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Breast-Fed Infants Process Speech Differently From Bottle-Fed Infants: Evidence From Neuroelectrophysiology

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Numerous studies report positive effects of breast-feeding on infant development. Such effects are apparent early in development as well as in later years. Recently, elements in breast milk, polyunsaturatred fatty acids (PUFAs), have been identified as having great potential for increasing nutritional benefits. PUFAs are long-chain fatty acids containing two or more double bonds. While some scientists are enthusiastic about the long-term benefits of PUFAs on brain and cognitive development, many of the positive pharmacological effects attributed to PUFAs remain unsubstantiated. The present study investigated the differential impact of breast-feeding vs. PUFA-enriched formula in a small but well-matched population of 12 infants tested at 6 months of age. Event-related potential (ERP) and a range of behavior measures were recorded. ERP waveforms identified marked differences between the breast-fed and PUFA-fed infants by 6 months of age. When a range of biological, perinatal, and cognitive factors were equated between the two groups, only the ERPs recorded from breast-fed infants changed throughout their recorded period (700 msec), differentiated between all speech sounds, and generated differences in scalp recordings across all regions recorded across both hemispheres. Such differences in the range of their brain responses could signal an advantage for the breast-fed infants for later linguistic and cognitive development.

Previous research indicates that breast-feeding during infancy provides benefits that influence cognitive processing and developmental outcomes (Quinn et al., 2001). Numerous studies report positive effects of breast-feeding on infant development. Such effects are apparent early in development as well as in later years.

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Amanda and Singh (1992) found that those children who were breast-fed for more than 4 months during infancy scored higher on a mental ability test than those who were bottle-fed during the first 4 months of life. In their investigations into long-term effects, Gomez-Sanchiz, Canete, Rodero, Baeza, and Avila (2003) found that infants that were breast-fed for more than four months scored higher than bottle-fed infants on the Mental Developmental Index of the Bayley Scales of Infant Development (Bayley, 1993) at 18 months of age, a study replicated and extended by Rogan and Gladen (1993).

Additional research suggests that breast-feeding provides long-term cognitive benefits up to 7-years of age. Fergusson, Beautrais, and Silva (1982) reported higher scores among those children who were breast-fed during infancy at 3, 5, and 7 years of age on the Wechsler Intelligence Scales for Children, Peabody Picture Vocabulary Test, and the Reynell Developmental Language Scales. Rogan and Gladen (1993) replicated these findings and further found that developmental differences during infancy carried over into not only cognitive performance but later academic performance as well at 3, 4, and 5 years of age.

More recently, elements in breast milk, polyunsaturated fatty acids or PUFAs, have been identified as having great potential for increasing nutritional benefits. PUFAs are long-chain fatty acids containing two or more double bonds. While some scientists are enthusiastic about the long-term benefits of PUFAs on brain and cognitive development, many of the positive pharmacological effects attributed to PUFAs remain unsubstantiated.. Furthermore, although commercial businesses have released PUFA-enriched infant formula and claimed both neurological and cognitive long-term benefits, the importance of PUFA in infant feeding formula as compared with the documented advantages of breast milk remains unclear.

There continues to be a need to assess and contrast the impact of PUFA-enriched formula vs. breast-feeding on subsequent brain and behavior development. If breast-feeding is superior to PUFA-enriched formula in facilitating brain development, measures of brain processing should reflect such differences. However, if there are no such advantages, these same brain measures should fail to detect differences. To address this issue, a technique was needed that would be effective in identifying early brain response. For this reason we selected the event-related potential (ERP). ERPs are a portion of the ongoing electroencephalography (EEG) activity of the brain that is time locked to the onset of some stimulus event (Molfese, Molfese, & Kelly, 2001). An extensive literature indicates that ERPs are sensitive to changes in cognitive processing while infants are engaged in a variety of cognitive-related tasks (Molfese & Molfese, 1979; Molfese, Molfese, & Pratt, in press).

