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Original Article

ORIGINAL ARTICLE

Functional Maps at the Onset of Auditory Inputs in Very Early Preterm Human Neonates

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Abstract

During the last trimester of human gestation, neurons reach their final destination and establish long- and short-distance connections. Due to the difficulties obtaining functional data at this age, the characteristics of the functional architecture at the onset of sensory thalamocortical connectivity in humans remain largely unknown. In particular, it is unknown to what extent responses evoked by an external stimulus are general or already sensitive to certain stimuli. In the present study, we recorded high-density event-related potentials (ERPs) in 19 neonates, tested ten weeks before term (28–32 weeks gestational age (wGA), that is, at an average age of 30 wGA) by means of a syllable discrimination task (i.e., a phonetic change: ba vs. ga; and a voice change: male vs. female voice). We first observed that the syllables elicited 4 peaks with distinct topographies implying a progression of the sensory input along a processing hierarchy; second, repetition induced a decrease in the amplitude (repetition suppression) of these peaks, but their latencies and topographies remained stable; and third, a change of stimulus generated mismatch responses, which were more precisely time-locked to event onset in the case of a phonetic change than in the case of a voice change. A hierarchical and parallel functional architecture is therefore able to process environmental sounds in a timely precise fashion, well before term birth. This elaborate functional architecture at the onset of extrinsic neural activity suggests that specialized areas weakly dependent on the environment are present in the perisylvian region as part of the genetic endowment of the human species.

Key words: brain development, EEG, ERP, language, MMN, premature

Introduction

As in other primates, sensory development starts prenatally in humans and stimulus-dependent responses have been reported from the sixth month of gestation in premature infants (Weitzman and Graziani 1968; Rotteveel et al. 1987; Colonnese et al. 2010) and fetuses (Fulford et al. 2003; Draganova et al. 2005; Jardri et al. 2008). However, functional studies remain scarce at this age due to the difficulties in testing extremely frail infants, who are unable to survive without intensive care after a preterm birth, or who are not readily accessible to imaging techniques in the case of a normal intrauterine pregnancy. Human functional development is therefore often inferred from animal models, mainly rodents, in which the chronology of brain development is very different to that observed in the human species, which is mostly postnatal. Nevertheless, complex cognitive capacities, such as recognition of native language features (Mehler et al. 1988; Moon et al. 2013) and recognition of the mother's voice (Decasper and Fifer 1980), observed in human neonates at term birth suggest the development of a sophisticated cortical organization able to already perform complex and specific learning during pregnancy. Given the importance of language development,

speech learning, and social cognition (which obviously also relies on speech cues) in the human species, the study of the first stages of cortical functional organization should shed light on the neural mechanisms promoting the emergence of these capacities in humans. Furthermore, the long-lasting language difficulties reported in children born prematurely, even in the absence of visible brain lesions (Barde et al. 2012; Van Noort-Van Der Spek et al. 2012), suggest a possible role of the auditory environment during the last weeks of pregnancy in language acquisition.

Human gestation normally lasts 40 wGA, but premature births (before 38 wGA) occur in 9.6% of births (WHO bulletin, 2009) and, as a result of long and intensive care, neonates now commonly survive after a gestational age of 28 wGA, and sometimes as early as 23 wGA. Brain imaging studies in these neonates may provide insight into the initial organization of the human brain. The last trimester of gestation is characterized by the development of the cortical plate and its afferent and efferent connections. Neurons migrate to reach their final destination according to radial and tangential pathways, now identified in postmortem structural magnetic resonance imaging (MRI) in human infants (Kolasinski et al. 2013). A large number of neurons are located in the subplate, creating a hyperintense layer underneath the cortical plate, which is clearly visible on MRI (Corbett-Detig et al. 2011). This layer gradually disappears, but may persist until the end of the first year in frontal regions (Kostovic and Judas 2010). Before sensory systems and brainstem are fully developed, thalamic afferents reach the subplate, but only start to invade the cortical plate at about 28 wGA and then provide the first inputs to the developing cortical circuitry (Kostovic and Judas 2010). Short-range connectivity rapidly expands to complete long-range tracts that have been developing since the second trimester. Neural activity can be recorded on the scalp, but is initially highly discontinuous and asynchronous. Long periods of low voltage activity that may last several tens of seconds are interrupted by bursts of high voltage activity with an amplitude of about 300 μV. Discontinuity decreases, and different states become progressively recognizable from 28–30 wGA consisting of short periods of awakening, quiet and agitated sleep. Awakening becomes more clearly observable around 32–35 wGA, when thalamic generators become able to continuously drive the cortical activity (Lamblin et al. 1999). Frontal sharp waves, delta brushes, theta temporal activities, transiently recorded at definite preterm ages (Wallois 2010), attest the progressive organization, with abrupt changes, of the underlying neural networks.

How does this immature brain react to its environment and is it able to process speech? The primate auditory cortex is organized in several parallel and hierarchical streams, which process different aspects of a sound: its location, intensity, timbre, etc. (Kaas and Hackett 2000; Tian et al. 2001). In the case of speech, recovering the message and the messenger requires complex representations that are progressively elaborated along different functional processing streams located in the superior temporal regions beyond the primary auditory cortex (Wessinger et al. 2001; Dehaene-Lambertz et al. 2006). These streams can be distinguished according to the temporal window of analysis: for phonetic analyses, the very rapid temporal variations of the signal should be taken into account, whereas voice and emotion representations are computed from slower variations of the signal. Differences in temporal sensitivity of the left and right hemispheres have been proposed to be at the origin of the left hemispheric advantage for linguistic processing and of the right for voice and emotion processing (Zatorre and Belin 2001; Boemio et al. 2005; Giraud et al. 2007). How do these pathways develop? Although the hypothesis that the rough cortical specialization is already determined during neuronal multiplication in the embryonic ventricular zone (Rakic 1988; Rakic et al. 2009) is now fairly consensual, the degree of precision of the cortical maps at the onset of neuronal activity in humans needs to be clarified (Sur and Rubenstein 2005).

At term, apart from recognition of their mother's voice (DeCasper and Fifer 1980) and the prosody of their native language (Mehler et al. 1988), neonates also recognize sound-patterns (stories, words, and melodies), which have been repeatedly presented close to the maternal womb during pregnancy (Granier-Deferre et al. 2011; Partanen, Kujala, Naatanen et al. 2013a; Partanen, Kujala, Tervaniemi, et al. 2013b). However, these studies cannot assess when cortical development is sufficient to allow learning (i.e., infants may have learned to recognize these sounds only during the last weeks/days of pregnancy). DeRegnier et al. (2002), using event-related potentials (ERPs), observed that neonates born between 35 and 38 wGA did not distinguish between the word "baby" spoken by their mother and the same word said by another woman, contrary to those born between 39 and 42 wGA, whereas Granier-Deferre et al. (2011), using heart rate recording, reported postnatal memories in 1-month-old full-term infants of melodies that were presented only between 35 and 37 wGA. Krueger and Garvan (2014) monitored, every 2 weeks from 28 to 38 wGA, the fetal responses to rhymes told by the mother between 28 and 34 wGA. Responses were assessed by heart rate recording. The experimental group who listened to the rhyme attributed to their mother was compared with the control group who listened to a "new" rhyme. All rhymes were recited by an unknown speaker. Significant differences between the 2 groups were observed only at 38 wGA, suggesting that fetuses in the experimental group only slowly accumulated their experience across sessions with some memory of the mother's recitation.

Most functional studies before term have been conducted at 34–35 wGA, when preterms with no medical complications are sufficiently autonomous to be discharged from hospitals. At this age, cochlear functions are relatively mature and infants are able to discriminate various sounds (Bisiacchi et al. 2009), including vowels (Cheour-Luhtanen et al. 1996), displaying a slow mismatch response when a change occurs in a series of repeated sounds. Discrimination responses are also observed in fetuses of the same age (Draganova et al. 2005, 2007; Muenssinger et al. 2013) and functional MRI (fMRI) confirms a cortical origin of auditory responses: activation in the temporal lobe is observed in fetuses close to term hearing external sounds (Moore et al. 2001) but also at 33 wGA (Jardri et al. 2008).

