Infants exposed to antibiotics after birth have altered recognition memory responses at one month of age

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Abstract

Background: Neonatal exposure to antibiotics, in the absence of infection, results in abnormal learning and memory in animals and is linked to changes in gut microbes. The relevance of early-life antibiotic exposure to brain function in humans is not known.

Methods: Recognition memory was assessed at 1 month of age in 15 term-born infants exposed to antibiotics (with negative cultures) and 57 unexposed infants using event-related potentials (ERPs). Linear regression analysis, adjusting for covariates, was employed to compare groups with respect to ERP features representing early stimulus processing (P2 amplitude) and discrimination between mother and stranger voices.

Results: Infants exposed to antibiotics exhibited smaller P2 amplitudes for both voice conditions ($p = 0.001$), with greatest reductions observed for mother's voice in frontal and central scalp regions ($p < 0.04$). Infants exposed to antibiotics showed larger P2 amplitudes to stranger's as compared to mother's voice, a reversal of the typical response exhibited by unexposed infants. Abnormal ERP responses did not consistently correlate with increased inflammatory cytokines within the antibiotic-exposed group.

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Parental consent of subjects was required prior to enrollment in this study.

Category of study: Clinical research

Conclusion: Otherwise healthy infants exposed to antibiotics soon after birth demonstrated altered auditory processing and recognition memory responses, supporting the possibility of a microbiota-gut-brain axis in humans during early life.

INTRODUCTION

According to current estimates, ~10% of all newborns in the U.S. are treated with antibiotics immediately after birth, with many of these exposures being unnecessary (1,2). While many of these infants are discharged in a seemingly healthy state after a short hospitalization, there is a paucity of information about potential "non-target" effects of antibiotic exposure on infant health.

Antibiotic exposure during childhood has been linked to an increased risk of chronic diseases such as obesity, asthma, and inflammatory bowel disease (3–7). It has been hypothesized that these associated risks are mediated by the effects of antibiotics on gut commensal microbes. In particular, research in animal models supports a role for gut microbes in modulating brain function (the so-called Microbiome-Gut-Brain Axis), including cognition and behavior (8–10). The hippocampus, a brain structure critical to recognition memory and cognitive function, is the target of many of these gut microbial-mediated effects (11–13).

Early-life adverse exposures and stress are important risk factors for impaired neurodevelopment (14–16). Event-related potentials (ERP) have been used to evaluate early cognitive development and the effect of an exposure on brain function in pre-verbal infants. This technique has been widely used by cognitive neuroscientists to study discrimination, attention and memory (17). Studies of at-risk populations of infants, including preterm infants, infants of diabetic mothers and infants with neonatal encephalopathy, discovered disrupted memory function in the newborn period using ERPs (18–20). These studies showed that recognition memory can be evaluated in very young (newborn) infants and, importantly, that neonatal recognition memory is associated with later cognitive function (18, 21).

ERPs allow for early evaluation of the functional maturation of the hippocampus and other medial temporal lobe structures responsible for recognition memory in infants by recording the brain's electrophysiologic response to specific stimuli using electroencephalogram (EEG). The ERP waveform is the portion of the EEG that reflects cognitive processing. It is embedded in the raw EEG data and is extracted by averaging the EEG across multiple presentations of the stimulus (18). Studies of newborn auditory recognition memory have identified several electrophysiologic components that represent certain aspects of memory function that are thought to involve the hippocampus. An early peak around 150 – 400 ms (P2) represents the initial perceptual processing of the auditory stimulus. Cognitive processing of the stimulus is represented by a slow wave occurring after about 1000 ms (a negative slow wave (NSW) indicating novelty detection).

The purpose of this study was to explore the presence of a Microbiome-Gut-Brain axis in infants. We evaluated brain function by comparing ERPs obtained in response to an auditory

stimulus in infants exposed to antibiotics as newborns as compared to non-exposed infants. We hypothesized that antibiotic exposure would be associated with altered recognition memory responses at 1 month of age.

METHODS

Subjects and Study Procedures

This study was approved by the University of Minnesota Institutional Review Board. Antibiotic exposed infants (n=18) were recruited from the University of Minnesota Masonic Children's Hospital Neonatal Intensive Care Unit and the University of Minnesota Medical Center newborn nursery from 2017 – 2018, and unexposed infants (n=57) were recruited prenatally from Health Partners Clinics and Research Institute, Minneapolis, MN. The unexposed infants were delivered at several different hospitals. Similar to the delivery hospital for the antibiotic-exposed group, the control delivery hospitals were all within the Twin Cities metro area. The control and antibiotic-exposed infants were all recruited within the same 12 month time frame. Infants in both groups were enrolled after obtaining informed parental consent. Infants exposed to antibiotics were otherwise healthy newborns of 36 weeks gestation who were treated with intravenous antibiotics after delivery due to concern for infection. All infants in this group received Ampicillin 100 mg/kg every 12 hours and Gentamicin 3.5 mg/kg every 24 hours for empiric antibiotic treatment of suspected early onset sepsis. Duration of antibiotic therapy is shown in Table 1. Control infants not exposed to antibiotics (pre- or postnatal) were healthy and born at 37 weeks. Exclusion criteria for both groups included history of maternal diabetes, maternal recreational and/or chronic CNS-active drug use, diagnosis of neonatal encephalopathy, intrauterine growth restriction, and congenital or chromosomal abnormalities that would affect neurodevelopment including failed newborn hearing screen (as this would affect auditory ERP assessment). Clinical information was extracted from birth medical records and questionnaires administered at one month of age, including maternal antibiotic use during pregnancy, birth history, gestational age of infant, laboratory evaluation for infection, factors related to severity of illness during birth hospitalization, infant diet, and maternal and infant antibiotic exposure after discharge.

