Understanding variability in newborns' NIRS data: the impact of birth weight and gestational age on infants' speech perception abilities

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Abstract (max. 200 words)

Significance: Language acquisition is a complex process already influenced by prenatal neural development and auditory experiences. From the third trimester fetuses perceive sounds already influencing the fetal brain.

Aim: The study investigates how the length of intrauterine language exposure, indexed by gestational age (GA), and overall maturation, indexed by birth weight (BW), affect newborns' brain activations to linguistic stimuli.

Approach: Data from 14 near-infrared spectroscopy (NIRS) studies testing responses to different auditory sound patterns in 192 1-4-day-old newborns were pooled together and analyzed to assess the impact of GA and BW on changes in oxygenated (HbO) and deoxygenated hemoglobin (HbR).

Results: Results showed that, for HbO, activations in both considered conditions were larger in the Temporal than in the Frontal areas, irrespective of BW or GA. For HbR, increased BW led to more positive, thus less strong, activations in the frontal and more negative, thus stronger, activations in the temporal regions of the left hemisphere, while in the right hemisphere the opposite pattern was observed. Besides, when considering effect sizes reflecting discrimination abilities, these were more strongly associated with BW in the frontal regions, while in the temporal regions they were more strongly associated to GA. Critically, HbR was overall more strongly correlated with prenatal experience and maturation than HbO, challenging the exclusive use of HbO in newborn studies.

Conclusions: The findings suggest a differential impact of BW and GA on brain activations, reflecting their roles in biological maturation and auditory discrimination, respectively. Overall, the study suggests that both the length of prenatal experience and maturation play significant roles in shaping newborns' hemodynamic responses.

Keywords: newborns, perinatal physiological measures, prenatal language experience, hemodynamic response, NIRS.

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1 Introduction

1.1 Measuring Neural Responses in Newborns: Near-Infrared Spectroscopy

Functional near-infrared spectroscopy (fNIRS) is a particularly useful technique to measure newborns' brain responses, specifically the changes in the concentrations of oxygenated and deoxygenated hemoglobin accompanying focal neural activation, underlying complex cognitive

processes, such as language acquisition. Therefore, it can enhance the understanding of perceptual and cognitive abilities that are already in place at birth. It is indeed a method of choice for studying newborns and very young infants' abilities by non-invasively measuring changes in the hemodynamic response of the infant brain[1]. Newborns and infants have thinner skin, skull and minimal hair, allowing near-infrared light to penetrate deeper into the cortex and reducing noise and artifacts by promoting better contact between the head and the sensors[1–5]. For these reasons, NIRS is a suitable technique for brain imaging in developmental populations.

The increasing use of fNIRS in developmental research greatly enhances the possibility to study brain responses to sensory stimuli from the earliest stages of life. Yet, the developmental trajectory of the shape of the hemodynamic response function (HRF) is not fully understood, in particular in the youngest infants when the brain and the neurovascular coupling are the least mature. A critical unanswered question is how the early HRF is shaped by factors such as maturation and prenatal learning. In newborn infants, individual differences in gestational age and birth weight are particularly important, as they are directly associated with overall brain development, functional maturation and later developmental outcomes. Indeed, even within typical development, gestational age and birth weight span a large range. Understanding how these factors influence the HRF is essential for interpreting NIRS data and drawing accurate conclusions about early neural functioning and development. Therefore, the current study sets out to investigate the influence of birth weight and gestational age on brain hemodynamics in a variety of auditory and speech perception tasks, many of which have been shown to be robust and replicable[6], in 1-5-day-old newborn infants.

1.2 The Effects of Maturation and Learning

When investigating how the above-mentioned perinatal physiological factors influence newborns' brain responses, speech perception is a highly relevant cognitive ability that can be used as a model. The auditory system becomes functional around the 18-24th week of gestation, when the cochlea and the peripheral structures of the auditory system establish neural connections with the relevant subcortical and cortical areas[7,8]. Thus, fetuses gradually start hearing sounds that are present in the womb. These include the mother's heartbeat, blood flow and breathing, digestive noises, the sounds generated by the mother's movements, the mother's speech and loud external sounds[9–11]. These sounds are filtered by the maternal tissues and the amniotic fluid, so they have different acoustic characteristics inside the womb than outside.

The mother's voice reaches the fetus in different ways, as it's transmitted through air when coming from the outside, while through the inside it's transmitted more directly through tissues and fluids, as well as in the form of vibrations, so it reaches the fetus more effectively than many other sounds[10]. So, speech and language are some of the primary sounds a fetus encounters during the intrauterine period. This makes newborns' perception of speech sounds particularly relevant when testing the effects of experience and learning on the developing brain. As gestational age determines the duration of prenatal exposure, it is a useful proxy for assessing the impact of experience on the HRF and on NIRS measures of early linguistic abilities. As for birth weight, it is a commonly used indicator of overall prenatal health and maturation, and the single best predictor of later developmental outcomes[12–14]. It is thus also important to understand how birth weight may impact HRF. These two important measures, gestational age (GA) and birth weight (BW) are easily available information when testing newborns, and can be the root of significant physiological variability even across full-term, typically developing infants: treating this group as

if it was homogenous may underlie some of the hitherto unexplained variability in developmental studies.

In general, GA and BW are significant predictors of later development due to their impact on early neurodevelopment. Infants born preterm or with low birth weight are at increased risk for developmental impairments, including cognitive, motor, and language deficits[12]. Children born very preterm or with very low birth weight often perform lower in expressive and receptive language skills compared to their full-term peers, highlighting the importance of the last trimester for critical neurodevelopmental processes[15–17]. This period is crucial for brain growth, and low birth weight is associated with reduced volumes of cortical gray and white matter essential for cognitive functions[16]. The additional time for growth and development in utero supports better brain maturation, which is reflected in better cognitive performance and fewer developmental delays[18]. The lung also begins its final maturational stage at about 36 weeks of gestation[19] contributing to more mature neurovascular coupling, brain oxygenation and HRF in older full-term newborns[19,20]. Infants with longer gestational age and higher birth weight, even within the full-term norm (37–42 weeks), thus benefit from extended prenatal maturation and exposure to linguistic stimuli, enhancing overall brain development. These findings underscore the importance of a full-term, nurturing prenatal environment.

From a neurobiological perspective, while the adult brain's language system, primarily involving the superior temporal, inferior frontal, and parietal lobes in the left hemisphere, is well-characterized [21,22], its developmental trajectory in the infant brain is much less well understood.

