response (from −40 to 0 ms), computing the mean of the obtained values and calculating the square root of the obtained average.

Spectral amplitude at F_0. Spectral amplitude at F_0 (113 Hz) was considered as a measure of the magnitude of the neural phase-locking at the specific chosen frequency, obtaining an indicator of the response strength (Arenillas-Alcón et al., 2021a; Ribas-Prats et al., 2019, 2022; White-Schwoch et al., 2015). It was computed using a fast Fourier transform (FFT) (Cooley & Tukey, 1965) to obtain the neural response frequency structure, and calculating the mean amplitude within a ± 5 Hz window centered at the stimulus F_0 peak.

2.6 | Statistical analysis

Statistical analyses were performed on SPSS 25.0 (Corp.). Descriptive statistics include the mean, standard deviation (SD), median, first (Q_1) and third (Q_3) quartiles, interquartile range (IQR) and minimum and maximum values of the computed parameter for each group of newborns (DE; NDE). The Shapiro-Wilk test was selected to check the normal distribution of the samples. Depending on the normality of the samples, two-tailed independent samples *t*-tests or *Mann-Whitney U* tests were computed to check for significant differences in comparisons between groups, reporting Cohen's *d* for effect size. Results were considered significant when $p < 0.05$.

A univariate general linear ANOVA was conducted to examine and control for different effects depending on the type of stimulus used. To assess effects of Group (DE and NDE), this variable was introduced in the ANOVA as fixed factor; for the effect of Stimulus (/da/ and /oa/), the variable was taken as random factor. Partial eta squared effect ($\eta^2{}_p$) sizes are reported.

3 | RESULTS

The ABR and the FFRs elicited by the /da/ (Figure 1a) and the /oa/ (Figure 1b) stimuli were successfully collected from a total of 60 newborns, which were divided into two groups according to their musical exposure during the last trimester of pregnancy (Daily musical exposure: DE; Not-Daily musical exposure: NDE). Neonatal FFR parameters (neural lag, pre-stimulus RMS, spectral amplitude at stimulus F_0) were analyzed in a section of both stimuli identical in duration (113 ms) and fundamental frequency (113 Hz). Table 2 reports the descriptive statistics for the FFR parameters analyzed separately in both groups; statistics for each stimulus separately can be found in Table S2 (/da/) and Table S3 (/oa/).

ABR. From the ABRs, the wave V could be identified in all newborns recruited. For the daily musically exposed (DE) group, the mean latency of wave V was 8.582 ± 0.336 ms, and its mean amplitude was $0.132 \pm 0.062\ \mu V$. The not-daily musically exposed (NDE) group exhibited a wave V latency of 8.482 ± 0.368 ms, and a mean amplitude of $0.096 + 0.091\ \mu V$. In Figure S2b the grand-average of the ABR waveform is shown; violin plots of the group distribution for wave V latency and amplitude are depicted in Figure S2c,d, respectively. No significant group differences were found for wave V latency ($U = 347.000$, $p = 0.129$, Cohen's $d = 0.400$) or amplitude ($U = 329.000$, $p = 0.075$, Cohen's $d = 0.473$).

Neural lag. DE and NDE groups did not exhibit significant differences in neural transmission delay values ($t_{(58)} = 0.459$, $p = 0.648$, Cohen's $d = 0.119$).

Pre-stimulus root mean square (RMS) amplitude. No different background neural activity was found across groups ($U = 356.000$, $p = 0.167$, Cohen's $d = 0.363$).

Spectral amplitude at F_0. Spectral representation of the neonatal FFR extracted from the equivalent analyzed sections of each stimulus is

TABLE 2 **Descriptive statistics for DE** (N = 29) **and NDE** (N = 31) **groups in FFR parameters**: Neural lag, Root-Mean-Square from pre-stimulus section, spectral amplitude at F_0 peak, computed for the steady pitch section of each stimulus (/da/; /oa/)