At earlier ages, only rare and less conclusive data have been reported in the literature. Colonnese et al. (2010), comparing sensory responses in infantile rats and preterm human infants, described a sudden switch in evoked responses to visual flashes after 12 days of life in rats from an immature "all or none" response to mature visual evoked responses. These authors suggested a similar switch in human preterms around 35 wGA. A similar shift from an undifferentiated response to noxious and tactile stimuli to a specific ERP after 35 wGA has also been reported for the somatosensory system (Fabrizi et al. 2011), and for the auditory system between tone and voice stimuli (Chipaux et al. 2013). However, these nonspecific responses to external stimuli contrast with fetal discrimination responses between 2 tones (500 vs. 700 Hz) obtained with magnetoencephalography (MEG) in fetuses from 27 wGA onward (Holst et al. 2005). No abrupt switch in evoked responses has been reported between preterm and full-term responses, but rather a continuity mainly

marked by acceleration of response latency and decreased variability, observed in both fetuses (Schleussner and Schneider 2004; Holst et al. 2005) and preterms (Weitzman and Graziani 1968). However, in monkeys (Tian et al. 2001) and human adults (Wessinger et al. 2001), pure tones activate only the core area of the auditory cortex, whereas more complex stimuli are processed in the belt and parabelt regions which may not be sufficiently mature to process complex sounds such as speech syllables at this early age. In a previous study (Mahmoudzadeh et al. 2013) based on functional optical imaging (NIRS) in 30 wGA neonates, we showed that these neonates discriminated a change of syllable (/ba/ vs. /ga/) by means of a network of temporal and frontal areas fairly similar to that described subsequently, whereas the response to a novel voice (male vs. female voice), although present, was more limited. These results demonstrated the presence of cortical responses with spatial organization and functional specialization in the preterm brain, as different networks responded to different features of a syllable (i.e., voice quality and phonetic category). However, this study provided no information about the type of neural responses ("all or none" vs. specific) and the processing mechanisms that encode auditory stimuli or their dynamics.

In summary, cortical processing and learning are indubitably observed during the last month of pregnancy, and possibly from 34 to 35 wGA. This age, at which electroencephalography (EEG) becomes continuous and different states (wakefulness, active, and quiet sleep) are clearly identified, represents a milestone in cortical development. Results concerning discrimination capacities of stimuli presented according to the same modality are inconsistent before 35 wGA. To address this issue, we performed EEG recordings in nineteen 30-wGA-preterm infants during their first week of life (mean age at test: 3 days, range: 1–6 days, corresponding to 28–32 wGA). CV syllables were presented by series of 4 to constitute either standard trials (4 repetitions of the same syllable, e.g., ba ba ba ba) or deviant trials (3 repetitions followed by a change, e.g., ba ba ba ga), which were presented randomly. This AAAX paradigm has been successfully used in a large number of groups of different ages (infants: Dehaene-Lambertz and Dehaene (1994) and adults: Wacongne et al. (2011)), level of consciousness (comatose patients: Bekinschtein et al. (2009) and sleeping subjects: Strauss et al. (2015)), attentive and inattentive subjects, etc., as it can be used to examine how the brain responds to repetition (i.e., responses to trials of the first 3 syllables) and how it detects novelty (i.e., responses to the deviant last syllable). Repetition generally leads to a decreased neural response, called repetition suppression, whereas detection of a change induces recovery of scalp voltage amplitude, resulting in a mismatch response when deviant and standard trials are subtracted. In adults and post-term infants, this response is automatic, recorded even in inattentive subjects and mainly originates in the posterior superior temporal regions. It can be followed by a later second response (P300 in adults, late slow wave in infants), particularly when subjects are attentive to the trial structure (Dehaene-Lambertz and Dehaene 1994; Bekinschtein et al. 2009; Basirat et al. 2014).

At the age tested here (mean: 30.8 wGA), EEG is discontinuous with long periods of low-voltage activity (around 10 μV) interrupted by bursts of high-voltage activity (around 300 μV). A transient circuitry is in place with connections of thalamic afferents with both subplate neurons and pyramidal cells in the cortical plate (Kanold and Luhmann 2010), a different involvement of layer 1 (Rakic and Zecevic 2003; Marín-Padilla 2011; Kirischuk et al. 2014), whereas the 6-layer cortical organization is difficult to identify (Mrzljak et al. 1988), as neuronal migration is still underway. We therefore wondered whether this immature brain was able to follow a rapid succession of syllables, one syllable every 600 ms, or whether the first syllable presentation exhausted the responding neurons, as postulated by Colonnese et al. (2010) for example. We then investigated the characteristics of the response to novelty at this age by using 2 different auditory changes (voice and consonant change) to examine the specificity of the mismatch responses and consequently the encoding of speech stimuli along different time-scales. The b/g discrimination requires a fine temporal resolution to follow the rapid variations of the frequency pattern, which is not required to perceive voice differences. In 2-month-old post-term infants, voice and phoneme are processed in parallel by different sets of regions and a change of one of these features induces mismatch responses with different topographies but at similar latencies (Dehaene-Lambertz 2000; Bristow et al. 2009). Finally, after analyses of event-related responses, we also considered time–frequency analyses because jitter in the latencies of the responses may be present in immature networks and reduce signal-to-noise ratio in ERPs, whereas time–frequency analyses may reveal changes in frequency power.

Materials and Methods

Participants

Nineteen preterm neonates (11 males, 8 females; mean gestational age [GA] at birth: 30.4 weeks GA ± 1.4) were tested while sleeping at night during their first week of life (mean recording age: 3 days (1–6 days) corresponding to 30.8 wGA ± 1.4). All infants had appropriate birth weight, size, and head circumference for their term age, an APGAR score >6 at 5 min, normal auditory and clinical neurological assessments and were considered to be at low risk for brain damage (normal ultrasonography and normal EEG for term age, see Supplementary Table 1). Parents were informed about the study and provided their written informed consent. The study was approved by the local ethics committee (CPP Nord-Ouest II).

Experimental Paradigm

Four syllables (/ba/ and /ga/, produced by a male and female speaker, Fig. 1) were matched for intonation, intensity, total duration (285 ms), prevoicing, and voiced formant transition duration (40/45 ms). They were presented at a comfortable hearing level (≈70 dB) via speakers placed at the infant's feet, by series of 4 syllables (SOA = 600 ms) to form 3 types of trials (standard, deviant voice, and deviant phoneme trials). In standard trials, the same syllable was repeated 4 times. In deviant trials, the last syllable changed relative to the first 3 syllables either according to the voice dimension, or according to the phonetic dimension.

To compare results obtained in ERPs and optical imaging (Mahmoudzadeh et al. 2013), trials were presented by blocks of 5 separated by an intertrial interval of 1600 ms. Each block (duration 20 s) was followed by 40 s of silence. This design resulted in stimulation blocks followed by an interval of silence necessary to elicit a robust hemodynamic response, whereas the trial structure within a block was similar to that used in our previous experiments (Dehaene-Lambertz and Dehaene 1994), that is, SOA and intertrial interval were similar except that a long 40-s interval of silence was observed every 5 trials. Standard blocks, in which all trials were standard trials, necessary for optical topography analyses, were not pertinent for the present study and

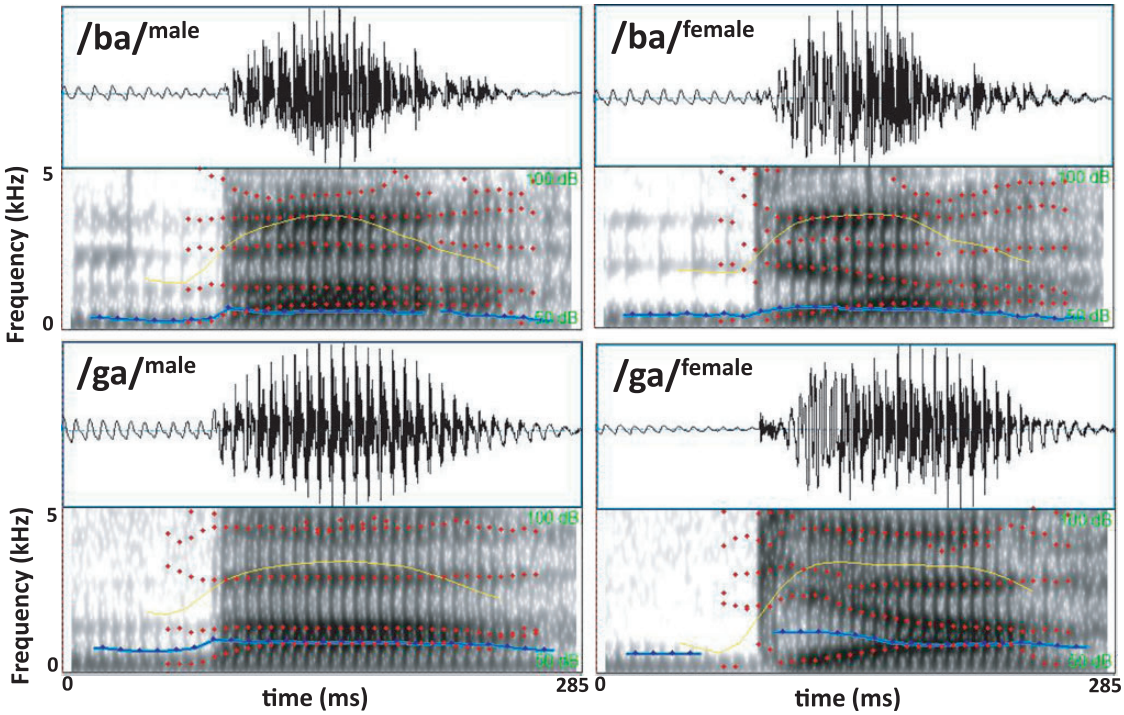

Figure 1. Time waveforms and spectrograms of the 4 syllables used as stimuli were analyzed with Praat sound analysis software. The pitch, formants, and mean-energy contrasting /ba/ and /ga/, male and female voices are underlined with blue, red, and yellow dotted lines, respectively.

