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Original Article

Breastfeeding and early brain development: the Generation R study

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Abstract

Breastfeeding during infancy is associated with a range of short- and long-term health benefits. We examine whether breastfeeding in the first 2 months of life is associated with structural markers of brain development in infants from the general population. This study was embedded within the Generation R study. Cranial ultrasounds were obtained at approximately 7 weeks post-natal age. The diameter of the gangliothalamic ovoid, corpus callosum length, ventricular volume and head circumference were measured. Maternal reports of breastfeeding were obtained at 2 months of age. We examined associations in relation to current breastfeeding practices (exclusively breastfed, $n = 318$, breast- and bottle-fed, $n = 119$, and bottle-fed, $n = 243$). Analyses were adjusted for head size and relevant covariates. Secondary analyses were conducted for breastfeeding history (exclusively breastfed, $n = 318$, breast- and bottle-fed, $n = 281$, and never breastfed, $n = 81$). Exclusive breastfeeding was associated with more optimal brain development compared with babies who were bottle-fed or never breastfed. Results were most consistent for gangliothalamic ovoid diameter. Larger gangliothalamic ovoid diameters were evident in babies who were exclusively breastfed compared with bottle-fed babies [difference between means (95% confidence interval) = 0.21(0.02, 0.39), $P = 0.02$]. Smaller ventricular volume and larger head circumference were also found for exclusively breastfed babies. Breastfeeding was not significantly associated with corpus callosum length. Maternal reports of breastfeeding are associated with more mature brain development within the first 2 months of life. Results are most consistent for gangliothalamic ovoid diameter, a subcortical structure rich in docosahexaenoic acid. Findings also pointed to non-specific neural developmental advantage for exclusively breastfed babies.

Keywords: early growth, brain function, breastfeeding, cohort study, epidemiology, infancy and childhood.

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Introduction

It is widely recognised by the World Health Organization (WHO) and by the American, Canadian and British paediatric societies, among others, that breastfeeding during infancy holds many short- and long-term health benefits (Singhal *et al*. 2004; Horta *et al*. 2007). Previous research has linked breast milk to more optimal neurodevelopment among preterm infants (Lucas *et al*. 1994; Tanaka *et al*. 2009) or infants born small for gestational age (Morley *et al*. 2004). Results of epidemiological studies have also provided support for breast milk to be associated with better cognitive development (mainly assessed through verbal and performance IQ) (Oddy *et al*. 2003; see also Anderson *et al*. 1999), although there is some

indication that this effect may be moderated by genetic or socio-economic factors (Jacobson *et al*. 1999; Caspi *et al*. 2007). A recent study also demonstrated links between breast milk, cognitive development and white matter volume among children aged 7–8 years, who were born prematurely (Isaacs *et al*. 2010). In this study, links between breast milk and white matter volume were particularly strong for boys who were born premature. From a biological perspective, the association between breast milk and optimal brain development is very plausible. The last trimester of pregnancy is characterised by the greatest period of brain growth such as a doubling of whole brain volume, a fourfold increase in cortical grey matter and a 70% increase in subcortical grey matter or basal ganglia (Huppi 2008). However, extensive neurodevelopment continues into the first 2 years of life (Isaacs *et al*. 2008), and brain development is at a critical stage soon after birth (Wang *et al*. 2003). Human breast milk contains long-chain polyunsaturated fatty acids such as docosahexaenoic acid (DHA) and arachidonic acid (AA). The rapid brain development just prior to birth and in the first 6–12 months after birth is associated with an increased incorporation of these long-chain polyunsaturated fatty acids into the brain, particularly the cerebral cortex (Makrides *et al*. 2000; Carlson 2001; Gibson & Makrides 2001; Reynolds 2001; Webb *et al*. 2001; Tanaka *et al*. 2009) and neural networks (Makrides *et al*. 2000). Studies in non-human primates indicate that DHA is an important component in neural development and is particularly rich in deeper brain structures, such as the basal ganglia, thalamus and midbrain (Diau *et al*. 2005; Hsieh *et al*. 2007). Furthermore, some research indicates that early infant nutrition, such as special formulas fed to preterm babies containing higher levels of fat and protein, can alter developing subcortical brain structures such as the caudate, particularly in boys (Isaacs *et al*. 2008). Results of neurophysiological measures of 30 exclusively breastfed and 23 exclusively formula-fed healthy infants pointed towards more mature neural development, indicative of increased myelination among breastfed compared with formula-fed babies (Khedr *et al*. 2004).