Newborn Auditory Recognition Memory Assessment

Both study group and control infants underwent auditory recognition memory assessment via ERP at the University of Minnesota Center for Neurobehavioral Development, as previously described (20). Infants were targeted for testing at 44 weeks post-conceptual age (i.e. 1 month post term), a target that was achieved (see Table 2). The range of postnatal (chronological) age was 3–6 weeks at testing. To ensure that there was not a gestational age effect, mean gestational age at the time of ERP was factored into the statistical analysis. Briefly, infant auditory recognition memory was assessed by recording ERPs in response to the mother's voice (familiar stimulus) randomly alternating with a stranger's voice (novel stimulus). Each stimulus type (familiar and novel) was presented 50 times per testing session. Interstimulus interval was 2500 ms. The auditory stimulus was the word "baby" digitized and edited to 750 ms in duration and 75 dB using Creative WaveStudio 5.00.10 software (Creative Technology, Singapore, Republic of Singapore). The stimuli were

presented via E-prime software (Psychology Software Tools, Sharpsburg, PA) through 2 speakers located 36 inches from the infant's head.

After measuring infant head circumference, ERPs were recorded with the Geodesic EEG system (Electrical Geodesics Incorporated (EGI), Eugene, OR) using a 64-electrode HydroCel Geodesic Sensor Net. During net placement, the reference electrode is placed at the vertex of the scalp, allowing for consistent placement of the electrodes on the scalp of the infant. Scalp impedances were measured using Netstation 4.4.2 software (EGI) and were accepted if <50 KU. EEGs were referenced to the vertex, amplified with 0.1–100 Hz bandpass and digitized at 250 Hz.

Serum Cytokine Determinations

Blood was obtained from infants exposed to antibiotics at the time of routine laboratory draws, at 24–48 hours of age. Inflammatory cytokines and proteins (tumor necrosis factor alpha (TNFα), interleukin 1 beta (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), and interferon gamma (IFNγ) were quantified by ELISA by The University of Minnesota's Cytokine Reference Laboratory (CLIA-88 licensed facility, #24D0931212).

ERP Data Analysis

Data were analyzed using Netstation 4.5.7 analysis software (EGI) as previously described (20). The two EEG electrodes below the infant's eyes (channels 62 and 63) and two of the electrodes on the outside canthii of the eyes (channels 61 and 64) were not used to avoid artifact due to blinking; this resulted in the use of recordings from a total of 60 electrodes. Data were filtered with a 30-Hz lowpass filter, segmented to 2100 ms periods starting 100 ms before the stimulus presentation, and baseline corrected to the average prestimulus voltage. EEGs were hand-edited for poor recordings, movement artifact and eye movements. The entire trial was excluded if more than 9 electrodes were rejected or if an eye blink, eye movement or other significant artifact had occurred. For acceptable trials, electrodes with unusable data were replaced using spherical spline interpolation. Participants with <20 acceptable trials per stimulus were excluded from further analysis. The average waveform at each electrode was calculated and re-referenced to the average reference.

We analyzed the ERP components reflecting auditory processing and recognition memory (17–19, 22). Mean P2 amplitude and latency were measured from a 150–400 ms window post-stimulus. Slow wave activity was analyzed by integrating areas under the curve measured from 900–2000 ms poststimulus. We analyzed mean data from adjacent groupings of electrodes (Figure 1) where we noted the most activity to stimuli, in the frontal, central, and posterior (slow wave only) scalp regions. These areas are consistent with the most prominent areas of activity in other studies of newborn auditory recognition memory (18–20, 22).

Statistical Comparisons

Infant clinical characteristics were summarized by groups. For between group comparisons, *p*- values were calculated by two-tailed *t*-tests. For ERPs, P2 amplitudes and latencies and NSW areas under the curve were analyzed using repeated measures Analyses of Variance

(ANOVAs) with antibiotic exposure as the factor. Significant findings were further evaluated by linear regression analysis to compare ERP features between the antibiotic-exposed and unexposed groups, adjusting for delivery mode, sex, and gestational age at the time of testing. The association of antibiotic exposure to ERP features is represented by beta coefficients (degree of change in ERP feature associated with antibiotic exposure) in Table 3. Box-cox (IL-6 and IL-1β) and log transformed (IL-8 and TNFα) cytokine values were used in linear regression analyses to determine associations between cytokine values and ERP features. The linear correlation coefficient (r) is used to represent the strength and direction of the relationship between cytokine values and ERP features in Table 4. A *p*-value of <0.05 was considered statistically significant for all data comparisons described above.