Existing studies have established that the fetus can learn from the prenatally heard linguistic input, which already modifies newborns' auditory sensitivities and their neural correlates. fNIRS studies revealed that the brain's specialization for language begins before birth, shaped

significantly by prenatal auditory experience. Newborns already exhibit stronger and/or more lateralized neural activity in the left temporal and frontal regions in response to native language stimuli[23–27] than to unfamiliar ones, which elicit more bilateral and more reduced responses[24]. This suggests that prenatal exposure drives the brain specialization for language, which then induces long-term changes in neural dynamics. EEG studies demonstrate that hearing their native language elevates newborns' neural activity during and after stimulation, with enhanced long-range temporal correlations in the theta band (4-8 Hz)[28,29]. This effect is absent when infants are exposed to rhythmically unfamiliar languages, indicating that the rhythm and auditory structure of the native language shape neural dynamics[28,29].

In summary, language plays a crucial role in shaping newborns' cognitive and neurodevelopmental pathways and their brain activations. The early exposure to speech and language influences auditory cortex development, establishes memory traces, and facilitates neural tuning. Understanding how perinatal factors, such as GA and BW, affect these processes provides valuable insights into early brain organization and developmental trajectories.

1.3 Current study

The current study aims to investigate the effects of perinatal factors on newborns' NIRS responses to speech-like and other sound stimuli with a meta-analytic approach pooling together data from 14 published and unpublished newborn fNIRS studies. In these studies, infants are presented with two or more conditions of different auditory stimuli, testing newborns' abilities to discriminate between the different sounds.

In our study, we hypothesize a positive correlation between newborns' GA/BW and their hemodynamic responses. Specifically, we correlated two different NIRS measures with the perinatal variables: (i) the absolute magnitude of the NIRS response, i.e. the size of the NIRS

response in a given experimental condition compared to baseline and (ii) the differential NIRS response, i.e. the difference between the responses to two conditions. We assume that the absolute magnitude of an infant's NIRS response is more closely related to the maturity of the infant's overall hemodynamic activity and neurovascular coupling, while the differential response between two conditions represents the infant's auditory discrimination ability and perceptual sensitivity and may thus be more specifically linguistic. Under this hypothesis, if gestational age and/or birth weight correlate with the overall magnitude of the NIRS response, it will be interpreted as a maturational effect, while if correlations are found between gestational age and/or birth weight and discrimination abilities, then more likely they are not driven by maturation alone, but also by the length of prenatal experience. We acknowledge, however, that maturation and prenatal experience are related to one another, and cannot completely be unconfounded in the studies included in our analysis, as they did not explicitly manipulate these factors. We simply use this approach as a suggestive interpretative framework.

2 Materials and Methods

2.1 Data

2.1.1 Studies

Data was assembled from 14 published and unpublished NIRS studies conducted in two different laboratories. The criteria for inclusion were (1) the examination of linguistic abilities of typically developing newborns (2) not older than 7 days (3) with the usage of NIRS brain imaging technique and (4) the availability of the infants' gestational age and birth weight. In the case of papers that included more than one study the individual experiments were considered as separate studies. This way, in the current research 14 studies were considered from the published papers of Abboub et

al., 2016 [30]; Bouchon et al., 2015 [31]; Martinez-Alvarez et al., 2023 [32]; and Benavides & Gervain, 2017 [33], from the PhD dissertation of Bouchon, 2014 [34]; and from an unpublished paper by the members of the BabyLab of the University of Padua, Marino et al. [35], which is currently under review. No other unpublished study that could be relevant is known. Overall, the included studies comprised data from 192 newborns, aged 1-4 days, tested in two different countries, Italy and France. Table 1 provides information about the individual sample sizes and descriptive information of each study. For further details, we refer the reader to each study's original publication.

All studies tested newborn infants' brain responses to different auditory or linguistic stimuli in a variety of tasks such as rule learning, prosodic grouping and the detection of prosodic violations. All studies used similar methodology testing solely auditory stimulation, with similar NIRS devices, optode configurations and experimental designs, which made them comparable.

Table 1. List of studies included in the current research.

ID	Study	Publication	Condition 1	Condition 2	Language	Modality of input	Type of input	Sample size	Lab
1	1-0m-FrenchProsody-ItaBabies	Marino et al. (under review)	French Standard	French Deviant	Italian	Auditory	Linguistic	25	Padua
2	2-AAB-ABC-0m-Speech-ReplicationNIRx	Bouchon 2014 (PhD thesis)	ABC	AAB	French	Auditory	Linguistic	24	Paris
3	3-ABB-ABC-0m-Speech-CV	Bouchon et al. 2015	ABC	ABB	French	Auditory	Linguistic	24	Paris
4	4-ProsodicGrouping-0m-Exp1_Duration-R=I	Abboub et al. 2016	No contrast	Iambic	French	Auditory	Linguistic	18	Paris
5	5-ProsodicGrouping-0m-Exp1_Duration-R=T	Abboub et al. 2016	No contrast	Trochaic	French	Auditory	Linguistic	18	Paris
6	6-ProsodicGrouping-0m-Exp2_Intensity-R=I	Abboub et al. 2016	No contrast	Iambic	French	Auditory	Linguistic	18	Paris
7	7-ProsodicGrouping-0m-Exp2_Intensity-R=T	Abboub et al. 2016	No contrast	Trochaic	French	Auditory	Linguistic	18	Paris
8	8-ProsodicGrouping-0m-Exp3_PitchMonolingual-R=I	Abboub et al. 2016	No contrast	Iambic	French	Auditory	Linguistic	18	Paris
9	9-ProsodicGrouping-0m-Exp3_PitchMonolingual-R=T	Abboub et al. 2016	No contrast	Trochaic	French	Auditory	Linguistic	18	Paris
10	10-ProsodicGrouping-0m-Exp4_PitchBilingual-R=I	Abboub et al. 2016	No contrast	Iambic	French	Auditory	Linguistic	18	Paris
11	11-ProsodicGrouping-0m-Exp4_PitchBilingual-R=T	Abboub et al. 2016	No contrast	Trochaic	French	Auditory	Linguistic	18	Paris
12	12-ProsodicViolation-0m-Martinezetal2023	Martinez et al. 2023	Standard	Deviant	French	Auditory	Linguistic	25	Paris
13	13-ProsodicViolation-0m-Benavides&Gervain2017-Exp1	Benavides & Gervain 2017	Standard	Deviant	French	Auditory	Linguistic	20	Paris
14	14-ProsodicViolation-0m-Benavides&Gervain2017-Exp2	Benavides & Gervain 2017	Standard	Deviant	French	Auditory	Linguistic	20	Paris

2.1.2 Materials

The studies examined different auditory and speech perception abilities included rule learning, prosodic grouping, and the detection of prosodic violations. Accordingly, the studies used different auditory and speech stimuli. The studies that tested rule learning compared repetition-based (e.g. ABB: "mubaba", "penana" etc.) and diversity-based (ABC: "mubage", "penaku" etc.) regularities [31,34]. The prosodic grouping study tested sequences of pure tone stimuli that were either consistent or inconsistent with the pitch, durational or intensity contrasts found in the prosody of the newborns' native language [30] . Studies assessing the detection of prosodic violations used word sequences with well-formed or ill-formed prosodic contours [32,33,35].