Measure	Mean	SD	Median	Q_1	Q_3	IQR	Minimum	Maximum
Neural lag (ms)								
DE	7.585	1.396	7.425	6.675	8.475	1.800	4.650	10.575
NDE	7.389	1.873	7.425	5.850	8.100	2.250	4.650	12.975
Pre-stimulus RMS (μV)								
DE	0.033	0.018	0.031	0.020	0.038	0.018	0.015	0.088
NDE	0.026	0.010	0.022	0.020	0.032	0.012	0.011	0.047
F_0 Spectral Amplitude (nV)								
DE	20.068	9.464	18.622	12.696	25.702	13.005	5.480	49.073
NDE	14.420	8.840	11.600	7.697	20.131	12.435	2.838	35.041

Note: Descriptive statistics in FFR parameters for each stimulus separately can be found in Table S2 (/da/) and Table S3 (/oa/).
Abbreviations: SD, standard deviation; Q_1, first quartile (25th percentile); Q_3, third quartile (75th percentile); IQR, interquartile range.

shown in Figure 2a. Violin plots and graphic bars illustrating the distribution of spectral amplitudes values at the fundamental frequency (F_0) for each group and stimulus are provided in Figure 2b,c. Statistical analyses revealed significant differences between groups, indicating that newborns exposed daily to music during the last trimester of pregnancy exhibited a larger spectral amplitude at the stimulus F_0 as compared to the less exposed group ($U = 275.000$, $p = 0.010$, Cohen's $d = 0.707$). Furthermore, in order to control for the influence of the stimulus type (/da/ or /oa/) on the stimulus F_0 spectral amplitude values, a univariate ANOVA was computed. A main effect of Group revealed significantly greater spectral amplitudes at stimulus F_0 for the DE newborns ($F = 6.750$, $p = 0.012$, $\eta^2{}_p = 0.108$). A significant main effect of Stimulus was also observed, caused by a larger spectral amplitude to the /da/ stimulus as compared to the /oa/ ($F = 14.152$, $p < 0.001$, $\eta^2{}_p = 0.202$). However, no interaction between the two factors was found ($F = 0.132$, $p = 0.718$, $\eta^2{}_p = 0.002$). Consequently, our data reveals that newborns exposed to music on a daily basis during the last trimester of pregnancy show a greater magnitude of neural phase-locking to speech stimuli F_0 than not-daily exposed neonates. Importantly, this effect was observed regardless of the type of speech stimulus, /da/ or /oa/, used to elicit the FFR, and despite the neural response was in itself remarkably different between stimuli (Figure 2c). An additional ANOVA excluding one outlier newborn in the DE group (with a F_0 spectral amplitude elicited to the /da/ stimulus equal to 2.18 times the interquartile range) yielded similar results, except for a Group effect p value marginally above the significance level (Group effect: $F = 3.919$, $p = 0.053$, $\eta^2{}_p = 0.067$; Stimulus effect: $F = 10.270$, $p = 0.002$, $\eta^2{}_p = 0.157$; Interaction: $F = 1.294$, $p = 0.260$, $\eta^2{}_p = 0.023$). Considering that the data was not normally distributed, we also conducted a non-parametric analysis in order to test for Group and Stimulus main effects, yielding significant differences in both factors (Group main effect: $U = 275.000$, $p = 0.016$, Cohen's $d = 0.587$; Stimulus main effect: $U = 200.000$, $p = 0.005$, Cohen's $d = 0.835$). Detailed descriptive statistics are reported in Table S4.

4 | DISCUSSION

In the current study, we disclose the effects of prenatal music exposure on speech stimuli F_0 encoding at birth, through the analysis of the neonatal frequency-following response (FFR) elicited by two different periodic speech stimuli (/da/ and /oa/) in stimulus sections of identical pitch. Our results indicate that daily exposure to music, during the last 3 months of pregnancy, is related to a stronger encoding of speech stimuli F_0 content at birth. This was evidenced as greater neural response amplitudes at F_0 in the daily exposed (DE) group, in absence of distinct background neural activity and regardless of the specific stimulus used to elicit the FFR. Moreover, no significant differences between groups were found in auditory neural transmission time measures, such as wave V latency or neural lag. Thus, the results of the present study suggest that the ability to perceive and process pitch at birth, that mainly depends on the neural encoding of the F_0 of the incoming sounds (Krizman & Kraus, 2019; Plack et al., 2014) is, to a certain extent, modulated by the auditory experiences while in the womb.