RESULTS

Study Cohort Characteristics

Seventy-two infants with acceptable ERP data were included in this data analysis (15 infants in the antibiotic-exposed group; 57 infants in the unexposed group). Of the 18 infants that met inclusion criteria for the antibiotic-exposed group, one was excluded due to excessive artifact in ERP recordings and 2 infants were not able to return for completion of 1 month ERP assessments. Details regarding delivery mode, admission location, reason for antibiotic treatment and duration of antibiotic course for infants in the antibiotic-exposed group are shown in Table 1. All antibiotic-exposed group infants received the same antibiotics (ampicillin and gentamicin). Reasons for urgent cesarean section included fetal intolerance of labor (including fetal tachycardia in the context of maternal fever) and failure to descend. Infants admitted with respiratory distress received short-term (range 2–24 hours) respiratory support via nasal cannula oxygen or nasal continuous positive airway pressure with or without oxygen. All infants in the antibiotic-exposed group had negative blood cultures. All but one infant had antibiotics stopped at (or shortly before) 48 hours. One infant was treated with 5 days of antibiotics for possible pneumonia due to precipitous delivery into the toilet and respiratory distress (but only required low flow nasal cannula support for 1 day and had a low CRP). All infants of mothers who were diagnosed with chorioamnionitis during labor were assigned an infection risk score on the basis of maternal risk factors and clinical exam after birth, according to the Kaiser Permanente Neonatal Early-Onset Sepsis Risk Calculator (23). Of infants treated with antibiotics due to maternal chorioamnionitis during labor, sepsis risk scores were less than 1.0/1000 live births, which according to risk stratification studies using the neonatal sepsis risk calculator, does not meet criteria for antibiotic treatment, but rather observation or blood culture and observation only. Thus, the risk scores of our infants were not high enough to meet criteria for empiric antibiotics based on sepsis risk score alone (24). Though none of the included infants who underwent evaluation for sepsis due to maternal chorioamnionitis during labor demonstrated clinical concern for infection or positive blood culture, in 4 cases, placental pathology revealed evidence of acute chorioamnionitis.

The clinical characteristics of the 2 groups are shown in Table 2. There were no group differences with respect to clinical features, except for 5 minute Apgar scores. All infants in both groups were receiving maternal breastmilk for at least a portion of their feedings.

Infant ERP Feature Comparisons

Grandmean ERPs for infants in both groups are shown in Figure 2. Each group showed similar waveform components, with a P2 response to both mother's and stranger's voices, indicating the presence of intact neuronal circuitry.

Analysis of overall mean P2 amplitude by group (including response to both mother and stranger voices) revealed a main effect of group (antibiotic-exposed vs. unexposed $p = 0.001$). This effect was due to lower overall mean P2 amplitude in the antibiotic-exposed group (2.08 mV vs. 3.84 mV in the unexposed group). There was also a significant interaction between scalp region and group ($p = 0.02$), with greater frontal (as compared to central scalp region) P2 amplitude in the unexposed group, whereas P2 amplitude was equal across scalp regions in the antibiotic-treated group, except for the right central region, where mean P2 amplitude was smaller.

Post-hoc analysis of associations of antibiotic exposure with mean P2 amplitude by scalp region (see Figure 1) and condition (response to mother vs. stranger voices) is shown in Table 3. Compared to control subjects, infants exposed to antibiotics had significantly smaller P2 amplitude in both frontal as well as the right central scalp regions in response to their mother's voice as well as significantly smaller P2 amplitude in response to stranger voice in left frontal and left central scalp regions (indicated by bolded negative B's in the Table). These associations persisted with adjustments for sex, gestational age and delivery mode (**Model 2 in** Table 3). Heat maps of P2 amplitude responses to mother's and stranger's voice in the two infant groups are shown in Figure 3.

Infants in the antibiotic-treated group demonstrated greater mean P2 amplitude in response to stranger's voice than mother's voice in all scalp regions (except left central) which is a reversal of the pattern seen in the unexposed group, where mother's voice elicited a greater P2 amplitude compared to stranger's voice in all regions (except left central) (Figure 4). To quantify and compare this change in pattern, a difference score was calculated for each group (mean P2 amplitude [familiar] - mean P2 amplitude [novel]) and compared between antibiotic-exposed and unexposed groups. The familiar-novel difference in mean P2 amplitude between antibiotic-treated and unexposed infants was statistically significant in the right frontal and left central regions (right frontal $p = 0.045$, 95% CI −3.4, −0.128; left central $p = 0.05$, 95% CI 0.20, 2.51).