For each study, activations and effect sizes were computed for three comparisons of interest: (1) Condition 1 versus the zero baseline, we designed as Condition 1 the less complex, less marked condition where lower activations were expected, i.e. the non-repetition / no contrast / control condition; (2) Condition 2 versus the zero baseline, Condition 2 being the experimental / repetition condition, and (3) Condition 2 versus Condition 1 [6].

2.1.3 Procedure

In all studies, newborns were tested using a NIRx NIRS device (Figure 1, Panel A) while auditory linguistic stimuli were presented via loudspeakers.

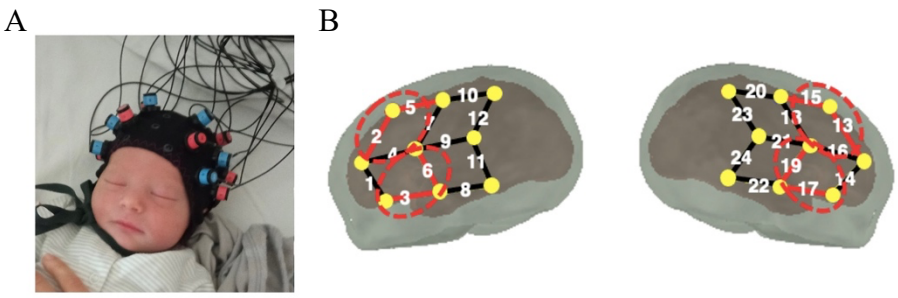

Figure 1. Panel A: NIRS headgear on a newborn. Panel B: Optode arrangement employed in the studies included 8 or 10 sources and 8 detectors, forming a total of 20 or 24 channels. The region of interest that this study focused on are the left temporal lobe, comprising channels 3 and 6; the left frontal lobe, comprising channels 2 and 5; the right temporal lobe, formed by channels 17 and 19; and the right frontal lobe, formed by channels 13 and 15. The relevant ROIs are highlighted in red.

Eight to ten sources and eight detectors were positioned on newborns' head bilaterally, with a source – detector distance of 2.5 to 3 cm, creating 10 to 12 channels on each hemisphere. Channel localization and numbering were consistent across the studies (Figure 1, Panel B). The exact anatomical localization of the resulting array is detailed in [30]. As the left temporal and frontal regions are critical for auditory and speech processing already at birth [23,33,36–38], the analyses focused on the left temporal lobe, defined as the cluster of channels 3 and 6, and the left inferior frontal lobe formed by channels 2 and 5 as well as the analogous right hemispheric regions (right temporal lobe: channels 17 and 19, right inferior frontal lobe: channels 13 and 15; Figure 1, Panel B). We opted to use predetermined, anatomically defined ROIs, as the areas responsible for auditory and speech processing in newborns are well known, and because all studies used the same headgear configuration, resulting in comparable channel configurations.

2.2 Data Analysis

2.2.1 NIRS preprocessing

NIRS data was pre-processed in the same way as in the original publications, which was similar across most studies. Briefly, light intensities were converted to optical densities, then to hemoglobin concentration changes using the modified Beer-Lambert Law with the absorption coefficients µa, mm-1 x mM-1: µa(HbO, 760 nm) = 0.1496, µa(HbO, 850 nm) = 0.2526, µa(HbR,

760 nm) = 0.3865 and μa(HbR, 850 nm) = 0.1798. The product of the optical pathlength and the differential pathlength factor was set to 1, resulting in concentration changes being expressed in mM x mm. A bandpass filter between 0.01 and 0.7 or 1 Hz – depending on the considered research paper – was applied to concentration changes using a *fft* digital filter. Then, as illustrated in [39], blocks of single-trial data were rejected if they contained motion artifacts or if the light intensity reached the saturation value, with motion artifacts defined as signal changes larger than 0.1 mM x mm over 0.2 sec. The artifact detection and trial rejection procedure were performed independently for each channel, and channels with less than at least two valid blocks were discarded from the analysis. Trial inclusion rate for each study ranged between 50 and 72% (across studies, mean: 60%, std: 8%). Finally, for the non-rejected blocks, a baseline was linearly fit between the mean of the 5 seconds preceding the onset of the block and the mean of the 5 seconds preceding the onset of the next one. Blocks were then averaged within each infant to obtain channel-wise block-averages for each condition as well as across infants to obtain grand averages. This pre-processing routine has been shown to yield an accurate recovery of the infant hemodynamic response [6,39].

2.2.2 Activation values and effect sizes

Individual trial data was employed to compute infant-level activation values, i.e. response magnitude, for the comparison of each condition against the zero baseline (Condition 1 vs. 0; Condition 2 vs. 0) as well as infant-level effect sizes, i.e. discrimination ability, for the between-condition comparisons (Condition 1 vs. Condition 2). Activation values for each condition were computed as the average of each non-rejected trial response within a time window starting at the onset of stimulation and lasting up to 15 seconds after the stimulus' end, thus the window length differed between studies as they employed different window. Obtained activation values, for each trial, were then averaged for each newborn, channel and hemoglobin component if at least two

valid, non-rejected trials for that baby/channel were available, otherwise that channel was rejected. For each newborn, activation values were then further averaged within the four ROIs.

Infant-level effect sizes of between-condition differential responses, i.e. discrimination, were obtained by calculating the difference between the average activations in Conditions 2 and 1 and dividing the result by the standard deviation of trial-wise activation values from both conditions (Figure 2). Then, for each baby and hemoglobin component, channel-wise effect sizes were averaged within the four ROIs.