Several factors support the value of the present results. First, the sample of newborns was homogeneous in terms of gestational age and birth weight, factors known to affect the FFR (Ribas-Prats et al., 2022). Second, we found no differences across groups segregated by musical exposure in background neural activity, as measured by pre-stimulus RMS amplitudes. This highlights the observed group differences in FFR spectral amplitudes elicited by speech stimuli. Third, auditory neural transmission time measures did not differ across groups, as evidenced by ABR wave V latencies and the FFR neural lag. This ensures that the processing of the acoustic input along the ascending auditory pathway is homogeneous across groups, being all newborns tested normal in terms of typical hearing screening measures implemented in hospital routines. Furthermore, this indicates that prenatal music exposure does not have an impact on neural transmission times immediately after birth, in contrast with the faster processing speed of auditory and speech stimuli reported in musically trained adults (Schochat et al., 2017). Finally, and most importantly, our study design featured two

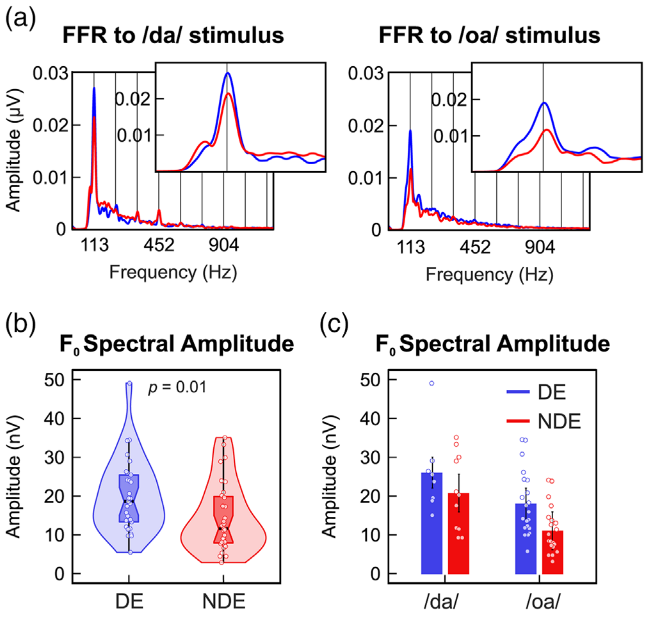

FIGURE 2 Spectral representation of the FFR and data distribution of the F_0 spectral amplitude for the DE (blue) and the NDE (red) groups. **(a) (left)**: FFR frequency spectra extracted from the equivalent /da/ analyzed section of the neural response. **(a) (right)**: FFR frequency spectra extracted from the equivalent /oa/ analyzed section of the neural response. **(b)** Violin plots of F_0 spectral amplitude. Horizontal black lines and vertical black lines indicate the median and 1.5 times the interquartile range (IQR), respectively. Dark colored boxes illustrate the IQR, while violin plots outlines show kernel probability density, that is, the proportion of data located there. Scatter plots show all tested participants in each group. **(c)** Vertical color bars represent the mean F_0 spectral amplitude separately for each group (DE; NDE) exposed to the /da/ (left) or the /oa/ (right) stimuli. Vertical black lines illustrate the mean standard error. Scatter plots show all tested participants in each group.

speech stimuli of different characteristics except for their periodicity, which were delivered to two different sets of newborns. Albeit FFR spectral amplitudes were different across stimuli, with the /da/ stimulus yielding overall higher spectral amplitudes at F_0 than the /oa/ stimulus, in line with previous studies (see spectral amplitudes for /da/ in Ribas-Prats et al. (2019) and for /oa/ in Arenillas-Alcón et al. (2021a)), we found no interaction between stimulus type and musical exposure. Therefore, regardless of the syllable that a newborn heard, fetuses that were daily exposed to music while in the womb showed a stronger neural encoding of speech F_0 at birth.