were not analyzed. Deviant blocks always began with a deviant trial, then 2 standard and 2 deviant trials were randomly intermixed. Deviant trials corresponded to the voice dimension in DV blocks, and the phonetic dimension in DP blocks. In each block, the repeated syllable was kept constant and was randomly chosen among the 4 possible syllables (ba^m, ga^m, ba^f, and ga^f) in order to present each syllable the same number of times in each condition and for each infant. The 12 types of blocks (4 syllables × 3 conditions) were randomly presented 9 times for a total duration of 108 min, providing a total of 360 useful trials (discarding the 180 trials presented in the standard blocks).

EEG Recording

EEG was recorded at the bedside using Ag/AgCl surface electrodes and a nasion reference at a sampling rate of 2048 Hz, amplified by A.N.T (Enschede, The Netherlands) and DC-50 Hz filtered. The electrode impedance was kept below 5 kΩ. The number of electrodes (31–61) was determined by the infant's head circumference in order to maintain a regular inter-electrode space (center to center) of about 1.5 cm. Because of rapid brain growth at this age, 4 caps were used to cover the normal range of head circumference (25–33 cm) during this period. In all infants, a minimum of 31 electrodes were placed on the classical 10–20 points and supplementary electrodes were placed on intermediate positions according to head circumference.

Data Processing and Statistical Analysis

Event-related Potentials

EEG was band-pass filtered (1–20 Hz), down-sampled to 512 Hz, segmented in 4-s epochs ([–0.5 + 3.5]) time-locked to the first syllable of the trial, and baseline corrected to the 200 ms before S1 onset. We choose to keep 1.7 s after S4 because late components up until 1 s (Csibra et al. 2008; Kouider et al. 2013) are commonly described in post-term infants and may be even further delayed in preterms. Because these long segments exposed to slow signal drifts that interfere with ERPs analysis, the high-pass filter was set to 1 Hz. After artefact rejection (see Supplementary Material for details), an average of 224 trials were kept for each infant (137–349 trials) corresponding to an average of 43/70/45/66 trials for stDV/DV/stDP/DP in each infant, respectively. The number of trials in deviant conditions was greater than in standard conditions because, in each deviant block, a first deviant trial was randomly followed by 2 deviant trials and 2 standard trials (see paradigm). Similar results to those presented were obtained for analyses of discrimination when the first (deviant) trial of the block was discarded to equalize the number of trials and the (un)predictability of the trial condition. Note that because of the design, all syllables were presented in all positions in each infant and the 4 syllables therefore similarly contributed to the standard and deviant conditions.

For statistical analysis, only the 33 most common electrodes were considered in this group of infants (AFz, Fz, FCz, Cz, CPz, Pz, Oz, AF4, F6, F4, F2, FC2, C4, C2, CP4, CP2, P6, P4, P2, O2, and the symmetrical left locations), discarding electrodes and their symmetric locations on the other hemisphere, for which data were available for less than 12 (/19) infants.

Syllable repetition analyses. Firstly, global field power (GFP), which corresponds to the spatial variation across channels at each time-point, was used to identify the 4 peaks at 84, 244, 378, and 576 ms following S1 onset (Skrandies 1990; Murray et al. 2008; Brunet et al. 2011). If the peak amplitude decreases with repetition, as described in older infants, the spatial variation between channels and therefore the GFP should also decrease in a similar fashion. We therefore averaged the GFP values in each infant across the 120-ms time-window centered on Peaks 2–3–4 and

submitted these values to ANOVA with repetition (S1, S2, S3) and peak (Peaks 2, 3, and 4) as within-subject factors. As syllables were presented every 600 ms and as the fourth peak was observed 576 ms after syllable onset, peak 1 for S2 and S3 was cancelled by the much larger Peak 4 evoked by the previous syllable preventing analysis of the repetition effect for peak 1.

Secondly, we examined whether the topography of the responses was reproduced at a fixed latency after each syllable by using a spatial filtering procedure (Schurger et al. 2013). We created 3 spatial filters corresponding to the 2D maps of the grand-average voltage averaged on a [−60 +60] ms time-window centered on Peaks 2, 3, and 4 following S1. Maps were normalized (Fig. 3C), resulting in the creation of 3 sets of coefficients (one for each map). Each infant's time series was then reduced to its projection on these filters, that is, at each time-point, we performed a regression of the infant's normalized vector of data (the voltage at each electrode site) on the set of coefficients corresponding to one map. In other words, the fluctuations of the regression reflect the distance of individual data from a given topography at each time-point. The grand-average was computed for each map (Fig. 3C) and the latency of the highest correlation point after each syllable ([0–600 ms] post-syllable onset) was determined.

Novelty response analysis. We used cluster-based statistics as implemented in the Fieldtrip Matlab toolbox (Oostenveld et al. 2011). Deviant and standard conditions were compared separately for the deviant phoneme and deviant voice blocks after S4 (i.e., on the 1800–3500 ms segment after S1), baseline-corrected to the 200 ms preceding S4. After computing the *t*-value of a comparison between standard and deviant conditions in each channel and for each time-point, a spatiotemporal cluster was defined as the sum of all *t*-values exceeding a limit set at $P = 0.05$ 2-tailed in the data points closely related in space and/or time. A similar procedure was performed for each of 10 000 random permutations of the conditions in order to establish the null distribution of cluster values in our population.

Time–Frequency Analysis

In order to further characterize the novelty response, original trials (i.e., not subjected to any offline filtering) from all electrodes were converted into the time–frequency domain using a complex demodulation approach (step = 1 Hz, frequencies = [1–20 Hz], epoch = [−500–4000] ms), implemented by BESA Source Coherence module version 6. Changes in spectral power were calculated relative to prestimulus baseline (−500 to 0 ms). Statistical significance between conditions was tested using a similar cluster-randomization procedure as for ERPs using BESA Statistics for TFRs (see Supplementary Material).

Results

ERPs Analyses

Adaptation to Repeated Stimuli

To characterize the neuronal response to syllables at this age, we first analyzed the auditory ERPs for all 3 conditions combined. As shown in Figure 2, a single peak was usually recorded after each syllable on each electrode and amplitude decay was observed