Analysis of P2 component latency (time from stimulus presentation to P2 peak) revealed an apparent longer latency in response to mother's voice than stranger voice (difference of 16 ms) in the control group, but this difference did not reach statistical significance. There were no differences in P2 latency in response to familiar or novel stimuli in the antibiotic-treated group nor were there statistically significant differences in P2 latency between the antibiotic-exposed and unexposed groups. In addition, there were no significant differences in NSW

areas in response to mother's versus stranger voice in either antibiotic-exposed or unexposed groups or between groups.

Association of Inflammatory Markers with ERP Features

Since the antibiotic-exposed study group includes infants who are at risk for inflammation related to infection or other processes, and inflammation has been shown to affect neurodevelopmental outcomes (25), levels of pro-inflammatory proteins were determined from blood obtained 24–48 hours after birth in that group. Associations of inflammatory marker levels with mean P2 amplitude by condition and scalp region (those that showed significant decrease in P2 amplitude associated with antibiotic exposure) for the infants exposed to antibiotics are shown in Table 4. If inflammation was a covariable for ERP results in this group, we would expect that high levels of pro-inflammatory proteins are correlated with lower P2 amplitudes in the leads previously identified (Table 3). Although high levels of TNF-α were associated with lower P2 amplitudes to mother voice in the left frontal lead, no other inflammatory markers were negatively associated with P2 amplitudes in relevant leads (for mother or stranger voices) and, of note, IL-6 was instead positively associated with P2 amplitudes (i.e. inflammation associated with higher P2 amplitude) in one lead (Table 4). Thus, in this exploratory analysis, inflammation does not appear to be a covariable for ERP results in the antibiotic-exposed group.

DISCUSSION

This study found that infants exposed to antibiotics soon after birth who are otherwise healthy following a negative evaluation for sepsis have altered auditory processing and discrimination responses at one month of age. Infants in this antibiotic-exposed cohort represent an at-risk group, not only due to "non-target" effects of antibiotic exposure, but also due to potential consequences of reason for antibiotic treatment, circumstances surrounding delivery, admission location, infant diet and maternal-infant bonding. The altered ERP responses seen in the antibiotic-exposed group included reduced P2 amplitude and reversal of the expected P2 amplitude pattern in response to familiar versus novel stimuli. This study is unique in its use of ERP, a tool that allows detection of differences in brain function in preverbal infants and allows for assessment of a specific at-risk neural processes close to the time of exposure.

Infants who were exposed to antibiotics shortly after birth demonstrated decreased discrimination processing (lower P2 amplitude) of both familiar and novel stimuli, when compared to unexposed infants. In addition, infants exposed to antibiotics exhibited a stronger discrimination response to novel as compared to familiar stimuli. These changes are visually apparent in the heatmaps in Figure 3, which demonstrate significant discrepancy between the maps of exposed and unexposed groups, suggesting different neural circuit function between the groups. Furthermore, these infants did not demonstrate differences in P2 latency (speed of processing) or NSW area under the curve (novelty detection) in response to novel versus familiar stimuli. Previous studies of healthy newborns with more than 1 week of ex-utero experience report that these infants demonstrate larger amplitude and longer latency of P2 to familiar stimuli (17,18). The infants exposed to antibiotics in the

study cohort failed to exhibit these patterns, suggesting that they may be processing familiar and novel stimuli differently than unexposed infants.

In addition to the overall decreased P2 amplitude seen in the antibiotic-exposed group, we also observed a scalp location-specific reversal of the P2 amplitude pattern in response to mother versus stranger voice in both groups of infants. While the unexposed infants demonstrated the expected pattern of greater P2 amplitude in response to mother's voice compared to stranger's voice in all except the left central scalp region, the infants exposed to antibiotics showed the reverse pattern, with greater P2 amplitude in response to stranger voice everywhere except the left central region. Given that the P2 represents a discrimination process that is modulated by attention, larger response to the mother's voice in the unexposed group may indicate greater attention allocation to the familiar voice (26). The exposed group's failure to exhibit greater amplitude P2 to the mother's voice may indicate deficits in the ability to encode and discriminate meaningful stimuli (mother's voice) from less meaningful stimuli (stranger's voice).

Systemic inflammatory cytokines are important mediators of host response to stress and infection. They have been implicated in neuroinflammation and have been shown to have effects on cognition (25, 27–28). In order to evaluate inflammation as a potential covariable with neurodevelopment, we analyzed associations between inflammatory marker levels and ERP features. Our sub-analysis of the association of P2 amplitude with inflammation failed to show a consistent trend in the antibiotic-exposed group. Furthermore, pathophysiologicially, we would expect that if inflammation was a covariable in the ERP changes we observed, speed of processing (P2 latency) differences would also be present between the exposed and unexposed groups. Latency measurements reflect speed of processing, which is a function of neural substrate, such as myelin, and synaptogenesis. Pro-inflammatory cytokines are toxic to oligodendrocytes (29) and can result in lower myelin content, and therefore, slower speed of processing. Therefore, the absence of observed P2 latency differences, along with the lack of association between inflammatory marker levels and the observed ERP changes, does not support inflammation as contributing to the ERP patterns seen in the antibiotic-exposed group.