Accurate F_0 tracking is fundamental to pitch processing, as pitch directly relates to the periodicity of a sound waveform (Plack et al., 2014). Sound pitch patterns organized in time are the basis of musical melody, and simultaneously sounding pitches produce musical harmony. In human oral communication, pitch is a crucial cue to recognize different speakers, segment speech in linguistically relevant units and segregate speech from a noisy background, but it is an essential feature in tonal language semantics and in linguistic prosody, which conveys intonational meaning as well as emotional content (Arenillas-Alcón et al., 2021a; Benavides-Varela et al., 2012; Cabrera & Gervain, 2020; Gervain, 2018; Musacchia et al., 2007; Partanen et al., 2013; Plack et al., 2014; Ribas-Prats et al., 2022). Moreover, pitch cues underlie the discrimination between infant-directed and adult-directed speech (Fernald & Kuhl, 1987). In fact, the acoustic characteristics of infant-directed speech, particularly regarding pitch (i.e., higher and more variable) and purer vocal timbres (Hilton et al., 2022), resemble those of music. Because infant-directed speech aids language acquisition (Ma et al., 2011; Trainor & Desjardins, 2002), we hypothesize that exposure to music might as well provide similar benefits. Unfortunately, our questionnaire did not collect any data on the amount of speech exposure while in the womb, nor whether speech was directed to the fetus. Nevertheless, pitch appears to be a very important feature of speech to babies, as its variations directly call for their attention, providing a substantial wealth of information and aiding language development in multiple ways.

It is worth noting that linguistic prosody and non-vocal musical patterns are readily available to fetuses due to the low-pass filter characteristics of the mother's womb (Gerhardt & Abrams, 2000; Jeng, 2017; McCarthy et al., 2019; Parga et al., 2018). These characteristics may explain why healthy newborns exhibit an adult-like neural encoding of speech F_0, while the encoding of other speech features, based on sound frequencies higher than the womb's low-pass cutoff, are still undeveloped (Arenillas-Alcón et al., 2021a). It is also the most plausible explanation for the wealth of studies showing the influence of prenatal acoustic experiences in shaping the sound preferences of newborns (Chorna et al., 2019; DeCasper & Fifer, 1980; DeCasper & Spence, 1986; Gervain, 2018; May et al., 2011; Moon et al., 1993, 2012; Partanen et al., 2013). Interestingly, if plasticity in the fetus' auditory system is mainly driven by F_0 variation information, our findings stress the importance of a shared pitch processing mechanism across speech and non-speech auditory domains that can be modulated before birth and assessed after birth through non-invasive FFR recordings.

Timing and rhythmic structure are other important characteristics of both music and speech which are also available to the fetus. However, we overlooked them in the present study as we focused only on F_0 neural representation. Both refer to the temporal organization of acoustic events (i.e., energy changes in the acoustic signal), and are crucial to find structure and meaning in speech and music (Iversen et al., 2008). Musical rhythm perception is associated with phonological awareness (Flaugnacco et al., 2014), with the production of complex syntax and reorganization of grammatical information (Gordon et al., 2015), and with developmental conditions such as dyslexia and developmental language disorders (for a review from an auditory neuroscience perspective, see Goswami, 2022). Moreover, training in temporal processing and rhythmic skills with musical material appears to exert a beneficial effect in children with developmental dyslexia (Flaugnacco et al., 2015). Therefore, future studies on prenatal musical exposure should measure as well the neural representation of musical and speech rhythmic patterns in newborns, and in longitudinal designs, relate them to language development.