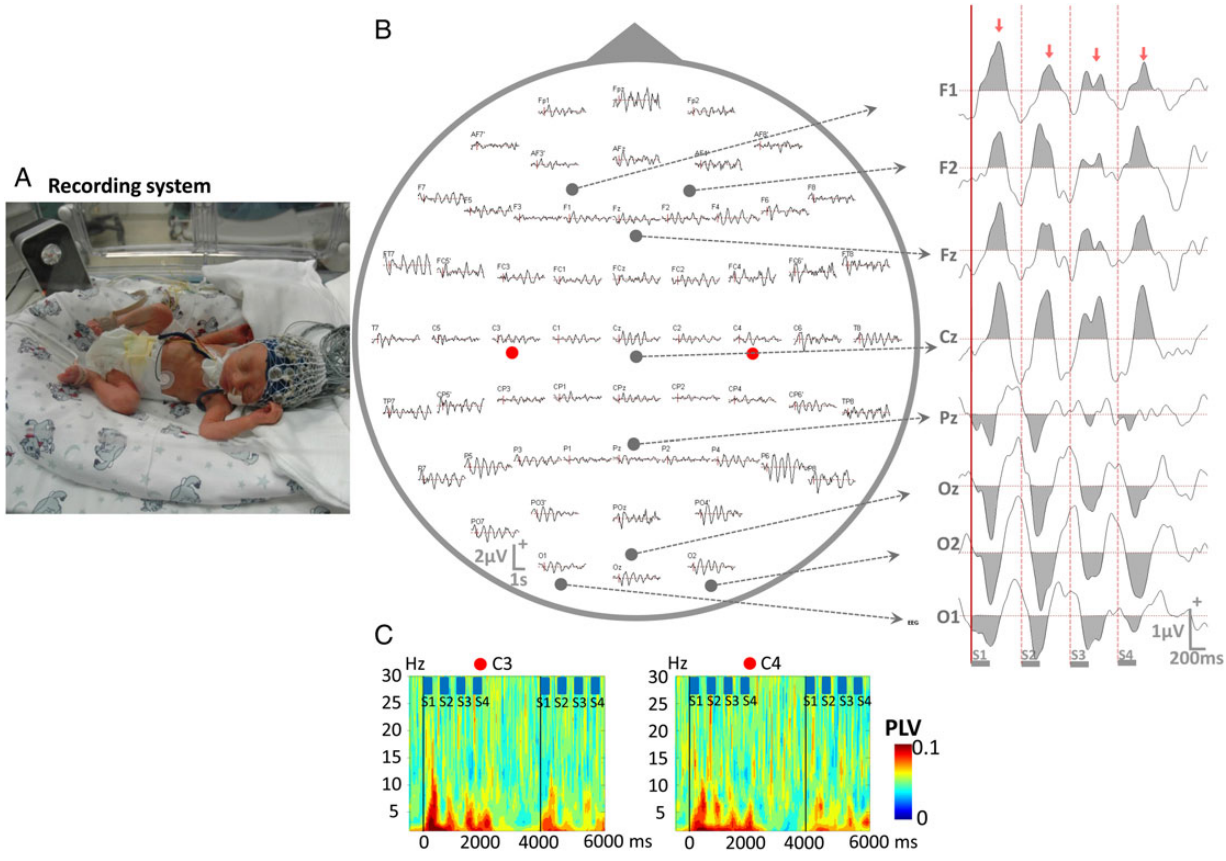

Figure 2. (A) Preterm neonate with high-density EEG cap (B) Grand average of auditory ERPs (all conditions combined). The evoked response to each syllable (S1, S2, S3, S4) induced peaks with complex topographies (see Fig. 3). Repetition decreased ERP amplitude. (C) Time–frequency representation (phase-locking value: PLV) of the grand-average over 2 successive trials in C3 and C4 (red dots in the central plot).

with repetition. The phase-locking value, which assesses the variability of the response across trials, was weak (around 0.1), but present for each syllable, as shown for C3 and C4 in Figure 2. The global field power (GFP, or spatial variance) computed at each time-point presented 4 maxima at 84, 244, 378, and 576 ms after S1 onset. At these maxima, the 2D voltage maps showed different topographies, indicating that the neural sources changed with time (Fig. 3A,B). With syllable repetition, the ERP amplitude decreased, as indicated by a reduced GFP (Fig. 3B, Repetition effect on GFP values averaged around the peaks for S1, S2, and S3: $F_{2,36} = 7.87$, $P = 0.0015$). The amplitude decrease was similar for all peaks 2, 3 and 4 (nonsignificant repetition × peak interaction: $F_{4,72} = 1.47$, $P = 0.22$). As described in older infants (Dehaene-Lambertz and Dehaene 1994), the repetition effect was notably observed between the first and second syllables (S1 vs. S2: $F_{1,18} = 7.78$, $P = 0.012$) with no further decrease between

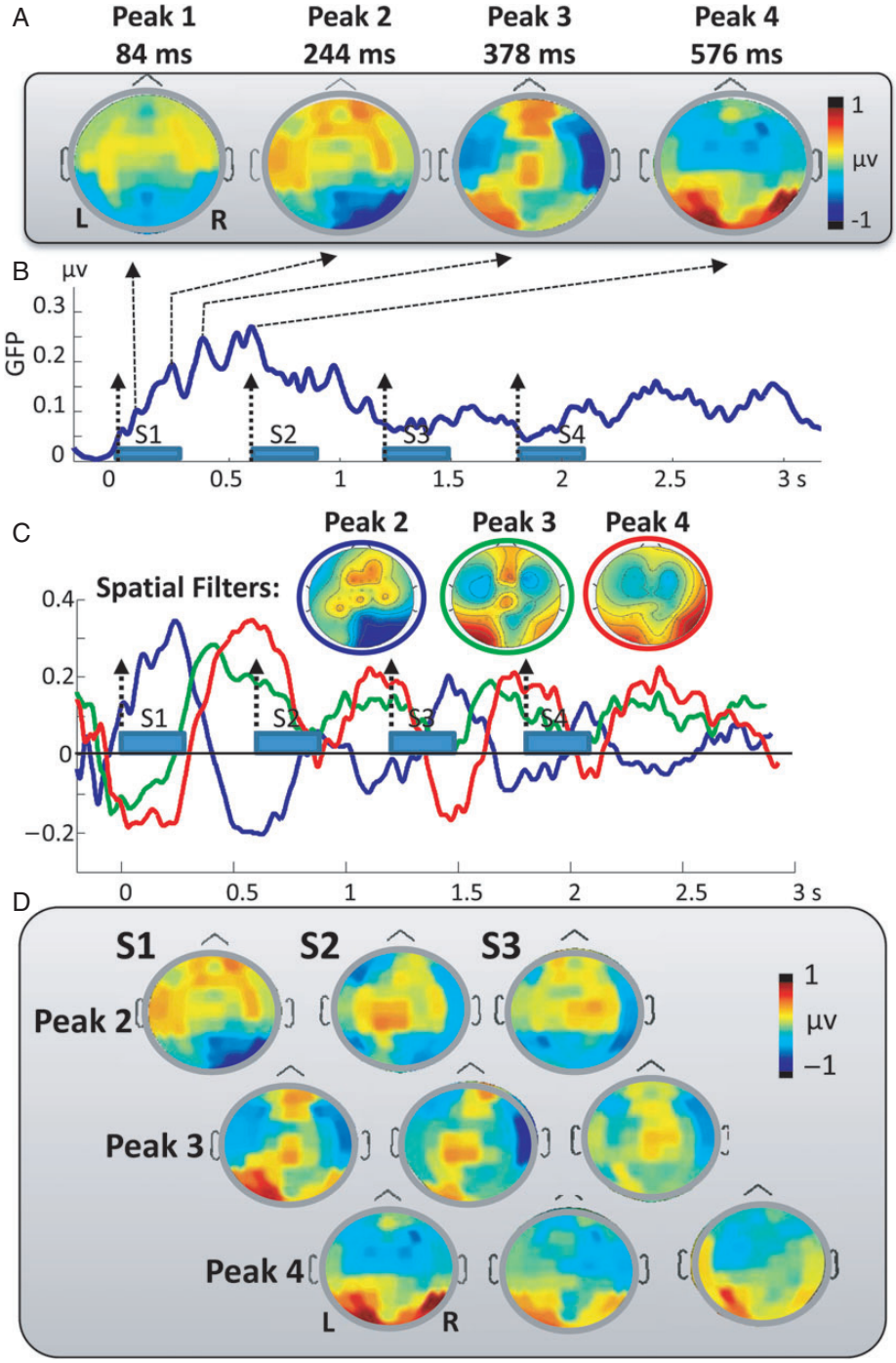

Figure 3. Auditory adaptation to syllable repetition: (*A*) 2D voltage maps at the maximum of the 4 peaks following S1. (*B*) GFP of the grand average during the entire trial illustrating the decrease of voltage amplitude during the trial. (*C*) Grand averages of the projection of the individual time-series on the 3 spatial filters corresponding to the topography of peaks 2 (blue line), 3 (green line), and 4 (red line). The order of the curves after each syllable remained constant and reproduced the same order of peaks. (*D*) 2D voltage maps at the maximum of the peaks after each repeated syllable showing the decreased response, but with preserved topography.

S2 and S3 ($F_{1,18} = 1.39$, $P = 0.25$). In both analyses, the peaks × syll interaction (S1 vs. S2 and S2 vs. S3) was not significant (both Ps > 0.15), indicating a similar repetition pattern for the 3 peaks (i.e., decreased amplitude between S1 and S2 and no further significant decrease from S2 to S3).