Antibiotic exposure during infancy causes dysbiosis, that is, a disruption in the usual composition of gut microbial communities (30). Existing animal model data indicate that gut microbes have effects on brain function, however, evidence of this relationship in humans, particularly during the relatively rapid period of brain development during infancy, are lacking. We postulate that perturbation of gut microbes is one potential reason for the observed ERP changes seen in infants exposed to antibiotics in our study. Several key studies indicate that antibiotic exposure changes gut microbial communities (characterized by 16S sequencing) at least transiently (30). These reports suggest that after relatively short courses of antibiotics, microbiome diversity recovers over the timespan of several weeks to several months (32–35). Given the evidence that brief courses of antibiotics alter the gut microbiome for weeks to months, our study results provide support for the existence of a Microbiome-Gut-Brain Axis in infants, and raises the possibility that disruption of this axis affects brain development. In the future, longitudinal studies are needed to test the

hypothesis that specific microbiome structural and functional features are linked to infant brain development.

ERPs are quite sensitive and can detect important differences between groups even with small sample sizes (17–19). Although we were able to detect ERP changes in underlying circuitry in the infants exposed to antibiotics, the small sample size and scope of this pilot study size precluded multivariate analysis of all potential covariables. Though there were 2 infants in the antibiotic-exposed group who were 36 to <37 weeks' gestation at birth, or late preterm, gestational age at the time of ERP did not statistically affect ERP performance. Inflammation and mode of delivery also did not affect ERP performance, based on our analyses. It remains possible that other clinical factors associated with the decision to treat with antibiotics and/or NICU admission are driving the ERP differences, separately or in addition to dysbiosis caused by antibiotics. For example, the mean 5 minute Apgar score was lower in the antibiotic-treated group, and this was likely part of the clinical factors that led the healthcare team to decide to treat with antibiotics. We used the Apgar scoring system as a measure of a newborn's condition at birth and response to resuscitation, but this scoring system was not designed to be used as a prediction tool for outcomes beyond the immediate postnatal period (31).

Diet is an additional covariable for both gut microbiome composition and neurodevelopment. While all infants in the study received at least partial feedings of maternal breast milk, detailed milk intake data was unavailable for the group of infants exposed to antibiotics, however all of them received a portion or all of their diet as breasmilk. All unexposed infants were exclusively breastfed at 1 month. An exclusive breast milk diet has been associated with faster speeds of processing for both auditory and visual stimuli as compared to formula diet (38). Because we did not observe any latency differences between the two groups (as would be expected with speed of processing differences), and the number of infants receiving at least a portion of their diet via breast milk did not differ between the groups, it is unlikely that diet differences significantly affected our results. Another potential covariable that we were unable to fully evaluate includes the possibility of disrupted maternal-infant bonding in the neonatal period related to antibiotic-treatment or NICU admission. While the majority of the infants in the antibiotic-exposed group required only brief (if any) NICU admission, this study did not have adequate power to control for the potential effects of disrupted maternal-infant bonding on infant neurodevelopment via breastfeeding patterns or other mechanisms (37), which, to our knowledge, has not been studied in human infants. Furthermore, the infants in this study were born at several different hospitals. Although all hospitals were within the Twin Cities metro area, we could not evaluate for possible differences related to specific delivery hospitals.

One implication of our study findings is that infants discharged following a negative sepsis evaluation after birth may have unrealized risk factors for altered brain development, including the clinical factors that increase suspicion for infection, and the factors that result from antibiotic treatment and hospitalization. While efforts are ongoing to improve antibiotic stewardship and precision of diagnostic tools, infections increase neonatal morbidity and mortality, and there are situations in which antibiotics must be used.

Clarifying the potential impact of specific risk factors in the perinatal and neonatal period and identifying early markers that help identify infants at risk will highlight opportunities to alter these exposures, or develop targeted interventions to alleviate potential impact.

In conclusion, our study shows that infants exposed to antibiotics during the birth hospitalization, and subsequently "ruled-out" for infection, demonstrate altered attentional perceptual discrimination responses at one month of age. While this group of infants was exposed to several risk factors, the observed changes in perceptual encoding provide support for the hypothesis that a healthy gut microbiota-brain axis contributes to brain development. Given the prevalence of perinatal and postnatal antibiotic exposure, there is a need for larger, prospective studies to determine the role of gut microbes and the potential long-term clinical significance of early-life antibiotic exposure.

Funding statement:

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Impact:

- Infants exposed to antibiotics after birth demonstrate altered auditory processing and recognition memory responses at one month of age

- Preclinical models support a role for gut microbiomes in modulating brain function and behavior, particularly in developing brains. This study is one of the first to explore the relevance of these findings for human infants.

- The findings of this study have implications for the management and follow-up of at-risk infants with exposure to gut-microbiome disrupting factors and lay foundation for future studies to further characterize the short- and long-term effects of gut microbiome perturbation on brain development.