The present study suggests potential implications for infants at risk for language development conditions. Children who experience language disorders and clinical conditions with affected linguistic functions exhibit a weaker neural encoding of F_0 (Banai et al., 2009; Basu et al., 2010; Chandrasekaran et al., 2009; Font-Alaminos et al., 2020; Hornickel et al., 2012; King et al., 2002; Lam et al., 2017; Otto-Meyer et al., 2018; Rosenthal, 2020). Moreover, musicians from different ages show, as compared to non-musicians, improved non-vocal musical processing, and crucially, speech processing (Bidelman et al., 2011; Deguchi et al., 2012; Magne et al., 2006; Musacchia et al., 2007; Schön et al., 2004; Thompson et al., 2003; Wong et al., 2007). Thus, prenatal interventions based on musical stimulation could prove beneficial to ameliorate future language conditions. However, much basic research is needed before designing any evidence-based intervention. Our results link prenatal musical exposure to F_0 neural encoding, but do not provide any causal explanation nor detail on the most relevant constituents of musical exposure. For instance, the type of music that the mother and the fetus are exposed to, and its intensity, are both important factors in determining the impact of the exposure on speech encoding abilities and their general well-being (Gerhardt & Abrams, 2000; Wright et al., 2022). Musical features such as tempo, meter, melodic frequency range, musical notes, syllabic contour and presence or absence of singing differ across music genres (Teie, 2016), and are based on acoustic features (i.e., pitch, intensity, timing…) that are readily available to the fetus despite the low-pass filter characteristics of the womb. The relative impact of these variables on fetal hearing development is currently unknown. Unfortunately, although we collected information about musical genres that the mothers who participated in this study mostly heard or sung, the small size of the sample precludes us to consider this intriguing variable. Thus, longitudinal studies controlling for the amount, intensity and genre/characteristics of musical exposure such as timing, assessing F_0 neural encoding at birth and at several developmental stages, and relating these variables to measures of language acquisition and brain development (as the critical developmental windows for neuroplasticity already start in utero (Gilmore et al., 2018)) should provide the needed evidence to adequately inform music-based interventions. Also, future longitudinal studies should also take into account the prenatal exposure to infant-directed speech, as its acoustic characteristics, especially regarding pitch, resemble those of music (Hilton et al., 2022).

Moreover, music-based interventions have extensively been used in numerous conditions of neurodevelopmental risk at neonatal intensive care units (NICU), especially in preterm newborns (Chorna et al., 2018; Lordier et al., 2019; Olischar et al., 2011; Palazzi et al., 2021; Partanen et al., 2022; Standley, 2012), proving their effectiveness on fetal and neonatal well-being, and in addition and reciprocally, on the mother's comfort (Brillo et al., 2021; Çatalgöl & Ceber Turfan, 2021; García González et al., 2017; He et al., 2021; Poćwierz-Marciniak & Harciarek, 2021). In fetal growth restriction (FGR), an obstetric condition that affects 6%–10% of all deliveries (Marsál, 2002), neural F_0 encoding was found attenuated in neonates right after birth (Ribas-Prats et al., 2022). Since babies at risk of FGR are routinely identified around the third trimester of pregnancy (Melamed et al., 2021), and as observed in the present study, musical exposure during the last trimester of pregnancy can enhance F_0 processing, future studies could aim to disentangle the effectiveness of prenatal musical interventions in this obstetric condition, in line with recent results showing the impact of environmental manipulations on stress reduction and prevention in FGR (Crovetto et al., 2022).

We here interpreted our findings as resulting from neural plasticity mechanisms occurring before birth due to a prenatal exposure to musical stimulation. However, there are alternative viewpoints to consider. First, and in line with fetal music-based interventions, a reduction of maternal and fetus stress due to a higher exposure to music could induce neuroplastic changes in the auditory system. Also, a recent FFR study demonstrated an increased neural representation of sound F_0 in noisy environments in individuals with better musical abilities, despite no musical training (Mankel & Bidelman, 2018). Considering the heritability of some musical abilities, with some genes linked to music perception, singing and music memory (Tan et al., 2014), our results might as well be explained in part by the possibility that mothers more prone to listen to music and sing, give birth to babies with better sound encoding abilities.