A spatial and temporal pattern was therefore clearly observed after the first syllable of the trial, but is the preterm brain able to follow the rapid succession of syllables every 600 ms or does the voltage decrease between the first and following syllables simply correspond to a return to background activity, as described in the rat immature cortex (Colonnese et al. 2010)? To answer this question, we used a spatial filtering procedure to estimate whether the same topography was repeated after each syllable (Schurger et al. 2013). Each infant time-series was reduced to its projection on the topography of Peaks 2, 3, and 4 as evoked by S1. The grand averages for each projection are presented in Figure 3C. The order of the curves after each syllable remained constant and reproduced the order of the peaks. For each spatial filter (i.e., Peak), the maxima of the projections presented a reproducible latency after each syllable onset (for S1–S2–S3–S4: mean latency for Peak 2: 246–277–261–268 ms, Peak 3: 410–396–454–391 ms, Peak 4: 572–505–552–598 ms), that is, an average of 263, 413, and 557 ms post-syllable onset.

Mismatch Responses to a Change in Sequence

To examine the specificity of the auditory coding in these "immature" networks, we examined whether they were able to react to a novel syllable and cluster-based statistics were used to compare deviant trials with the standard trials of the same blocks. For a change of phoneme, this analysis revealed 2 successive responses (Fig. 4). A first negative temporospatial cluster ($P_{cluster\text{-}cor} = 0.021$) extended from 90 to 530 ms post-S4 and comprised a mostly right frontal group of electrodes (AF4, F6, F4, F2, AFZ, FZ, AF3, F1). A left posterior positive cluster on P3-O1, synchronous with the frontal negative cluster was not sufficiently large to be significant at the $P_{cluster\text{-}cor}$ level. A second significant cluster ($P_{cluster\text{-}cor} = 0.024$) was observed between 1000 and 1500 ms post-S4 comprising almost the same right frontal electrodes (AF4, F6, F4, F2, AFZ, FZ, FCZ, F1) as the early cluster, but the positive pole of the topography was situated over the right posterior area (CP4 CP2 P4 P2 O2). This positive pole did not reach significance at the $P_{cluster\text{-}cor}$ level ($P_{cluster\text{-}cor} = 0.15$). Cluster-based analysis did not detect any significant cluster for a change of voice. Testing of the (deviant – standard) × (phoneme vs. voice) interaction identified a significant posterior cluster (comprising P1, P3, P5, and O1) during the first time-window (24–891 ms, $P_{cluster\text{-}cor} = 0.023$). An anterior cluster (AF4 AFZ F6 F4 F2 FZ F1 FC2) was also revealed during the late time-window (1069–1464 ms), but did not reach significance at the $P_{cluster\text{-}cor}$ level ($P_{cluster\text{-}cor} = 0.10$). Post hoc analyses did not reveal any significant difference between the 2 standard conditions, but one extended anterior cluster (AF4 AFZ AF3 F6 F4 F2 FZ F3 F1 F5 FC2 C3 CP3) was observed during the late time-window (989–1520 ms) when the 2 deviant conditions were compared ($P_{cluster\text{-}cor} = 0.01$).

Time–frequency Analyses

Variability in response latencies may be more marked at the onset of neural circuitry and analyses of evoked responses may

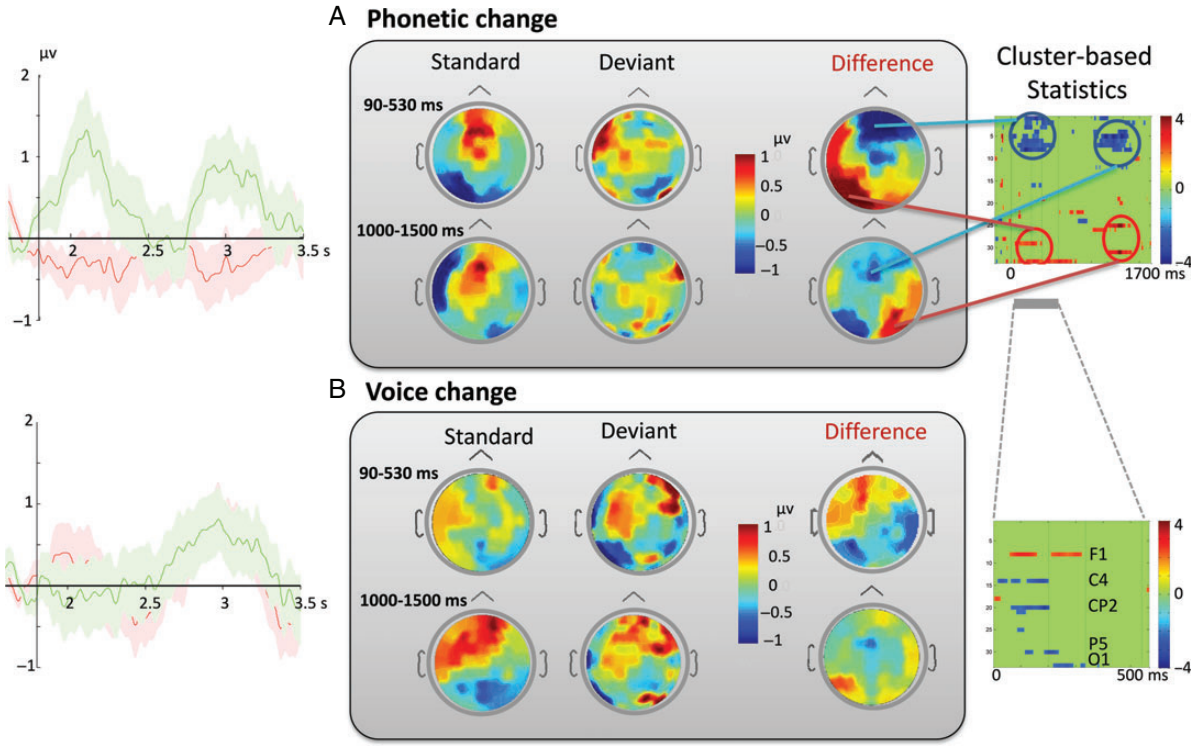

Figure 4. Novelty response for phonetic change (*A*) and voice change (*B*). On the right, results of the cluster-based permutation analysis (*t*-values over time (*x*-axis) and electrodes (*y*-axis), dotted lines are plotted at the maxima of peaks 2, 3, and 4). Analysis of phonetic change showed 2 significant clusters. The topographies of the responses during the identified time-windows are shown on the left for the standard and deviant conditions and their difference. No cluster was significant for voice change although some electrodes showed significant differences during the first 500 ms post-S4 onset ($P_{corrected} < 0.05$, magnified view on the bottom right). For comparison with *A*, topographies of the responses during the same time-windows are displayed on the left for the standard and deviant voice conditions and their difference. On the left, grand averages (mean and standard deviation) of the deviant and standard conditions for each type of change. Voltage was averaged across AF4, F6, F4, F2, AFZ, FZ, FCZ, and F1, forming the negative cluster on the 2D difference map.

miss some of the electrical activity induced by the stimuli, which is still not precisely time-locked to the stimuli. We therefore also performed time–frequency analyses. A greater power in oscillatory activity was recorded in the deviant trials for both types of changes in the 1–20 Hz frequency band. For a change of phoneme, a significant difference extended from 50 to 1150 ms after S4-onset ($P_{cluster\text{-}cor} = 0.003$) around the centroparietal electrode group of electrodes (c1 = Cz, CPz, Pz, C2, P2) and for a change of voice between 100 and 1750 ms post-S4 ($P_{cluster\text{-}cor} = 0.005$) at FCz and Cz. A second significant difference restricted to the frequency range [10–20] Hz was observed between 700 and 1700 ms ($P_{cluster\text{-}cor} = 004$) on P5, Pz, CP3, CP1, CPz, and C3 (c2 on Fig. 5B, left) for a change of phoneme and between 750 and 1350 ms ($P_{cluster\text{-}cor} = 0.01$) on a right anterior cluster (c2 = FC2, FCz, C2, C4) for a change of voice (c2 on Fig. 5B, right).

Discussion

The objective of this study was to characterize the neural response to speech syllables at an early preterm age (28–32 wGA), when the cortical plate begins to receive input from the external world as a result of maturation of the peripheral and subcortical auditory system and the arrival of thalamocortical fibers in the cortical plate. We wanted to determine whether the responses were general "all or none" responses with no stimulus specificity or whether they were already dependent on the auditory dimension tested, which would imply early definition of auditory processing streams. Because phonetic and voice analyses are based on 2 different temporal scales processed by distinct pathways at later ages, these 2 dimensions were contrasted by introducing either b/g or male/female voice changes in our series of repeated syllables.