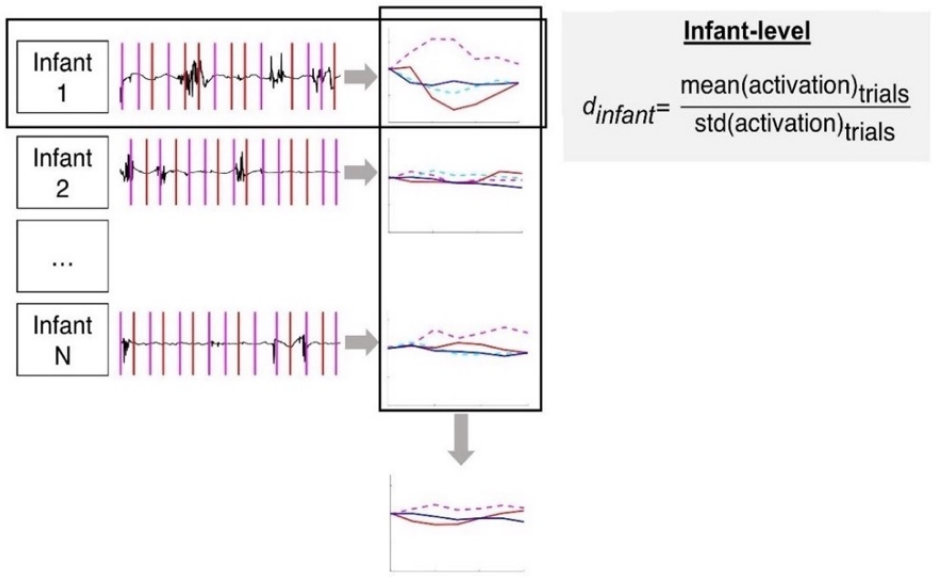

Figure 2. Schematic representation of infant-level effect size calculation. Activation refers to the Condition 1 vs. 0, Condition 2 vs. 0, and Condition 2 vs. Condition 1 contrasts computed as the average of the hemodynamic response function along its time course. Magenta and cyan indicate repetition trials (HbO and HbR, respectively), and red and blue indicate non-repetition trials (HbO and HbR, respectively) [6].

2.2.3 Extraction of individual gestational age and birth weight data

For each newborn, information about gender at birth, date of birth, date of test, gestational age and birth weight were collected. For the purposes of all subsequent analyses, birth weight was

244 registered in grams, gestational age in days. Some of this information was missing for 90
245 newborns, who thus needed to be excluded from the overall sample of 282 newborns, resulting in
246 a final dataset of 192 participants.

247 Gestational ages ranged from 255 to 298 days (M = 277.9, SD = 8.44), birth weight from 2235
248 to 4535 grams (M = 3308, SD = 387.25). As expected, there was a statistically significant
249 correlation between the two (rho = 0.3968, $p < 0.001$).

250 *2.2.4 Statistical analysis*

251 *2.2.4.1 Linear Mixed-Effects Modelling.* Linear mixed-effects models with fixed factors Gestational
252 Age, Birth Weight, ROI (temporal / frontal) and Hemisphere (LH/RH) were run separately over
253 the three dependent variables of interest (Condition 1 activation, Condition 2 activation, effect size
254 of the Condition 2 vs. Condition 1 differential response) using oxygenated and deoxygenated
255 hemoglobin (HbO and HbR, respectively). The random effects structure included random
256 intercepts for subject, study and lab. In case of failure to converge, the structure was gradually
257 simplified by removing the random intercept for lab first, followed by study, and then subject.
258 Fixed effects were added one at a time to the model, and the resulting models were compared. The
259 model with the lowest Akaike Information Criterion (AIC) value was selected as the best-fitting
260 model.

261 **3. Results**

262 *3.1 Oxygenated hemoglobin (HbO)*

263 For Condition 1 activations, the best fitting model included a random intercept for Subject and
264 fixed effects for Birth Weight and ROI. It yielded a significant main effect of ROI ($F(1, 695) = 7.73$, $p < 0.01$); the post-hoc comparison showed this was due to larger activations in the Temporal
265

area compared to the Frontal (mean difference = 0.00727, SE= 0.00262, t(690) = 2.778, $p < 0.01$, Figure 3).

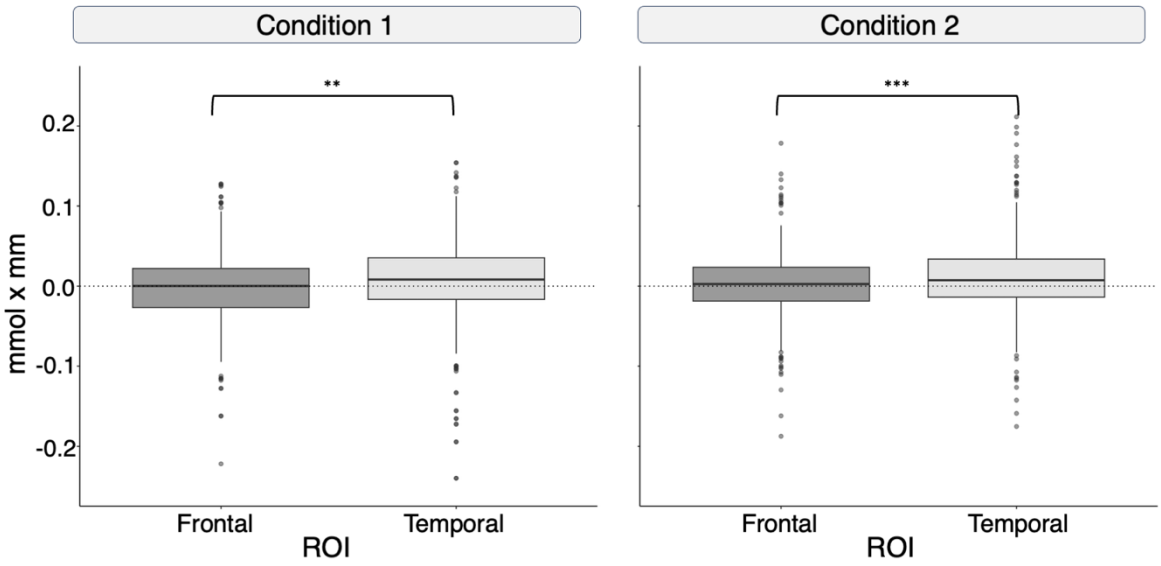

Figure 3: Distributions of HbO activations elicited by Condition 1 and Condition2 in the two ROIs, the Temporal and Frontal: for both, the analysis showed a main effect of ROI, driven by larger activations in the Temporal area compared to the Frontal area.

For Condition 2 activations, the best-fitting model included a random intercept for Subject and fixed effects for Birth Weight and ROI. This model also, similarly to that used for Condition 1, yielded a significant main effect of ROI ($F(1, 702)= 14.1$, $p < 0.001$), carried by greater activations in the Temporal area compared to the Frontal (mean difference = 0.00978, SE= 0.00261, $t(696)= 3.750$, $p< 0.001$, Figure 3).

For the effect sizes of the differential activation between Conditions 1 and 2, the best fitting model included a random intercept for Subject, and fixed effects of Gestational Age and ROI, but yielded no significant effects or interactions.

3.2 Deoxygenated hemoglobin (HbR)

For Condition 1 activations, the best fitting model included a random intercept for Subject and fixed effects for Birth Weight, ROI and Hemisphere. The model showed a significant interaction between ROI and Hemisphere ($F(1, 647) = 8.79$, $p < 0.01$), which did not hold after post-hoc comparisons, as well as a significant interaction between BW, ROI and Hemisphere ($F(1, 648) = 9.14$, $p < 0.01$) (Figure 4).