Figure 1.
Schematic of the 64-channel HydroCel Geodesic sensor net (Netstation, EGI) and clusters of electrodes (circled) used for data analysis of P2 waveforms. Leads 7, 9, and 12 make up the right frontal group; leads 16, 20, and 22 comprise the right central lead grouping; leads 3, 54, and 60 the left frontal and 49, 50 and 51 the left central lead grouping.
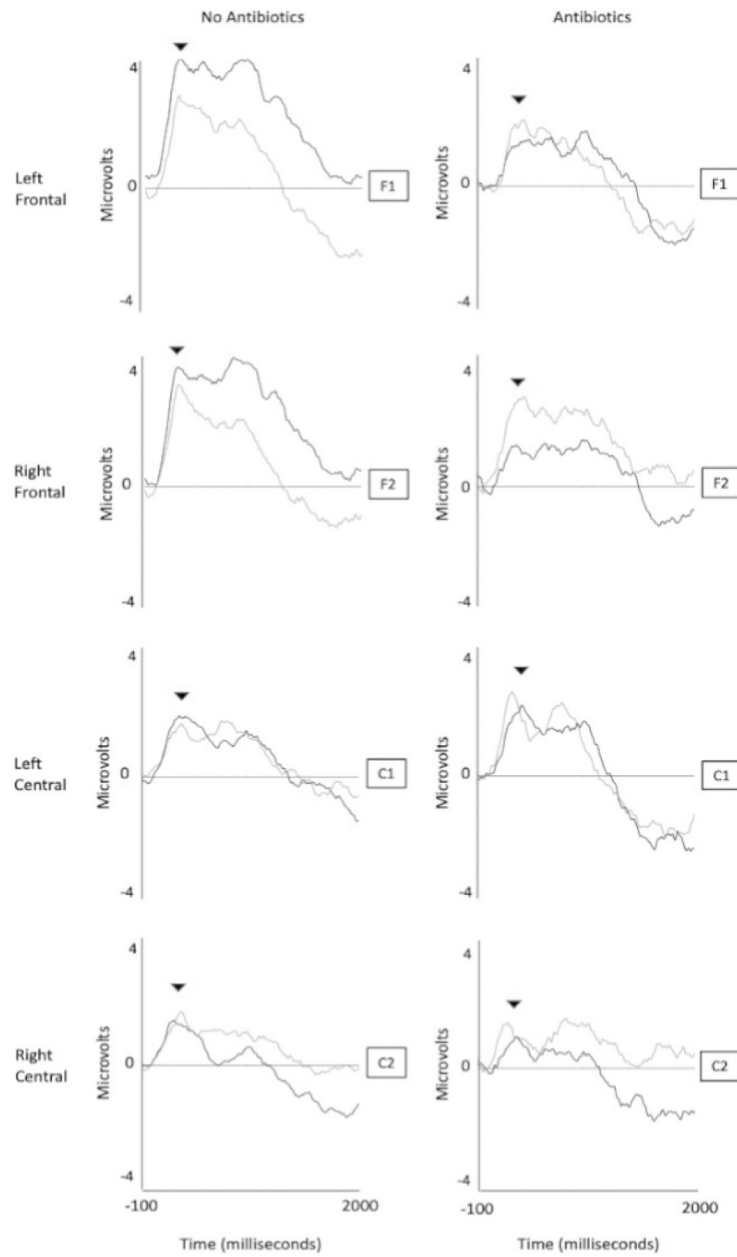
Figure 2.
Grandmean event-related potentials in response to mother's voice (black line) and stranger's voice (gray line). Each row contains a representative lead from each scalp electrode region cluster. Arrowheads denote P2. Equivalent of 10–10 scalp net system lead is indicated in the box to the right of each grandmean x-axis.