ERPs can provide information concerning the brain's speed of responding to the input of information through auditory and visual sensory systems. This information is usually coded in the latency of a component of a brain response. For example, the second large positive peak (P2) recorded from dyslexic children occurs up to 100 msec later in time than the same component recorded from advanced readers of the same age (Molfese, et al., 2006). In this case, better readers generated brain response components of shorter latency than children who have a reading disability. In addition to its sensitivity to processing speed, the procedure also provides information concerning the spatial distribution of these responses across the scalp as reflected in variations in ERP waveforms collected at different electrode sites over different hemispheres and brain regions (e.g., frontal vs. temporal). Such information has been effectively used to both identify infant processing capabilities as well as to predict developmental outcomes for such functions as language and reading (Molfese & Molfese, 1985; Molfese, 2000; Molfese et al., in press).

The present study investigated the differential impact of breast-feeding vs. PUFA- enriched formula in a small but well-matched population of infants tested at 6 months of age. In order to assess possible differences in brain processing, we decided to control for differences in cognitive development and only vary the feeding source. A strong form of the PUFA-enriched argument suggests that brain functions as measured by ERPs should be the same in children with comparable cognitive abilities, regardless of whether these infants were breast- or bottle-fed. On the other hand, if brain response differences do exist in infants who were breast-fed vs. those who received PUFA-enriched formula, it would be clear that the PUFA-enriched formula did not produce comparable levels of brain development. In controlling a range of biological, perinatal, and cognitive factors, we hypothesized first that breast-fed infants would be advantaged for later cognitive development and as a consequence would exhibit a mix of left and right hemisphere ERP component changes that were sensitive to speech stimuli (Molfese & Molfese, 1985; Molfese, 2000). Infants fed with PUFA-enriched formula, in contrast, were expected to generate stimulus-sensitive ERPs that were restricted to one hemisphere. A second hypothesis expected that breast-fed infants would generate larger ERP components to speech sounds than infants fed with the PUFA-enriched formula. The third hypothesis expected that components of the ERP from breast-fed infants would vary throughout the waveform while the responses of infants receiving the PUFA formula would be more restricted temporally in their responses.

METHODS

Participants

Twelve infants (six males, six females) participated in this study. The participants ranged in age from 5 months, 26 days to 7 months, 20 days (Mean = 6 months, 20 days; $SD = 15.95$ days). All infants participated in two phases of testing, a behavioral assessment session that evaluated the infants' developmental level, and an ERP portion, in which brain responses to auditory speech stimuli were recorded. The participants were divided into two groups based on feeding method from birth (exclusively breast-fed, with no formula supplement, and exclusively bottle-fed PUFA formula). The breast-fed group included 3 males and 3 females ($n = 6$) with a mean age of 6 months, 13 days ($SD = 10.68$ days). The bottle-fed group included 3 males and 3 females ($n = 6$), with a mean age of 6 months, 26.5 days ($SD = 18.41$ days). Analyses of variance revealed no significant differences between the groups in age, birth weight, apgar scores, gestational age, or maternal age. Characteristics of the infants are presented in Table 1. The maternal education level of both groups was comparable, with most mothers having some college education. Of the mothers who breast-fed their infants, three had masters degrees, two had bachelor degrees, and one completed high school. For mothers who bottle-fed their infants, two had masters degrees, two had bachelor degrees, one completed three years of college, and one completed high school.

Behavior Measures

The Mental Development Index (MDI) of the Bayley Scales of Infant Development, Second Edition (BSID-II) (Bayley, 1993) was administered as a tool to assess each infant's developmental level. The test was administered to each infant in the standard manner immediately prior to the electrophysiological portion of the testing session. All infants scored within the normal range of mental development according to scores on the BSID-II (Mean = 96.67, $SD = 4.54$, range = 86–102). The test revealed no significant differences in the developmental level between the two infant groups.

Stimuli

The auditory stimuli consisted of six consonant-vowel (CV) syllables adapted from the "transition-only" stimulus series employed by Stevens and Blumstein (1978). The consonants selected were /b, d, g/, and were paired individually with the vowels /a, u/. The tokens selected for the present study were the ones most