A post-hoc test on the slopes of activations versus Birth Weight was executed by conditioning within-ROIs slopes on Hemisphere, and another one conditioning within-Hemisphere slopes on ROI. It showed that the interaction was driven by a difference between the slopes in the temporal and in the frontal areas in the LH (mean difference Frontal-Temporal= 1.33e-05, $t(695) = 3.02$, $p < 0.01$): activations in the LH became more positive, i.e. less strong, with increasing BW in the frontal areas, but decreased, i.e. became stronger, in the temporal areas. The opposite was found in the RH, with a positive slope for the temporal (slope= 1.13e-06) and a negative one for the frontal regions (slope = -3.99e-06), although the direct post-hoc comparison within the RH did not yield a statistically significant difference. Conversely, the post-hoc analysis revealed a positive LH-RH difference between slopes in the Frontal areas (mean difference= 1.06e-05, SE= 4.64e-06, $t(660)= 2.27$, $p < 0.05$), and a negative LH-RH difference between slopes in the Temporal area (mean difference = -7.80e-06, SE= 3.97e-06, $t(654)= -1.96$, $p < 0.05$): the Temporal and Frontal areas display an opposite lateralization pattern of the slopes between activations and birth weight, with activations becoming stronger with increasing BW in the left temporal and right frontal areas, and becoming weaker with increasing BW in the right temporal and left frontal areas. These results were confirmed by correlations, which demonstrated a significant negative correlation between

activations and birth weight in the left temporal area (rho = -0.1265, $p < 0.05$) and in the right frontal area (rho = -0.1468, $p < 0.05$).

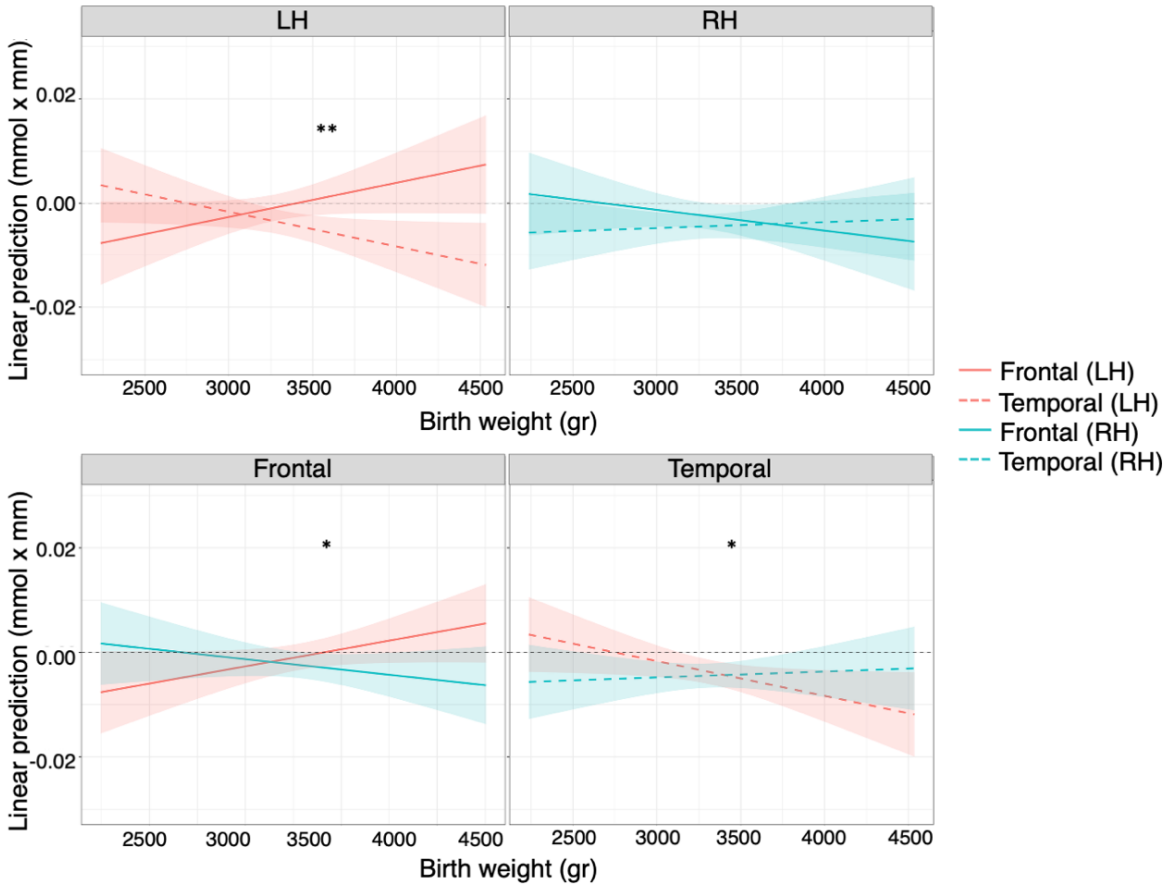

Figure 4. Significant interaction between ROI, Hemisphere and BW for Condition 1 activations obtained from HbR data. The figure on the top shows a significantly more positive slope for the frontal than for the temporal areas in the LH, with no differences between the ROIs in the RH. At the bottom, significant differences can be seen between LH and RH in both the frontal ($p < 0.05$) and temporal regions ($p < 0.05$).

For Condition 2 activations, the best fitting model included random intercepts for Subject and Experiment and fixed effects of Birth Weight and ROI but yielded no significant effects or interactions.

For the effect sizes of the differential response between Conditions 1 and 2, the best fitting model included a random intercept for Subject and Experiment and fixed effects for Birth Weight,

ROI, Hemisphere and Gestational Age. The model yielded a significant main effect of ROI ($F(1, 519) = 7.94$, $p < 0.01$), a significant BW X ROI interaction ($F(1, 549) = 11.50$, $p < 0.001$), and a significant ROI X GA interaction ($F(1, 523) = 12.22$, $p < 0.001$). The significant main effect of ROI was due to more positive effect sizes, i.e. less strong effects in the temporal than in the frontal areas, which however did not hold significance following post-hoc analyses. For the BW x ROI interaction, the post-hoc analysis revealed that the estimated slopes were found to be significantly more negative in the frontal than in the temporal ROIs with increasing birth weight (mean difference Frontal-Temporal= -0.000332, SE= 9.84e-05, $t(552) = -3.37$, $p < 0.001$) (Figure 5a). For GA X ROI, slopes were significantly more negative in the temporal than in the frontal ROIs with increasing gestational age (mean difference Frontal – Temporal= 0.0147, SE= 0.00423, $t(526) = 3.48$, $p < 0.001$) (Figure 5b).