Finally, despite being confident about our results due to the above-mentioned reasons, we are fully aware of an existing, important limitation of our study: musical exposure was assessed by a short (approx. 5 min answer time), retrospective questionnaire provided at the time of delivery, with a spoken description of the content of the questionnaire. This poses, at least, two factors not adequately controlled. First, the actual frequency in which mothers listened to music or sung, as we rely on their reports referring to the last trimester of pregnancy. The present study only documented number of days per month of exposure in closed categories (daily; weekly; twice a month; monthly; never) rather than in a continuous fashion. Furthermore, although a minimum period of exposure to music had to occur to be considered as valid, the questionnaire did not address the exact amount of music exposure within a day (e.g., 30 min vs. 3 hours per day). Second, the intensity of the music reaching the womb. Future studies should address these limitations. For instance, by collecting large amounts of data from a maternal diary of musical exposure during the last trimester of pregnancy, which could also inspect the music genre variable, and include an additional musical abilities test (such as PROMS (Law & Zentner, 2012)) to evaluate the putative link between F_0 encoding abilities in newborns and parental musical abilities. Additionally, a controlled experimental design in which the experimenter could define musical exposure (location and intensity of a loudspeaker, distance to the loudspeaker, amount of hours of exposure, etc.), could be implemented. Moreover, since our study used a cross-sectional design, we can only establish a link between the measured prenatal music exposure and F_0 neural encoding. Longitudinal and intervention work are of the essence to determine whether prenatal exposure to music, and no other possible contributing factors, is associated with neuroplasticity in utero and through early development, and its impact on future language development.

5 | CONCLUSION

In conclusion, acknowledging the aforementioned limitations, our findings support the idea that daily musical exposure during the last trimester of pregnancy is associated with enhanced encoding of low-frequency sound components, such as those typical of the fundamental frequency of human speech, that relate to pitch perception. However, future studies should address open questions, such as what acoustic and musical parameters provide greater benefits or what is the actual nature of the neuroplastic changes that the fetus undergoes with musical exposure, before music-based prenatal interventions are implemented to alleviate putative language disorders.

AUTHOR CONTRIBUTIONS

Sonia Arenillas-Alcón: Conceptualization, Methodology, Investigation, Formal analysis, Writing-Original draft, Writing-Review & Editing, Visualization. Teresa Ribas-Prats: Methodology, Investigation. Marta Puertollano: Methodology, Investigation. Alejandro Mondéjar-Segovia: Methodology, Investigation. María Dolores Gómez-Roig: Funding acquisition, Resources. Jordi Costa-Faidella: Conceptualization, Methodology, Writing-Original draft, Writing-Review & Editing, Supervision. Carles Escera: Conceptualization, Methodology, Writing-Review & Editing, Funding acquisition, Resources, Supervision.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study and materials employed are publicly available on OSF (https://osf.io/6avxq/).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX

QUESTIONNAIRE OF MUSICAL EXPOSURE IN PREGNANT WOMEN (English translation)

The following questionnaire aims to evaluate the amount of music your baby has been exposed to during the last trimester of pregnancy. To do so, we ask you to answer the following questions taking into account that all of them refer to musical exposure WITHOUT HEADPHONES. We remind you that any provided information will be treated with absolute confidentiality.

Musical practice

1. Did you play any musical instrument during the last trimester of pregnancy?

Yes / No

If yes, please answer the following questions. If no, continue with question 2.

1.1. Specify which instrument/s you played: _

1.2. Indicate how frequently you played each one of the instruments:

Daily / Weekly / Once every two weeks / Monthly

1.3. What type of music did you usually play? In case you played several musical genres, please enumerate them from most to least practiced (being 1 the one you spent most time playing).

Classical / Pop/rock / Children's songs or lullabies / Other/s: _

2. Did you sing during the last 3 months of pregnancy?

Yes / No

If yes, please answer the following questions. If no, continue with question 2.

2.1. Indicate how frequently you sang:

Daily / Weekly / Once every two weeks / Monthly

2.2. What type of music did you usually sing? In case you sang several musical genres, please enumerate them from most to least practiced (being 1 the one you spent most time singing).

Classical / Pop/rock / Children's songs or lullabies / Other/s: _

Musical exposure

Please, remember we ask you to answer the following questions taking into account the last trimester of your pregnancy.

3. Did you listen to music with speakers (WITHOUT headphones) during the last 3 months?

Yes / No

3.1. Indicate how frequently you listened to music WITHOUT headphones.

Daily / Weekly / Once every two weeks / Monthly

3.2. What type of music did you usually listen to? In case you listened to several musical genres, please enumerate them from most to least listened (being 1 the one you spent most time listening to).

Classical / Pop/rock / Children's songs or lullabies / Other/s: _