TABLE 1
Characteristics of Participant Population for This Study

Group	*N (m/f)*	*GA (wks)*	*Birthweight (g)*	*Mother's Age (yrs)*	*Apgar 1 min*	*Apgar 5 min*	*Age at Test*	*MDI Score*
Breast-fed	6 (3/3)	38.80 (1.30)	3330.45 (683.37)	33.00 (4.19)	8.67 (.52)	9 (0.0)	6 months, 13 days (10.68)	98 (3.79)
Bottle-fed	6 (3/3)	38.83 (1.17)	3462.54 (439.94)	29.50 (3.62)	8.5 (.55)	9 (0.0)	6 months, 26.5 days (18.41)	95.33 (5.16)

accurately identified by the adult subjects in the above study as members of their respective phonetic categories. These were stimulus tokens 1, 7, 14 from the /ba, da, ga/ continuum and 1, 7, 13 from the /bu, du, gu/ continuum, respectively. The five-formant CVs were synthesized on a Klatt (cascade) synthesizer, so that the amplitude of individual formants was modulated as a function. of the respective formant frequencies, as in natural speech. To further improve the naturalness of the stimuli, the vowel /u/ was slightly diphthongized. The central frequencies of the steady-state portion of the formants were kept constant across different consonants and only varied as a function of the vowel sounds. Duration of F1 transition ranged between 15 and 45 msec across tokens depending on the syllable-initial consonant as well as on the following vowel. Transition duration for all the other formants was always 40 msec and was followed by a 250 msec steady-state vowel. Rise and decay times were identical for all stimuli. Twenty-five orderings of the six syllables were presented to each infant. The speech stimuli were presented from a speaker 1 meter above and centered over the midline of each infant's head. Interstimulus intervals varied randomly (2.5–4.0 seconds) in order to reduce habituation and expectation effects.

Procedure

Each infant was individually tested in a sound-attenuated testing room. Once the infant was seated in his/her caregiver's lap, the circumference of the infant's head was measured to determine the appropriate size high density 128-electrode net. To assist in appropriate alignment of the electrode net, measurements were also made from nasion to inion and meatus to meatus, to locate the vertex of the head.

A 128-channel high-density array of Ag/AgCI electrodes embedded in soft sponges and arranged into a net (Geodesic Sensor Net, EGI Inc.) was used to record auditory ERPs. All electrodes initially were referred to vertex (Cz) during data collection and then re-referenced to an average reference for data analyses. Impedance levels remained under 40 kOHM throughout testing as measured before and after the testing session. The high pass filter was set to 0.1 Hz and the low pass filter to 30 Hz. Prior to application, electrodes were soaked in a warm potassium chloride solution (1.5 teaspoons of KCI, 1 liter of distilled water with three cc of Johnson's Baby Shampoo) that served as a conductor for electrical currents from the scalp to the electrodes of the net.

Electrophysiological data were recorded using Net Station 3.0 (EGI. Inc.) and stimulus presentation was controlled by E-Prime, v. 1.1. The infant's EEG and behavioral state was continuously monitored to determine when stimulus presentation should occur. Stimulus presentation was suspended during periods of motor activity or state change, and testing was resumed when the infant's motor activity returned to an acceptable level. Infants were in an awake state throughout testing. The data were continuously sampled every 4 msec over a one-second period (250 samples/sec) and included a 100 msec pre-stimulus baseline interval and a 900 msec post-stimulus interval. All ERPs were amplified 10,000 times during data collection.

Data Analysis

Based on past analyses (Molfese, 2000), final analyses were conducted on the 700 msec time interval following stimulus onset. The ERP data from all participants were re-referenced to the average of all electrodes, prior to all statistical analyses, and then subjected to artifact rejection, in which ERPs contaminated by motor movements were removed from further analysis. Individual trial ERPs were screened following standard procedures for eye related artifacts. An automated artifact rejected algorithm for artifacts (i.e., eye movements, eye blinks, motor artifacts, etc.) and bad channels (bad electrodes) was used. Trials with eye electrode differences in excess of +/-70 μV or more than 5% bad channels (defined as detecting voltage shifts in excess of 150 μV within and across trials) were rejected. Following artifact rejection, segmented data were averaged individually for each infant. Electrodes identified as having poor signal quality on 10% or more of the trials were replaced by interpolating their data from immediately adjacent electrodes. Subsequent to baseline correction and averaging procedures, all data from the 128 electrodes were clustered into 12 regions by averaging the data for electrodes within six anatomical regions for both hemispheres (frontal, central, parietal, occipital, anterior temporal, and posterior temporal). This approach reflected anatomically based boundaries and represented a modification of the clusters initially proposed by Curran (1999). The purpose of this clustering procedure was to reduce the number of variables in order to increase statistical power.