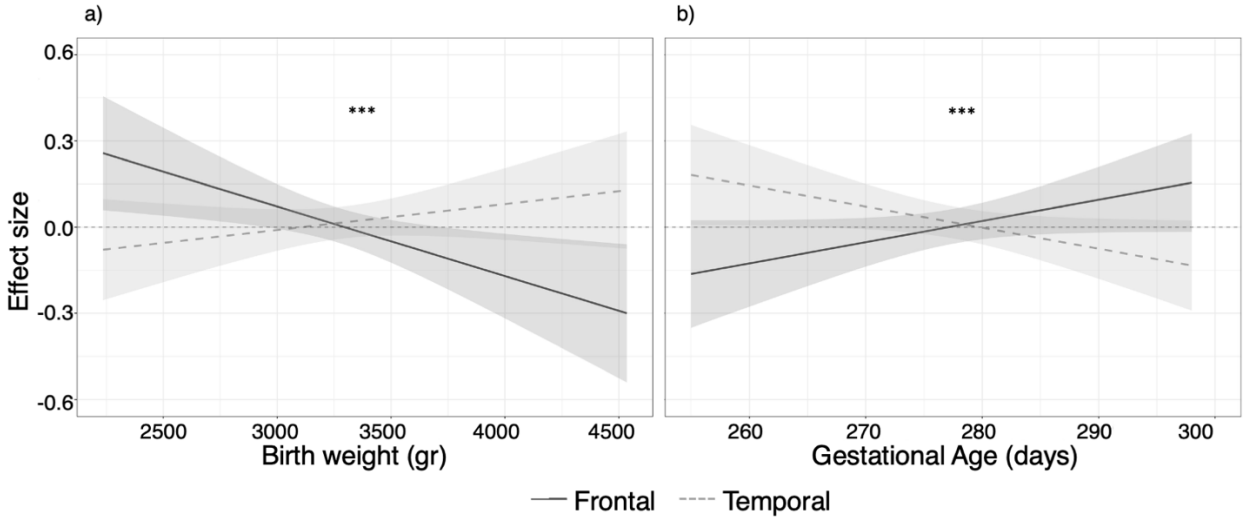

Figure 5. a) The interaction of BW and ROI showing a significantly more positive slope in the temporal than in the frontal areas ($p < 0.001$) for the effect sizes of the differential response between Conditions 1 and 2 in HbR. **b)** The

interaction of GA and ROI yielding a significantly more positive slope for the frontal than for the temporal areas ($p < 0.001$) for the effect sizes of the differential response between Conditions 1 and 2 in HbR.

4 Discussion

4.1 Effects of Birth Weight and Gestational Age on Brain Responses to Language

In the case of oxygenated hemoglobin activations to both Condition 1, i.e. the control, less complex auditory/linguistic condition, and Condition 2, i.e. the experimental condition, the analysis found no significant correlations with either BW or GA, but rather larger overall activations in the bilateral Temporal areas compared to the Frontal ones, a finding with ample convergence with the literature on newborns' early perceptual and linguistic abilities [27,36,40].

Interestingly, measures derived from HbR presented portray a more diversified account in terms of their relation with the considered perinatal factors. Condition 1 activations presented a different relation with Birth Weight in the temporal and frontal areas in the LH, which was less strongly observable in the RH. Specifically, HbR activations become more negative with increased BW significantly more in the temporal than in the frontal areas of the LH. This differential maturational pattern of the temporal and frontal regions is inverted between the two hemispheres, with activations getting stronger with increasing birth weight in the temporal as compared to the frontal area in the LH, and in the frontal as compared to the temporal area in the RH. These results were confirmed by showing significant negative correlations between activation and birth weight in the temporal left hemisphere and in the frontal right hemisphere. This hemispheric difference may be due to the LH's greater involvement in auditory and linguistic abilities, while the pattern found in the RH could reflect more generic maturational mechanisms.

This interpretation is further supported by results found on the effect sizes of the differential HbR responses between the two conditions, namely the discrimination task: in fact, they revealed statistically significant interactions between ROI and BW, and ROI and GA ,with increasing BW associated to stronger activations in the bilateral frontal areas, while increasing GA associated to stronger activations in the bilateral temporal areas. Following our interpretative proposal that that GA indicates the length of the prenatal experience, while BW represents the extent of biological maturation, these findings suggest that GA exhibits a stronger effect on a perceptual, auditory level affecting auditory discrimination mainly, hence it's significantly associated to stronger activations of the temporal areas where the auditory cortex is located. On the other hand, BW is suggested to reflect the maturation and development of more abstract, linguistic brain structures, i.e. Broca's area, located in the frontal region.

4.2 Implications

Most findings of the study highlight the effects of birth weight, but not gestational age. This may simply be a specificity of the dataset, as the two variables are correlated, and both reflect prenatal experience and maturation. However, it may also be that biological growth, indexed by birth weight is more important than the length of intrauterine experience. Gestational age may play a weaker role in the current context as the tested dataset included only full-term newborns. These infants reached at least 37 weeks of gestation, therefore they benefitted from prenatal exposure for the most part of the critical third trimester. The negligible effect of gestational age within the full-term range suggests that by 37 weeks of gestation the foundational aspects of prenatal learning already occurred. In contrast, birth weight may still reflect significant individual differences, thus, within a full-term sample, birth weight may serve as a more sensitive indicator of early

neurodevelopmental outcomes than gestational age. Indeed, birth weight is the most important predictor of later developmental outcomes for many cognitive abilities as well as pathologies [12].

Surprisingly, the results were stronger for deoxygenated hemoglobin compared to oxygenated hemoglobin. This is particularly relevant as many infant studies rely mostly on HbO. The reason for stronger effects in HbR may be that while HbO is more sensitive to the cognitive manipulations measured in typical experiments with newborns, HbR is more sensitive to overall biological maturation, growth and experience. Indeed, HbR correlates better with the BOLD response of MRI [41–43]. HbR is considered to exhibit a physiologically more robust response, therefore, it may also show a stronger correlation with prenatal experience and maturation than HbO. Based on these results, we suggest that HbR responses also be reported and investigated in fNIRS studies with newborns.

4.3 Limitations

In the current research, some limitations need to be acknowledged. The primary limitation arises from the diversity of the analyzed studies. Although, all of them used a consistent methodology and experimental design, due to the different targeted linguistic abilities the studies used different types of stimulation. This variability could affect the comparability of the results; however it may also contribute to the generalizability of the findings. The neural response to speech, on average, is robustly elicited across the dataset providing a solid foundation for investigating how individual level characteristics modulate such responses, but the diversity of

stimuli and language abilities allowed us to examine systematic responses and see how early neural processes may operate across a range of linguistic stimuli.