Although many of the studies outlined above focus on preterm babies who are more physically and neurologically immature at birth, research highlights the importance of early nutrition on brain development. Generally, results support the view that breast milk contains nutrients that play a role in optimal brain development. Furthermore, studies of both babies born preterm and of healthy-developing babies point towards a dose–response relationship between breast milk (i.e. percentage of breast milk in the baby's diet or duration of breastfeeding) and child outcome (Isaacs *et al*. 2010; Chiu *et al*. 2011; Guxens *et al*. 2011). Yet, greater in-depth knowledge is needed on associations between breastfeeding and infant brain development within healthy-developing babies. This knowledge would better inform us as to whether breastfeeding might be particularly beneficial for the optimal development of certain brain structures or rather contributes to a better overall brain development. It also is very important to conduct such an examination within the context of a longitudinal study, where important prenatal, perinatal and postnatal factors are evaluated prospectively and accounted for within the analyses.

Using data from the Generation R study, a prospective population-based cohort study following children from fetal life onwards, we sought to determine whether maternal reports of breastfeeding were associated with early infant brain development, as assessed by ultrasound measurements through babies' occipital fontanel obtained at approximately

Key messages

- Maternal reports of breastfeeding are associated with neural development in a large cohort of healthy infants.
- Breastfeeding was associated with a larger gangliothalamic ovoid, a DHA-rich subcortical structure, although general benefits were observed for ventricular volume and head size.
- Advantages for breastfeeding were particularly evident for exclusively breastfed babies compared with those who were bottle-fed or never breastfed.

seven weeks of age. More specifically, we address an important gap in the literature by examining these associations in relation to the specific brain structures of gangliothalamic ovoid diameter, length of corpus callosum and in relation to more general indices of brain development such as ventricular volume or head size. The gangliothalamic ovoid is an egg-like structure encompassing the basal ganglia and thalamus, and a smaller diameter has been linked to disorganised attachment (Tharner *et al*. 2011) and to elevated internalising behaviour problems (Herba *et al*. 2010) in young children. Structures encompassed within the basal ganglia and thalamus of the gangliothalamic ovoid have been shown both structurally and functionally to be associated with depressive disorders (Matsuo *et al*. 2008; Forbes *et al*. 2009), reward learning, and sensory and motor integration (Fareri *et al*. 2008; Haber & Calzavara 2009). Based on previous literature indicating that subcortical structures such as the basal ganglia, caudate and thalamus may be rich in nutrients such as DHA which is available in breast milk, we were particularly interested in the impact of breastfeeding on gangliothalamic ovoid diameter. We hypothesised that breastfeeding in the first 2 months of age would be associated with more mature early brain development, as indicated by a larger gangliothalamic ovoid diameter, after adjusting for head circumference as a proxy for total brain volume (Bartholomeusz *et al*. 2002) and other relevant factors that could influence a mother's choice or ability to breastfeed. To determine specificity of findings, we also assessed the associations between breastfeeding and corpus callosum length, one indicator of white matter development. Links between breast milk and white matter growth have been demonstrated in preterm children, with results particularly pronounced for boys born preterm (Isaacs *et al*. 2010). A smaller corpus callosum length has also been linked to poorer executive functioning (particularly inhibition and emotional control) at 4 years of age (Ghassabian *et al*. 2012). We thus also examined whether breastfeeding in the first 2 months of life would be associated with corpus callosum length in our sample of healthy-developing, mostly full-term, babies. Furthermore, to investigate whether breastfeeding was associated with more general brain development, we examined links between breastfeeding and total ventricular volume. Previous studies suggest that either extreme of ventricular volume could indicate risk for abnormal brain development and later temperament problems or developmental disabilities (Inder *et al*. 2005; Gilmore *et al*. 2008; Roza *et al*. 2008a). In all of these analyses, we adjust for general head size to examine the specific brain structure of interest. Breastfeeding practices at 2 months of age were obtained using maternal reports. Finally, given previous studies suggesting a dose–response relationship between breastfeeding and child outcome, we hypothesised that breastfeeding would be associated with more optimal brain development for exclusively breastfed babies, mid-range for babies who were both breast- and bottle-fed, and lowest for bottle-fed babies. Earlier research in both animals and humans have demonstrated that breastfeeding in the first several weeks after birth is associated with changes in brain structure and function (Makrides *et al*. 1995; Wang *et al*. 2003; Heird & Lapillonne 2005; Caspi *et al*. 2007). Thus, although our follow-up period is relatively short, we nevertheless expected to be able to detect significant differences in the size of brain structures of interest in relation to breastfeeding.