The data were then submitted to a Principal Components Analysis with Varimax rotation to identify regions of variability in the ERP, using a Scree criterion (Cattell, 1966) followed by an ANOVA to determine whether such variability occurred systematically in relation to the variables under investigation. This analysis sequence followed the procedures used by Molfese and others since 1976 (Molfese, Nunez, Seibert & Ramanaiah, 1976) and used extensively in programmatic research across numerous laboratories (Brown, Marsh, & Smith, 1979; Chapman, McCrary, Bragdon, & Chapman, 1979; Donchin, Teuting, Ritter, Kutas, & Heffley, 1975; Ruchkin, Sutton, Munson, Silver, & Macar, 1981; Segalowitz & Cohen, 1989). Moreover, findings replicate across laboratories (e.g., Gelfer, 1987; Segalowitz & Cohen, 1989). The analysis approach has proven successful both in identifying ERP regions where most of the variability occurred across ERPs and participants, and subsequently in determining if the variability characterized by the different PCA extracted factors results from systematic changes in the independent variables under investigation (Rockstroh, Elbert, Birbaumer, & Lutzenberger, 1982, page 63). When questions are raised regarding misallocation of variance in a PCA analysis across immediately adjacent peaks (Wood & McCarthy, 1984), even Wood and McCarthy noted that traditional amplitude and latency approaches are "no less subject to the problem of component overlap" (page 258, see also Chapman & McCrary, 1995). Furthermore, when sufficient power is available the likelihood of misallocation is marginalized. "To sum up ... the results of Wood and McCarthy (1984) were due to an excessive and unrealistic statistical power. Moreover, baseline-to-peak measures are not superior to PCA with respect to variance misallocation and thus, this comparison supports the use of the PCA-Varimax strategy for ERP component analysis" (Beauducel & Debener, 2003, page 112). Given our current power estimates, we consider the PCA approach reasonable for the present investigation.

Once the PCA identified where within the ERPs most of the variability occurred, the ANOVA was employed to identify the source for this variability. Scores from each rotated factor served as the dependent variables in the repeated measures ANOVA. The ANOVA design included Groups (2: Breast-fed, PUFA formula) × Consonants (3: b, d, g) × Vowels (2: a, u) × Electrode Region (6: frontal, central, occipital, parietal, anterior temporal, posterior temporal) × Hemisphere (2: left, right). Significant findings were investigated further using post-hoc analyses.

RESULTS

The PCA identified five factors that accounted for 93.15% of the total variance. Factor 1 accounted for 33.18% of the total variance, and corresponded to the time portion of the ERP between 368 and 700 msec following stimulus onset. The maximum region of variability for this factor occurred at 640 msec. Factor 2 encompassed the temporal range between 176–544 msec and accounted for 27.87% of the total variance (maximum peak latency = 352 msec). Factor 3 accounted for 16.12% of the variance, and characterized the ERP region between 40 and 216 msec (maximum peak latency 128 msec). Factor 4 (8.84% of the variance) characterized the ERP variability in the initial portion of the ERP between 8 and 104 msec (maximum peak 24 msec). Factor 5 accounted for 7.14% of total variance between 168 and 280 msec (maximum peak = 224 msec). The effects below are reported in the order in which they occurred temporally.

An Electrode × Hemisphere × Group interaction for Factor 4, $F(5,50)$ 2.788, $p <$ .027, power = .788, identified ERP differences that occurred from 8 to 104 msec between the two infant groups over left central, $t(10) = 2.470$, $p = .033$, left anterior temporal $t(10) = 2.616$, $p = .026$, and left posterior temporal electrode sites, $t = (10) = -2.564$, $p = .028$. Breast-fed infants generated larger amplitude ERPs over left central and left anterior temporal regions, while bottle-fed infants displayed larger ERP amplitudes over the left posterior temporal area.

A Vowel × Hemisphere × Electrode × Group interaction was also noted for Factor 4, $F(5,50) = 2.9$, $p < .023$, with breast-fed infants generating larger amplitudes over the left central region, $t(10) = 2.443$, $p < .04$ in response to the vowel, /a/, while bottle-fed infants displayed greater left parietal $t(10) = -2.521$, $p < .03$, left occipital, $t(10) = -2.999$, $p < .02$, and left anterior temporal ERP amplitudes, $t(10) = -3.666$, $p < .01$, to this same vowel.