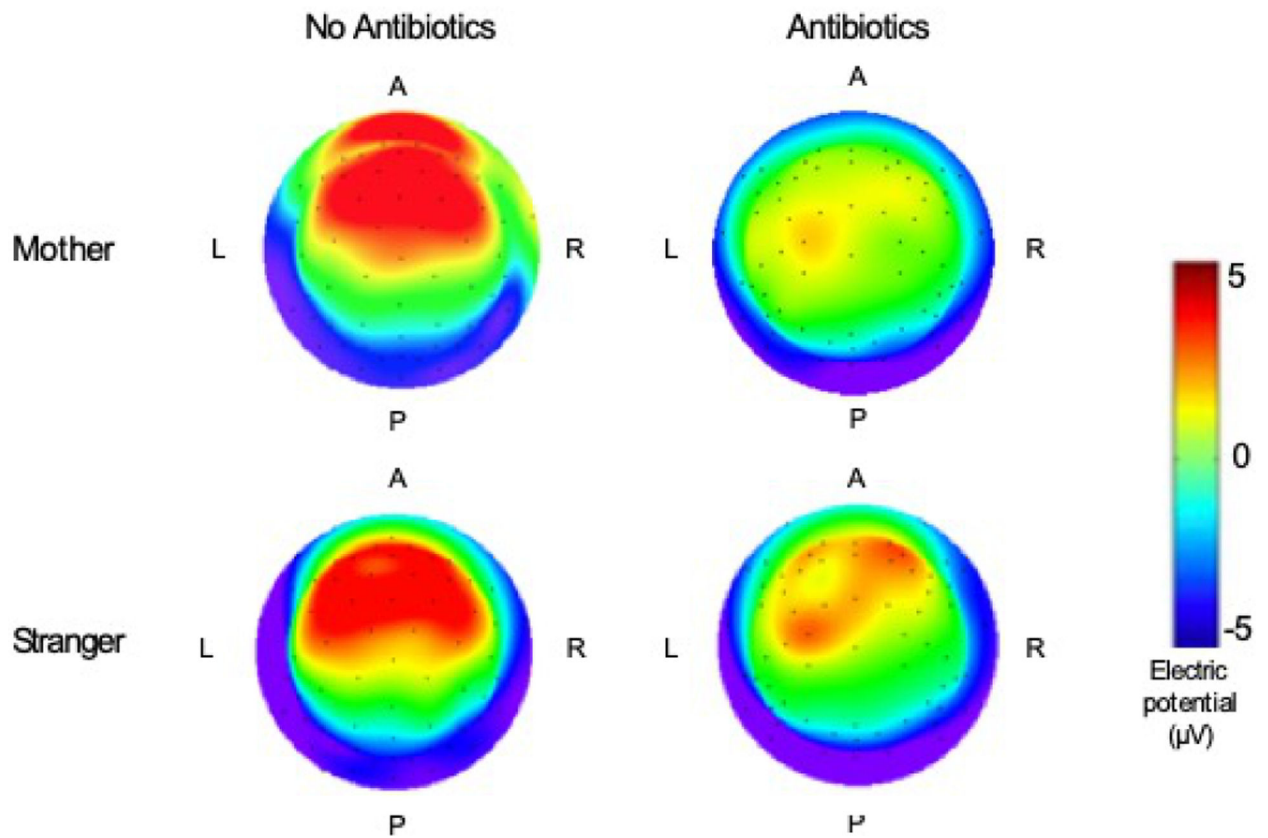
Figure 3.
Heatmaps of P2 amplitude responses (electrical potentials depicted as a color gradient) to mother and stranger voice in infants exposed to antibiotics or not. Cranium orientation indicated by letters (A: anterior, P: posterior, L: left, R: right). Black dots represent individual scalp electrode locations.
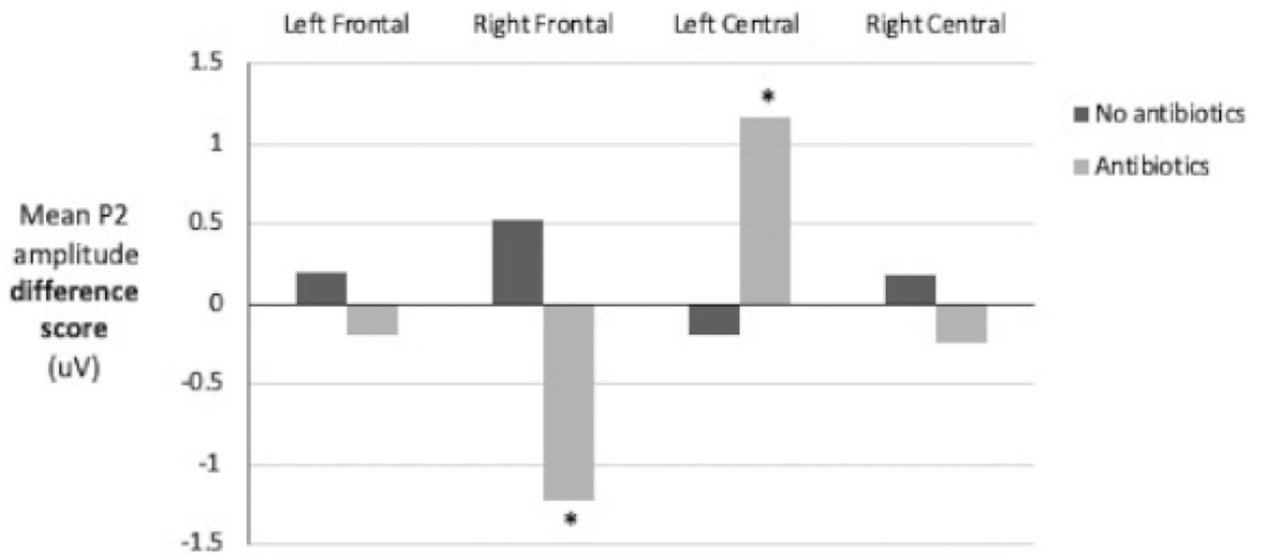
Figure 4.
Difference in P2 amplitude (familiar – novel) by scalp region. Dark gray, control group; light gray, antibiotic-treated group. *p <0.05.