To summarize our results, we first recorded temporally precise peaks with a succession of different topographies in response to syllables. Secondly, this temporal and spatial sequence was maintained when the syllables were repeated despite using relatively rapid stimulation (one syllable every 600 ms). These 2 results suggest an already robust and reproducible processing pathway for complex sounds at 7 months of pregnancy. Thirdly, the deviant voice and the deviant consonant induced "mismatch" responses, which were better time-locked to syllable onset in the case of a deviant consonant. Significant differences for the deviant voice were only observed in time–frequency analyses, whereas phonetic changes also affected ERPs. The differences in evoked pattern (time-locked or not time-locked) and topography (differences were more anterior for the deviant voice than for the deviant phoneme in time–frequency analyses) indicate that the syllabic features were coded along different processing streams, which displayed different sensitivities to novelty. These results will be discussed successively.

A Reproducible Sequence of Auditory Responses

Contrary to the "all or none" generic response reported in some studies (Colonnese et al. 2010; Fabrizi et al. 2011; Chipaux et al. 2013), in this study, we identified a succession of precise voltage topographies after each syllable at reproducible latencies, demonstrating that several neural generators were already successively involved in syllable perception at this age (28–32 wGA). Previous studies using tones and clicks have reported a main

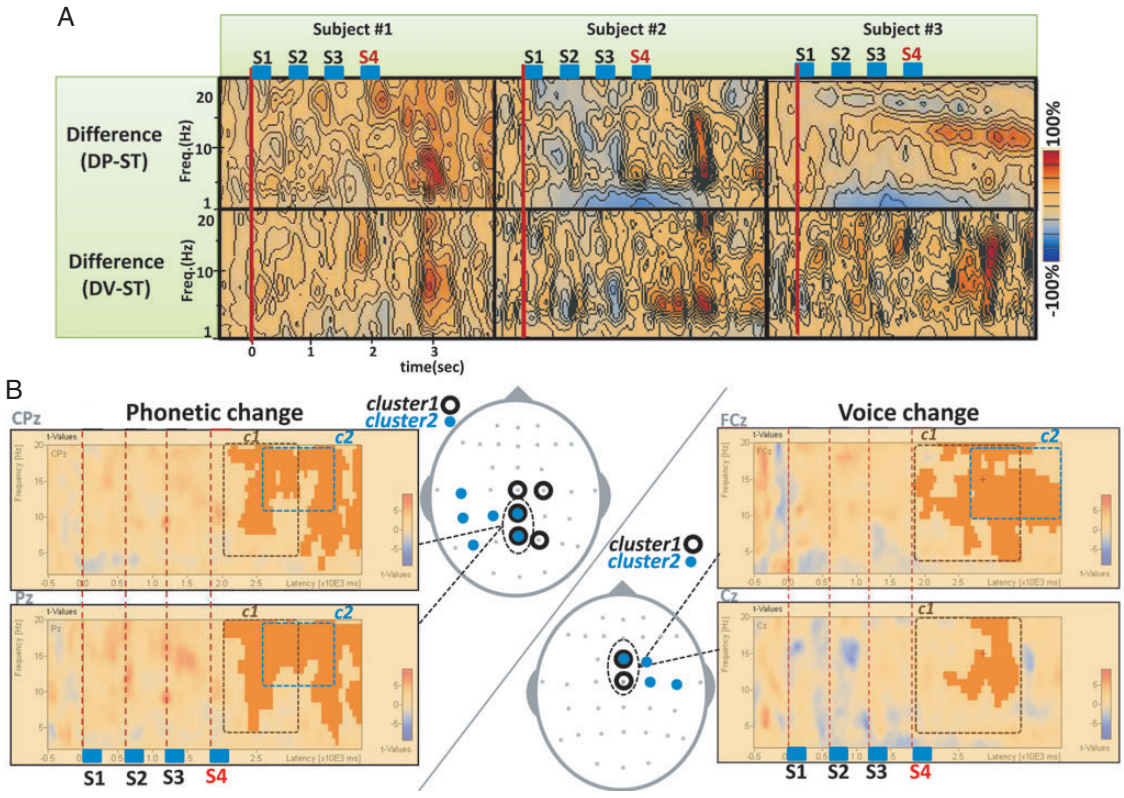

Figure 5. Time–frequency analyses (A) Examples in 3 individual infants of the relative power differences between ST and DV/DP conditions (DV-ST, DP-ST) on Cz. Color bar represents the percent change from baseline (i.e., normalized power). (B) Results of the cluster-based permutation analysis (x-axis: t-values over time and y-axis: frequencies) showing the 2 significant clusters c1 (cluster1) and c2 (cluster2) on CPz and Pz on the left for DP-ST and on FCz and Cz on the right for DV-ST.

auditory component with a latency ranging from 180 to 300 ms (Weitzman and Graziani 1968; Schleussner and Schneider 2004; Holst et al. 2005), followed by a long-lasting response of reverse polarity (Weitzman and Graziani 1968). Our higher electrode density can explain the finer description of auditory ERPs and, although the tones and clicks used in these studies may elicit faster latencies than our syllables due to their earlier energy peaks, these 3 published descriptions correspond to our peak 2 (244 ms) followed by peaks 3 and 4 (378 and 576 ms), which both presented reverse polarities relative to peak 2. The absence of an exact physical model of preterm tissue conductivities (e.g., fontanel vs. skull) and brain gyrification, as well as the lack of an accurate description of the underlying circuitry at this age when cortical columns are not yet well defined and transient connections between cortical plate and subplate neurons are predominant, prevented us from performing source reconstructions. Nevertheless, the succession of maps at precise latencies combined with our previous results using NIRS, which revealed extensive activation of the perisylvian regions, suggest progression of the input throughout a hierarchy of cortical processing stages, which may finally reach frontal areas as demonstrated by optical imaging (Mahmoudzadeh et al. 2013). This progression is compatible with the feed-forward connectivity, which is established prenatally in primates with well-defined specificity (Kennedy et al. 2007).

Auditory Adaptation to Syllable Repetition and Novelty Responses to a Change

Repetition of the same syllable was associated with a decrease in amplitude, mainly between the first and second syllables with no subsequent decrease. The reproducibility of peak topography and latencies after each syllable demonstrated that repeated stimuli were still processed according to the same functional stages with no depletion of brain activity after the first response, as could have been expected in such an immature brain. A decreased MEG response to repeated tones was similarly observed in older 35 wGA fetuses (30–39 wGA, Muenssinger et al. 2013) with recovery of the amplitude when a new tone was introduced. In the present study, a change of phoneme also evoked a long mismatch response with a sustained dipole configuration consisting of negativity over frontal areas synchronous with positivity over temporal areas. This pattern of repetition suppression associated with a mismatch response demonstrates that, by an average age of 30 wGA, 10 weeks before term, at an age when neurons are still migrating, neural mechanisms able to process the auditory environment are already in place and are similar to the habituation–discrimination pattern subsequently reported in 3-month-old post-term infants (Dehaene-Lambertz and Dehaene 1994) and adults (Dehaene-Lambertz et al. 2005).

Current models of these responses in adults emphasize the role of predicting coding relative to simpler models based on neural adaptation and refractory period. Attenuation/recovery of the voltage pattern is explained by an error signal that declines with repetition and that reaches a maximum when an unexpected sound is introduced (Friston 2005; Wacongne et al. 2011). These models imply complex cortico-cortical connectivity, notably involving supragranular layers. At an age when neurons of the more superficial layers and inhibitory interneurons are still migrating to reach their final location, local adaptation may still provide a major contribution to the pattern observed here. The possibility of predictive coding at such an immature age is an important point that should be tested more directly in future experiments in order to clarify the role of the various cortical layers in predictive coding.

Colonnese et al. (2010) suggested that preterm infants before 35 wGA were still at an immature stage of sensory development corresponding to nonspecific "all and none" responses in rats, and would therefore be unable to follow rapid stimulation (Chipaux et al. 2013). These authors reported evoked responses consisting of delta oscillations nested in a slow negativity of large amplitude (from −60 to + 60 μV) before 35 wGA, contrasting with the −1 to +1 amplitude of our ERPs and with the auditory-evoked responses reported in the literature (Weitzman and Graziani 1968; Schleussner and Schneider 2004; Holst et al. 2005). Due to signal-to-noise ratio, we excluded trials with bursts of high voltage activity and our analyses were therefore performed on the "silent" period of "discontinuous activity" characteristic of this age. How this stage can be compared with the clearer active–quiet sleep cycle observed at older ages remains to be studied with a larger combination of physiological and cognitive markers. In any case, the organized pattern of responses obtained in this study, with a clear pattern of repetition suppression and mismatch response, combined with our previous functional optical imaging results, demonstrate that at least the perisylvian area in 30 wGA human neonates is already at a stage corresponding to the "mature" stage in the rat, when the response becomes time-locked to the stimulus with a sharp and reproducible shape and an amplitude modulated by the stimulus characteristics. This early sensory development is a characteristic of primate development, complicating rodent modeling of human development (Levitt 2003). It cannot be excluded that a sudden and unexpected stimulus may also elicit a general, nonspecific response, for example, an avoidance response.