A further limitation stems from the characteristics of the sample. The studies included only naturally conceived newborns. In natural, non-medically assisted pregnancies gestational age is an estimate, which may lack precision, potentially varying by 1-2 weeks. This imprecision could influence the estimate of the developmental stage of the newborns at the time of the study, thereby affecting the interpretation of its significance with respect to the linguistic abilities and maturation.

4.4 Future Directions

This study highlights the different roles of birth weight and gestational age in shaping newborns' neural responses to language, suggesting several directions for future research. First, examining preterm and low birth weight newborns could further enhance the understanding of the effects of the length of prenatal experience and biological maturation. Besides, longitudinal studies could examine the developmental outcomes in regard of the examined physiological measures at birth. Finally, generalizability of these findings may be tested by expanding the research to more diverse linguistic and cultural contexts.

Importantly, given the stronger effects observed in HbR, the current study suggests reporting both HbO and HbR results in future newborn brain studies.

5 Conclusions

The increasing use of fNIRS in developmental research affords the opportunity to investigate brain responses in several perceptual domains from the earliest stages of life. Yet, a critical unanswered issue is whether and how the shape of the early HRF is modulated by perinatal factors such as birth weight (BW) and gestational age (GA). We found that the magnitude of NIRS de-

oxygenated responses (HbR) was significantly associated with BW in the left temporal and right frontal areas. Moreover, when investigating newborns' discrimination abilities, we found that increasing BW was associated to activations getting stronger in the bilateral frontal areas; these findings together support the role of BW in reflecting the extent of biological maturation. Further, increasing GA was associated to activations becoming stronger in the bilateral temporal areas, suggesting that duration of prenatal experience significantly modulates auditory discrimination abilities. Relations with perinatal factors were only significant when using measures derived from the HbR component, indicating its higher sensitivity, compared to HbO, to overall biological maturation and growth.

Understanding how perinatal factors influence newborns' HRF may provide a better understanding of the unexplained variability in developmental studies, thus contributing to achieving a better interpretation of newborns' NIRS data and to drawing more accurate conclusions about early neural functioning and development.

Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

Code, Data and Materials

The code and data presented in this study are available upon reasonable request to the authors.

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Author Contributions

Szeberényi, N: Data collection, Data curation, Formal analysis, Writing – original draft & editing; **Gervain J**: Conceptualization, Methodology, Data curation, Formal analysis, Funding acquisition, Resources, Writing – original draft, review & editing; **Gemignani J:** Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft, review & editing, Funding acquisition.

References

1. J. Gervain et al., "Using functional near-infrared spectroscopy to study the early developing brain: future directions and new challenges," Neurophoton. **10**(02) (2023) [doi:10.1117/1.NPh.10.2.023519].
2. R. N. Aslin and J. Mehler, "Near-infrared spectroscopy for functional studies of brain activity in human infants: Promise, prospects, and challenges," Journal of Biomedical Optics **10**(1), 11009 (2005).
3. S. Lloyd-Fox, A. Blasi, and C. E. Elwell, "Illuminating the developing brain: The past, present and future of functional near infrared spectroscopy," Neuroscience and Biobehavioral Reviews (2009).
4. H. Ayaz et al., "Optical imaging and spectroscopy for the study of the human brain: Status report," Neurophotonics **9**(S2), 24001 (2022).
5. J. Gervain et al., "Near-infrared spectroscopy: A report from the McDonnell infant methodology consortium," Developmental Cognitive Neuroscience **1**(1), 22–46 (2011) [doi:10.1016/j.dcn.2010.07.004].
6. J. Gemignani et al., "Reproducibility of infant fNIRS studies: a meta-analytic approach," Neurophoton. **10**(02) (2023) [doi:10.1117/1.NPh.10.2.023518].
7. J. C. Birnholz and B. R. Benacerraf, "The development of human fetal hearing," Science (New York, N.Y **222**(4623), 516–518 (1983) [doi:10.1126/science.6623091].
8. J. J. Eggermont and J. K. Moore, "Morphological and Functional Development of the Auditory Nervous System," in Human Auditory Development. Springer Handbook of Auditory

Research **42**, L. Werner, R. Fay, and A. Popper, Eds., Springer, New York, NY (2012) [doi:10.1007/978-1-4614-1421-6_3].
9. K. J. Gerhardt and R. M. Abrams, "Fetal exposures to sound and vibroacoustic stimulation," Journal of Perinatology: Official Journal of the California Perinatal Association **20**(8 Pt 2), 21–30 (2000) [doi:10.1038/sj.jp.7200446].
10. G. Dealessandri and M. Vivalda, "The mother's womb acoustic environment: study of the original sounds and replication for pre-term infants," Journal of Physics: Conference Series **1075**(1), 012056, IOP Publishing (2018).
11. J. J. Parga et al., "A description of externally recorded womb sounds in human subjects during gestation," PLoS ONE **13**(5), 0197045 (2018) [doi:10.1371/journal.].
12. M. Hack, N. K. Klein, and H. G. Taylor, "Long-term developmental outcomes of low birth weight infants," The Future of Children **5**(1), 176–196 (1995) [doi:10.2307/1602514].
13. J. D. Boardman et al., "Low birth weight, social factors, and developmental outcomes among children in the United States," Demography **39**(2), 353–368 (2002).
14. M. Cortese, D. Moster, and A. J. Wilcox, "Term birthweight and neurodevelopmental outcomes," Epidemiology (Cambridge, Mass **32**(4), 583–590 (2021) [doi:10.1097/EDE.0000000000001350].
15. N. Barre et al., "Language Abilities in Children Who Were Very Preterm and/or Very Low Birth Weight: A Meta-Analysis," The Journal of Pediatrics **158**(5), 766-774 1 (2011) [doi:10.1016/j.jpeds.2010.10.032].
16. M. Wade et al., "Normal Birth Weight Variation and Children's Neuropsychological Functioning: Links between Language, Executive Functioning, and Theory of Mind," Journal of the International Neuropsychological Society **20**(09), 909–919 (2014) [doi:10.1017/s1355617714000745].
17. A. B. Zerbeto, F. M. Cortelo, and É. B. C. Filho, "Association between gestational age and birth weight on the language development of Brazilian children: a systematic review," Jornal de Pediatria **91**(4), 326–332 (2015) [doi:10.1016/j.jped.2014.11.003].
18. B. R. Vohr, C. Garcia-Coll, and W. Oh, "Language and neurodevelopmental outcome of low-birthweight infants at three years," Developmental Medicine & Child Neurology **31**(5), 582–590 (1989) [doi:10.1111/j.1469-8749.1989.tb04043.x].
19. I. Copland and M. Post, "Lung development and fetal lung growth," Paediatric Respiratory Reviews **5**, 259–264 (2004).
20. A. A. Hislop, J. S. Wigglesworth, and R. Desai, "Alveolar development in the human fetus and infant," Early Human Development **13**(1), 1–11 (1986).
21. A. D. Friederici, "The cortical language circuit: From auditory perception to sentence comprehension," Trends in Cognitive Sciences **16**(5), 262–268 (2012) [doi:10.1016/j.tics.2012.04.001].
22. D. Poeppel, "The neuroanatomic and neurophysiological infrastructure for speech and language," Current Opinion in Neurobiology **28**, 142–149 (2014).
23. M. Peña et al., "Sounds and silence: An optical topography study of language recognition at birth," Proceedings of the National Academy of Sciences of the United States of America **100**(20), 11702–11705 (2003) [doi:10.1073/pnas.1934290100].
24. Y. Sato, Y. Sogabe, and R. Mazuka, "Development of hemispheric specialization for lexical pitch – accent in Japanese infants," Journal of Cognitive Neuroscience (2010).