Subsequently in time, an Electrode × Group interaction (Factor 3) characterized ERP changes between 40 and 216 msec, $F(5,50) = 4.610$, $p < .002$, power = .959. Posthoc analyses indicated that breast-fed infants generated larger amplitude ERPs over frontal, $t(10) = 2.611$, $p < .05$, and occipital electrode sites, $t(10) = -3.781$, $p < .01$. No effects were noted for the PUFA-fed infants.

A significant Hemisphere × Group interaction for this time period, $F(1,10) = 6.266$, $p < .031$, power = .618, indicated that the breast-fed group produced larger amplitude ERPs over the right hemisphere, $t(10) = 2.956$, $p < .02$. No hemisphere differences occurred at this latency for the PUFA-fed infants.

A Vowel × Hemisphere × Group interaction was also noted within the same time period, $F(1,10) = 11.101$, $p < .008$, power = .851, with posthoc analyses indicating that breast-fed infants generated larger amplitude ERPs in response to /a/ over right hemisphere electrode regions, $t(10) = -3.463$, $p < .01$, while bottle-fed infants produced larger amplitudes over left hemisphere electrode sites, $t(10) = 4.022$, $p < .01$. Subsequently, ERP variations between 176 and 544 msec (Factor 2), produced a significant Consonant × Electrode × Group interaction, $F(10,50) = 2.128$, $p < .029$, power = .88. Paired samples t-tests indicated that breast-fed infants produced larger amplitude ERPs over posterior temporal electrode sites in response to the consonant /d/, $t(10) = 2.477$, $p < .05$. No such effects were noted for PUFA-fed infants.

At the end of the ERP region under analysis (368–700 msec), posthoc analyses of a Vowel × Hemisphere × Group interaction (Factor 1), $F(1,10) = 7.07$, $p < .024$, power = .67, indicated that bottle-fed infants generated larger brain ERPs over the left hemisphere, $t(10) = -2.826$, $p < .02$, while the breast-fed group displayed larger amplitudes over right hemisphere electrode sites, $t(10) = 2.400$, $p < .04$.

DISCUSSION

As noted above, a strong form of the PUFA-enriched argument advances the position that brain functions as measured by ERPs should be the same regardless of whether these infants were breast- or bottle-fed. However, analyses of the ERP waveforms identified marked differences in the ERPs recorded from these two groups. Indeed, throughout the 700 msec recording period, multiple differences in the ERPs recorded from the two groups differed within and between hemispheres and in response to specific consonant and vowel features. Infants receiving the PUFA-enriched formula did not generate similar levels of brain activation as breast-fed infants. These results support our hypothesis that breast-fed infants are advantaged for later cognitive development and as a consequence exhibit a mix of left and right hemisphere ERP component changes that are sensitive to speech stimuli (Molfese & Molfese, 1985; Molfese, 2000). Infants fed with PUFA-enriched formula, in contrast, were expected to generate stimulus-sensitive ERPs that were restricted to one hemisphere. In fact, this result was found. Larger amplitude ERP differences were found for the breast-fed infants. This supports our second hypothesis that breast-fed infants would generate larger overall ERP components to speech sounds than infants fed the PUFA-enriched formula. Data supported our third hypothesis that components of the ERP from breast-fed infants would vary throughout the waveform, while the responses of the infants receiving the PUFA formula would be more restricted temporally.

The results of the present study clearly indicate that PUFA-enriched formula did not produce comparable levels of brain development in the two groups of infants studied at 6 months of age. When a range of biological, perinatal, and cognitive factors were equated between the two groups, only the ERPs recorded from breast-fed infants changed throughout their recorded period (700 msec). These differences occurred in the breast-fed infants over both the left and the right hemisphere electrode sites while those receiving PUFA-enriched formula generated differences restricted to the left hemisphere. Moreover, breast-fed infants generated responses to the stimuli across all electrode regions, that is, over frontal, temporal, posterior-temporal, central, parietal, and occipital regions while the responses of infants receiving the PUFA-enriched formula were restricted to more posterior electrode regions—temporal, parietal, and occipital sites. Such differences in the range of their brain responses could signal an advantage for the breast-fed infants for later linguistic and cognitive development.

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