In older infants and adults exposed to a change in series of repeated sounds, a first automatic response may be followed by a late response, typically a P300 in adults and a late frontal negativity in infants (Basirat et al. 2014). These late responses are generally attributed to orientation of attention to the deviant stimulus because they are modulated by the subject's level of attention toward the deviant stimulus and their vigilance. In the present study, we recorded a second significant difference 1 s after onset of the deviant syllable. This response had a dipole topography with a similar negative anterior pole to that of the early response, but synchronous with a right, rather than a left positivity on occipitoparietal electrodes. Although functional optical imaging data demonstrated that the discrimination response was not limited to sensory regions, but also involved a large network already comprising inferior frontal cortices, other experiments are certainly needed to determine whether this second response corresponds to an automatic response, or more intentional, attentional orientation. Given its topography on the scalp, this second response may also correspond to a delayed response in the right hemisphere, as hemodynamic discrimination responses recorded with optical imaging were slower on the right hemisphere than on the left hemisphere, notably in the posterior temporal region in the case of a phonetic change (Mahmoudzadeh et al. 2013). Although hemodynamic and neural delays do not present the same order of magnitude, these 2 results might be interpreted as poorer right hemisphere efficiency to process this type of novelty.

Specialized Streams in the Immature Brain

We used 2 deviant conditions (ba/ga and male/female voice) to examine whether, at this early age, mismatch responses were generic—preterm neonates reacting to any stimulus reaching a threshold ("all or none" response; Colonnese et al. 2010)—or

already presented characteristics suggesting that syllabic features are processed along different streams of processors. Although these syllables are sufficiently different to elicit mismatch responses in 4-month-old post-term infants (Dehaene-Lambertz 2000) and in rats (Mahmoudzadeh et al. submitted (in preparation)), no significant difference was observed in the present study for a voice change in ERPs analyses contrasting with the MMR to the consonant change. However, both changes evoked significant clusters on time–frequency analyses. This pattern suggests that the voice mismatch responses were less time-locked to syllable onsets compared with the phonetic mismatch response, which could be explained by delayed maturation of the neural network involved in voice discrimination. Microstructural differences have been described in the gray matter of the perisylvian regions, and notably between the left and right superior temporal areas. These differences in MRI signal are related to water movement, which is blocked and oriented by the membranes present within the voxel and have been explained by a heterogeneous calendar of maturation across cortical areas (Leroy et al. 2011; Ball et al. 2013). Our result might correspond to the functional counterpart of these maturational differences in temporal areas. Furthermore, the significant differences between deviant and standard trials were not observed on the same clusters of electrodes in time–frequency analyses, suggesting different underlying sources. A response pattern depending on which syllabic feature is changed is congruent with our previous optical imaging study. We observed a weaker and less extended response to a change of voice than to a change of phoneme: only the right inferior frontal region showed discrimination responses, contrary to the large response to a change of phoneme recorded in several temporal channels and including both inferior frontal regions (Mahmoudzadeh et al. 2013). The differences between the 2 deviant conditions suggest that these features were not encoded in the same circuits, thus revealing early organization of the auditory system in specialized areas by 7 months of gestation. This result is consistent with the prenatal organization of the visual system described in monkeys, before any exposure to structured visual stimuli (Rakic 1976, 1977) and with the results of recent resting-state fMRI studies in preterm infants (Doria et al. 2010; Smyser et al. 2010) and fetuses (Thomason et al. 2014), demonstrating a modular structure in early preterms.

The stimuli used in this study were natural syllables that were globally equalized, but which may have differed locally in terms of certain features, although it is commonly admitted that b/g discrimination in humans predominantly relies on the brief formant transition, and more generally that phonetic coding needs a rapid sampling of the auditory signal, whereas voice discrimination is based on a larger window of integration of the signal to recover the F0 and its harmonics (Zatorre and Belin 2001; Giraud et al. 2007). Phonetic coding and voice recognition are complex percepts that are realized in higher-level auditory regions in the superior temporal lobe with a left hemispheric advantage for phonetic processing and a right hemispheric advantage for voice recognition in adults (Belin et al. 2000) and even in infants during the first semester of life (Dehaene-Lambertz et al. 2010; Blasi et al. 2011). Although voice discrimination may appear to be a simple task, human infants have difficulties discriminating voices speaking a foreign language (Johnson et al. 2011) or producing different vowels, whereas they easily identify the phonetic categories of syllables across different voices (Kuhl and Miller 1982; Nazzi et al. 1998; Dehaene-Lambertz and Pena 2001). Moreover, infants' preferences for their parents' voices are generally tested with passages (e.g., Decasper and Fifer 1980) and might be based on specific rhythm, emphasis pattern, and other supra-segmental cues rather than on timbre per se because the infants' performances decline when the mother's voice is monotonous (Mehler et al. 1978). In 35–38 wGA preterms, DeRegnier et al. (2002) did not observe differences in the ERPs evoked by the word "baby" produced either by their mother or by the previous baby's mother. A difference was observed only in older infants (39–42 wGA), whereas, as reported in the introduction, preterm infants at the same age (35 wGA) discriminate vowels (Cheour-Luhtanen et al. 1996) and CV syllables (Dehaene-Lambertz 1997). This facility to encode phonetic information compared with voice might be related to biases to encode fast temporal modulations. This hypothesis has been tested in full-term neonates by Telkemeyer et al. (2009). Using NIRS, these authors showed that the highest amplitude over bilateral auditory cortices was recorded for the 25 ms modulated complex sound, whereas the response to slower modulation (165 and 300 ms) was very focal, recorded only in the right superior temporal location. Thus, as in other primates, the human infant auditory cortex appears to be organized into parallel processing streams, which filter the incoming acoustic information in different ways. An early and persistent advantage to process fast modulations is observed, a property particularly useful to process the linguistic message.

Conclusion

In conclusion, our results show an early functional arealization of the human cortex. Although the exact onset of thalamic inputs, and therefore of stimulus-dependent activity, is difficult to determine in humans, the discrimination responses observed 10 weeks before term, for a difficult phonetic contrast based on a fine-grained temporal coding suggest that the phonetic capacities described in full-term neonates are not the consequence of we exposure to the mother's voice in the womb, but that human infants possess a strong genetic endowment to process speech features. In particular, the timely precise neural sequence induced by the rapid presentation of complex stimuli underscores the early abilities of the human brain to process temporal information. These early networks may represent the functional intrinsic envelope, which is subsequently refined by exposure to a specific linguistic environment or impaired in the case of deprivation or inappropriate stimulation such as incubator noise (Chang and Merzenich 2003). Further studies should specify how language development could be modified by early exposure to the extrauterine world (Gonzalez-Gomez and Nazzi 2012; Pena et al. 2012). Indeed, several studies have highlighted the persistent linguistic difficulties observed in preterm infants (Van Noort-Van Der Spek et al. 2012) and the impact of prematurity on the left superior temporal region (Aeby et al. 2013), a key region of the language system (Dewitt and Rauschecker 2012). Given the early onset of intrinsic and extrinsic cortical activity during pregnancy and the protracted postnatal maturational calendar, more structural and functional data acquired in human infants are necessary to precisely describe the ontogeny of the complex cognitive functions observed in our species. The development of noninvasive brain imaging techniques allows infants to be tested at a very immature stage with no harmful effects and should help to bridge the gap between animal models and human cognitive capacities.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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Notes