Table 1.

Characterization of antibiotic-exposed study cohort

Subject number	Delivery mode	Admission location	Reason for antibiotic treatment	Blood culture result	Duration of antibiotics
1	Urgent cesarean section	NICU	Respiratory distress	Negative	48 hours
2	Vaginal	NICU	Respiratory distress	Negative	48 hours
3	Urgent cesarean section	Nursery	Maternal chorioamnionitis	Negative	48 hours
4	Vaginal	Nursery	Maternal chorioamnionitis	Negative	48 hours
5	Vaginal	NICU	Respiratory distress	Negative	48 hours
6	Scheduled cesarean section	NICU	Respiratory distress	Negative	48 hours
7	Urgent cesarean section	NICU	Respiratory distress	Negative	36 hours
8	Vaginal	NICU	Respiratory distress	Negative	48 hours
9	Vaginal	NICU	Respiratory distress	Negative	120 hours
10	Vaginal	NICU	Respiratory distress	Negative	48 hours
11	Vaginal	Nursery	Maternal chorioamnionitis	Negative	48 hours
12	Urgent cesarean section	Nursery	Maternal chorioamnionitis	Negative	48 hours
13	Urgent cesarean section	NICU	Respiratory distress	Negative	36 hours
14	Vaginal	NICU	Respiratory distress	Negative	48 hours
15	Vaginal	NICU	Respiratory distress	Negative	48 hours

Table 2.

Infant characteristics by group

Newborn Characteristic	No antibiotics (N=57)	Antibiotics (N=15)	p
Sex - Female n (%)	30 (52.6)	10 (66.7)	0.33
Birth gestational age - mean ± SD (Range) (weeks)	39.82 ± 1.06 (37.1 – 41.7)	39.26 ± 1.87 (36 – 42)	0.13
Birth weight - mean ± SD (kg)	3.53 ± 0.43	3.35 ± 0.52	0.17
Corrected gestational age at 1 month visit - mean ± SD (weeks)	44.3 ± 1.1	43.8 ± 1.8	0.23
Mode of delivery - Cesarean n (%)	11 (19.3)	9 (60)	0.09
Maternal race - Caucasian race n (%)	50 (87.7)	13 (86.7)	0.91
5 min Apgar mean ± SD	8.9 ± 0.2	7.3 ± 2.0	0.006

Table 3.

Association of antibiotic exposure with mean P2 amplitude by lead grouping

Condition	Region	Unadjusted model		Model 1[a]		Model 2[b]	
		B[c] (SE)[d]	p	B (SE)	p	B (SE)	p
Mother voice	Left frontal	**−2.865** (0.816)	**0.0008**	**−2.583** (0.814)	**0.002**	**−2.585** (0.844)	**0.003**
	Right frontal	**−3.313** (0.794)	**<0.0001**	**−3.081** (0.799)	**0.0003**	**−3.054** (0.829)	**0.0005**
	Left central	−0.350 (0.712)	0.62	−0.208 (0.722)	0.77	−0.497 (0.735)	0.50
	Right central	**−1.445** (0.571)	**0.014**	**−1.377** (0.586)	**0.022**	**−1.288** (0.606)	**0.037**
Stranger voice	Left frontal	**−2.333** (0.888)	**0.011**	**−1.884** (0.854)	**0.031**	**−2.035** (0.883)	**0.024**
	Right frontal	**−1.495** (0.845)	**0.008**	−1.091 (0.820)	0.19	−1.251 (0.846)	0.14
	Left central	**−1.768** (0.717)	**0.016**	**−1.614** (0.727)	**0.030**	**−1.511** (0.752)	**0.049**
	Right central	−1.071 (0.644)	0.10	−0.862 (0.645)	0.19	−0.842 (0.669)	0.21

[a]Adjusted for sex and gestational age at time of ERP

[b]Adjusted for sex, gestational age at time of ERP and delivery mode

[c]B, Beta coefficient

[d]SE, standard error

Significant inverse associations are in bold

Table 4.

Associations of inflammatory markers with mean P2 amplitudes in antibiotic-exposed infants

		IL - 6		IL - 8		TNFα		IL1 beta	
Condition	Region	r[a]	p	r	p	r	p	r	p
Mother Voice	Left Frontal	0.49	0.06	−0.36	0.18	**−0.56**	**0.03**	−0.03	0.93
	Right Frontal	**0.54**	**0.04**	−0.28	0.31	−0.21	0.43	0.04	0.88
	Right Central	0.49	0.06	−0.36	0.19	−0.14	0.62	−0.24	0.39
Stranger Voice	Left Frontal	0.40	0.14	−0.06	0.83	−0.34	0.22	0.14	0.63
	Left Central	−0.04	0.88	−0.15	0.59	−0.23	0.41	−0.46	0.09

[a]Linear correlation coefficients representing association of pro-inflammatory protein measurements to mean P2 amplitude by regional scalp lead grouping Significant correlations are in bold