25. L. May et al., “Language and the newborn brain: Does prenatal language experience shape the neonate neural response to speech?,” Language Sciences **2**, 222 (2011) [doi:10.3389/fpsyg.2011.00222].
26. P. Vannasing et al., “Distinct hemispheric specializations for native and non-native languages in one-day-old newborns identified by fNIRS,” Neuropsychologia **84**, 63–69 (2016) [doi:10.1016/j.neuropsychologia.2016.01.038].
27. L. May et al., “The specificity of the neural response to speech at birth,” Developmental Science **21**(3) (2017) [doi:10.1111/desc.12564/full].
28. B. Mariani et al., “Prenatal experience with language shapes the brain,” Science Advances **9**(47) (2023) [doi:10.1101/2023.05.25.542259].
29. M. C. Ortiz Barajas, R. Guevara, and J. Gervain, “Neural oscillations and speech processing at birth,” iScience **26**(11) (2023).
30. N. Abboub, T. Nazzi, and J. Gervain, “Prosodic grouping at birth,” Brain and Language **162**, 46–59, Elsevier Inc. (2016) [doi:10.1016/j.bandl.2016.08.002].
31. C. Bouchon, T. Nazzi, and J. Gervain, “Hemispheric asymmetries in repetition enhancement and suppression effects in the newborn brain,” PLoS ONE **10**(10), 1–17 (2015) [doi:10.1371/journal.pone.0140160].
32. A. Martinez-Alvarez et al., “Newborns discriminate utterance-level prosodic contours,” Developmental Science **26**(2), 13304 (2023) [doi:10.1111/desc.13304].
33. S. Benavides-Varela and J. Gervain, “Learning word order at birth: A NIRS study,” Developmental Cognitive Neuroscience **25**, 198–208, Elsevier Ltd (2017) [doi:10.1016/j.dcn.2017.03.003].
34. C. Bouchon, “Functional asymmetry between consonants and vowels from birth to 6 months of age - Cerebral imaging and behavioral data,” PhD thesis, Université Paris Cité (2014).
35. C. Marino et al., “Do infants detect prosodic violations in an unfamiliar language at birth?” ((Under review)).
36. J. Gervain et al., “The neonate brain detects speech structure,” Proceedings of the National Academy of Sciences **105**(37), 14222–14227 (2008) [doi:10.1073/pnas.0806530105].
37. J. Gervain, I. Berent, and J. F. Werker, “Binding at birth: The newborn brain detects identity relations and sequential position in speech,” Journal of Cognitive Neuroscience **24**(3), 564–574 (2012) [doi:10.1162/jocn_a_00157].
38. S. Telkemeyer et al., “Sensitivity of newborn auditory cortex to the temporal structure of sounds.,” The Journal of neuroscience : the official journal of the Society for Neuroscience **29**(47), 14726–14733 (2009) [doi:10.1523/JNEUROSCI.1246-09.2009].
39. J. Gemignani and J. Gervain, “Comparing different pre-processing routines for infant fNIRS data,” Developmental Cognitive Neuroscience **48**, 100943 (2021) [doi:10.1016/j.dcn.2021.100943].
40. J. Gemignani and J. Gervain, “Brain responses to repetition-based rule-learning do not exhibit sex differences: an aggregated analysis of infant fNIRS studies,” Sci Rep **14**(1), 2611 (2024) [doi:10.1038/s41598-024-53092-2].
41. S. Punwani et al., “Correlation between absolute deoxyhaemoglobin [dHb] measured by near infrared spectroscopy (NIRS) and absolute R2 as determined by magnetic resonance imaging (MRI,” Advances in Experimental Medicine and Biology **413**, 129–137 (1997).
42. S. Punwani et al., “MRI measurements of cerebral deoxyhaemoglobin con- centration [dHb]—Correlation with near infrared spectroscopy (NIRS,” NMR in Biomedicine **11**, 281–289 (1998).

43. G. Strangman et al., “A quantitative comparison of simultaneous BOLD fMRI and NIRS recordings during functional brain activation,” NeuroImage **17**(2), 719–731 (2002) [doi:10.1016/S1053-8119(02)91227-9].

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Caption List

Fig. 1. Panel A: NIRS headgear on a newborn. Panel B: Optode arrangement employed in the studies included 8 or 10 sources and 8 detectors, forming a total of 20 or 24 channels. The region of interest that this study focused on are the left temporal lobe, comprising channels 3 and 6; the left frontal lobe, comprising channels 2 and 5; the right temporal lobe, formed by channels 17 and 19; and the right frontal lobe, formed by channels 13 and 15. The relevant ROIs are highlighted in red.

Fig. 2. Schematic representation of infant-level effect size calculation. Activation refers to the Condition 1 vs. 0, Condition 2 vs. 0, and Condition 1 vs. Condition 2 contrasts computed as the average of the hemodynamic response function along its time course. Magenta and cyan indicate repetition trials (HbO and HbR, respectively), and red and blue indicate non-repetition trials (HbO and HbR, respectively) (Gemignani et al., 2023).

Fig. 3. Distributions of HbO activations elicited by Condition 1 and Condition2 in the two ROIs, the Temporal and Frontal: for both, the analysis showed a main effect of ROI, driven by larger activations in the Temporal area compared to the Frontal area.

Fig. 4. Significant interaction between ROI, Hemisphere and BW for Condition 1 activations obtained from HbR data. The figure on the top shows a significantly more positive slope for the frontal than for the temporal areas in the LH, with no differences between the ROIs in the RH. At the bottom, significant differences can be seen between LH and RH in both the frontal ($p < 0.05$) and temporal regions ($p < 0.05$).

Fig. 5. The interaction of BW and ROI showing a significantly more positive slope in the temporal than in the frontal areas ($p < 0.001$) for the effect sizes of the differential response between Conditions 1 and 2 in HbR.

607 **Table 1.** List of studies included in the current research.