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Supplementary Material

Table S1. Clinical features of the tested infants

Infant No.	Gender	GA at birth (wk)	GA at test (wk)	Birth Weight (g)	Apgar (1 min)	Apgar (5 min)	Brain US	EEG Cap*	Delivery	Presentation	Clinical conditions (Etiology)
1	M	32	32 2/7	1530	9	7	N	3	Vaginal	Transverse	Twin
2	F	28 1/7	28 5/7	1490	9	10	N	3	cesarean	Cephalic	RPH
3	M	32	32 2/7	1550	0	6	N	3	cesarean	Cephalic	Preeclampsia
4	M	32	32 3/7	2300	10	10	N	4	Vaginal	Cephalic	PROM
5	M	30 6/7	31 1/7	990	8	9	N	2	cesarean	Cephalic	Preeclampsia
6	F	31	31 4/7	1260	10	10	N	2	cesarean	Cephalic	Preeclampsia
7	F	31	31 2/7	1560	8	10	N	3	cesarean	Breech	Anoxia-ischemia
8	M	29	29 6/7	1610	10	10	N	3	Vaginal	Cephalic	Twin, preeclampsia, PROM
9	M	31 1/7	31 4/7	2300	10	10	N	4	Vaginal	Breech	Twin
10	F	28	28 3/7	995	8	8	N	1	Vaginal	Cephalic	Twin, preeclampsia
11	M	28	28 4/7	760	7	10	N	1	cesarean	Cephalic	Preeclampsia
12	M	30 2/7	30 5/7	1440	9	10	N	3	Vaginal	Cephalic	PROM, chorioamnionitis
13	M	32	32 1/7	1200	10	10	N	2	Cesarean	Cephalic	Twin
14	M	30 2/7	30 5/7	1730	7	8	N	3	Vaginal	Cephalic	PROM
15	M	28 2/7	28 3/7	1340	8	8	N	2	Cesarean	Cephalic	Twin
16	M	29 5/7	30 2/7	1560	7	8	N	3	Vaginal	Cephalic	Twin
17	F	30 3/7	30 6/7	1630	10	10	N	3	Cesarean	Cephalic	Twin
18	M	31 4/7	31 6/7	1090	10	10	N	2	Cesarean	Cephalic	Preeclampsia
19	F	31 1/7	31 4/7	2120	9	9	N	4	Vaginal	Breech	PROM

M: Male, **F**: Female, **GA**: Gestational Age, **EEG**: ElectroEncephaloGram, **Brain US:** Brain ultrasonography, **N**: Normal, **RPH**: RetroPlacental Hematoma, **PROM**: Premature Rupture Of Membranes, ***** EEG Cap (circumference): *#1* (22cm) *#2* (24cm) *#3*(28cm), *#4* (32cm)

1) **Data processing**: **Artefact rejection:**

A) For Event-Related Potentials analyses:

At this age, background EEG is marked by long periods of low-voltage activity (around 10 µV) interrupted by bursts of high-voltage activity with an amplitude of about 300 µV. We used an automatic artefact-rejection procedure to discard these high-activity periods. Individual channels were rejected when their absolute amplitude exceeded 50 µV or when a local amplitude jump between ten successive time-points exceeded 30 µV. A channel was rejected for the entire

session if it was rejected on more than 70% of the trials. The entire trial was rejected if more than 15% of the channels were rejected. An average of 312 trials were included for each infant (141 to 510 trials). The syllable identity (ba^m, ga^m, ba^f and ga^f) was not considered and epochs were averaged under four conditions for each infant: (standard (st) and deviant trials (D)) x (DV and DP blocks). We obtained an average of 43/70/45/66 trials for stDV/DV/stDP/DP in each infant, respectively.

B) For Time-Frequency analyses

As Time-frequency representations (TFR) are less sensitive to sharp local deviations than ERPs, we first rejected high-activity periods, and artefacts were then easily detected visually as a sudden increase in amplitude, in which case the entire trial was rejected (On average 97/65/72 trials for standard (ST), deviant voice (DV), and deviant phoneme (DP) conditions, respectively)

2) Data processing: TFR computation

Artifact-free trials were transformed into the time–frequency domain using a complex demodulation approach (resolution: 1.0 Hz, 50 ms), implemented by BESA Source Coherence module version 6.0 (Papp and Ktonas 1977, Hoechstetter et al., 2004), and the resulting spectral power estimations per electrode were averaged over trials to generate time-frequency plots of mean spectral density. These data were normalized by dividing the power value of each predetermined time-frequency bin by the respective bin's baseline power, which was calculated as the mean power during the −0.5 to 0s time period. The TFRs of post-stimulus activity (A(t,f)) were expressed as the change relative to baseline activity (i.e. -500 to 0 ms before the first syllable of the trial):

$$\mathrm{TFR} = \frac{\mathrm{A(t,f)} - \mathrm{A_{baseline}\,(f)}}{\mathrm{A_{baseline}\,(f)}} . 100\%,$$

with $A(t,f)$ = activity at time t and frequency f (absolute amplitude) and $A_{baseline}(f)$= mean activity at frequency f over the baseline epoch.

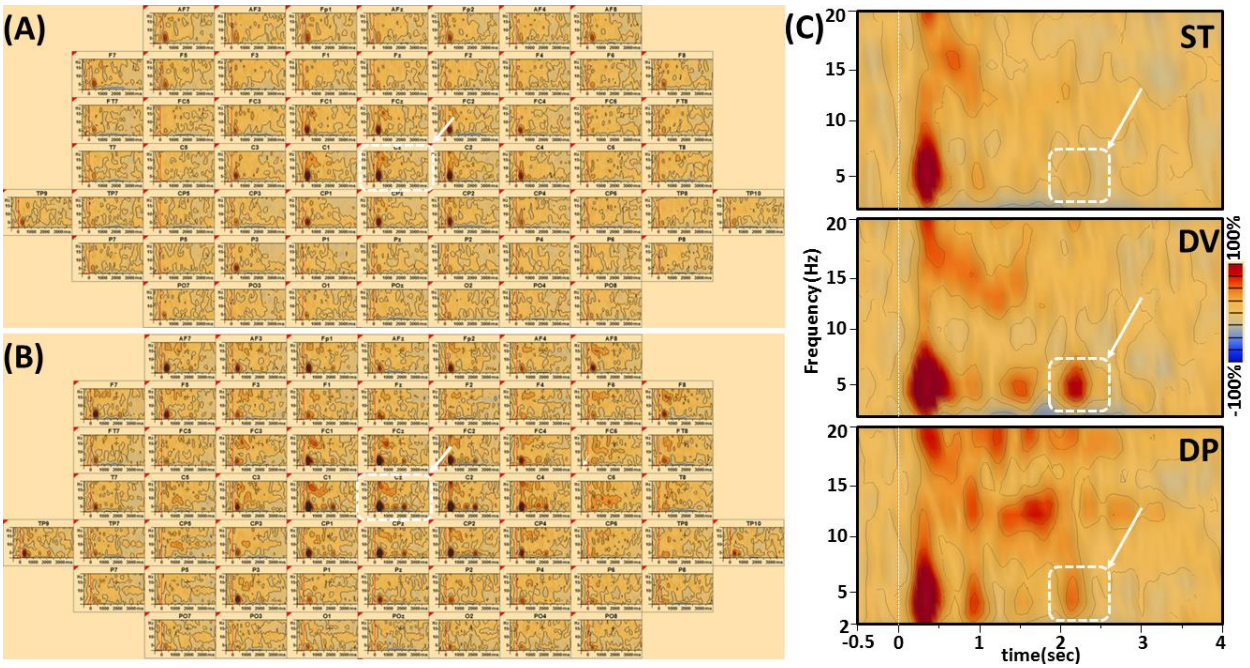

Fig. S1: (A) Time-frequency representations (TFRs) of relative power changes for the ST condition in one preterm infant (age: 30 weeks GA). (B) DP condition. (C) EEG Channel: Cz; In each panel, TFRs were calculated by averaging all trials belonging to the same condition (ST/DV/DP); the same power scale applies to TFRs. Color bar (Right middle) is the percent change from baseline (i.e., normalized power), with red representing increased power and blue representing decreased power.

3) Time-Frequency statistics

A preliminary Student's t test between conditions per data point was calculated using BESA Statistics® (v1.0, BESA Software, Gräfelfing, Germany). Parameter-free permutation testing in combination with data clustering was then used to obtained corrected p-values for multiple comparisons. In each permutation analysis, the standard and the tested deviant condition data were randomly reordered across subjects. T-tests were then calculated for all electrode sites (31 electrodes) and each time-frequency to identify electrodes with a significant difference between the reordered data. Clusters were identified by considering only those (at least four) contiguous electrode sites (with neighboring distance: 40 mm) with a p-value <.05, and the sum of the t-values was recovered for each cluster. The cluster with the highest sum of t-values was used in each permutation. This procedure was used to obtain the null distribution from the data and to compute the corrected p-values.

4) Data analyses: ERPs

As the first trial of a block was always deviant (a constraint imposed for optical imaging recordings), we also performed the same analyses after excluding these trials. The results remained similar with, in the phoneme change analyses, two significant time-windows showing a dipole configuration with a negative pole over frontal areas and reversed polarity over occipitoparietal areas (early cluster on frontal electrodes p_{cor}=.054; and late cluster also on frontal electrodes p_{cor}=.015).

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