Materials and methods

Participants

Participants included children who were involved in the Generation R population-based cohort of individuals followed from fetal life onwards. Our investigation was based on a subsample of children from the overall study who were followed in detail. This subgroup of Dutch children is ethnically homogeneous to exclude confounding or effect modification by ethnicity. No other inclusion or exclusion criteria were defined for participation in this subgroup. All children were born between February 2003 and August 2005 and form a prenatally enrolled birth cohort. Details of the Generation R study and this subsample have been described in previous papers (Hofman *et al*. 2004; Jaddoe *et al*. 2006, 2010). Briefly, 1232 women participated in detailed prenatal assessments, and gave birth to 1244 live births (including 15 twin pregnancies, 1

intrauterine fetal death, and 2 neonatal deaths). Of these mothers, 904 (73% of the sample) participated in post-natal assessments, which took place between 4 and 12 weeks post birth (see also Roza *et al*. 2008b). Data for 19 children were excluded as they were a twin. Twins were excluded because we were concerned that associations between breastfeeding and brain development could be different for twins compared with singletons. The ultrasound techniques are described below. Post-natal cranial ultrasounds of sufficient quality were available for the gangliothalamic ovoid in 774 infants, for the ventricular volume in 759 infants and for the corpus callosum in 781 infants at approximately 7 weeks of age [mean age (standard deviation, SD) = 6.82 (1.81) weeks gestation]. Missing data for post-natal cranial ultrasounds were due mainly to infant movement or restlessness, the unavailability of a trained sonographer or an anterior fontanel that was too small due to the baby being older than 3 months of post-natal age. For the assessment of the gangliothalamic ovoid, images for nine children were excluded due to poor quality (i.e. both raters noted the image was too poor to measure). For the assessment of the corpus callosum, images for three children were excluded due to poor quality. Among those children with at least one brain measurement available (for gangliothalamic ovoid diameter, corpus callosum or ventricular volume) and excluding data from twins, maternal reports of breastfeeding were available for 680 children. Analyses were conducted separately for each brain measure outcome of interest; thus, the number of participants for each separate brain analysis may have differed slightly depending on the measure assessed.

This study was conducted in accordance with the guidelines proposed in the World Medical Association Declaration of Helsinki and has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all participants.

Maternal reports of breastfeeding: determinant

Maternal reports of breastfeeding were obtained using a postal questionnaire at the child's age of 2 months, coinciding with the time of the ultrasound assessment (approximately 7 weeks post-natal age). Mothers were asked whether or not they had ever breastfed their child (yes/no), for how many weeks they had breastfed their child until that point and in which way their baby was fed at that time (i.e. breastfeeding only, bottle-feeding only, as much breast as bottle, more than half breastfeeding, and more than half bottle-feeding; for more details on breastfeeding variables on this cohort of children, see van Rossem *et al*. 2009). Throughout the paper, we refer to breastfeeding rather than breast milk, as this more closely reflects the questions mothers were asked. We did not have more specific data on whether any children might have been exclusively bottle-fed using breast milk. We examined our data in relation to the current breastfeeding practices reported at the child's age of 2 months. Children were allocated to one of the three groups: exclusively breastfed ($n = 318$), breast- and bottle-fed ($n = 119$) or bottle-fed ($n = 243$). Babies in this bottle-fed category could have been breastfed at some point in the first 2 months of life; they were simply not currently being breastfed. Data were available for 680 women. We also conducted supplementary analyses to examine whether findings would be replicated for a slightly different categorisation of breastfeeding, for which we more specifically examined those babies who had never been breastfed. Thus, for this breastfeeding history variable, we categorised babies as to whether they were exclusively breastfed ($n = 318$), breast- and bottle-fed ($n = 281$) or never breastfed ($n = 81$) during the first 2 months of life.

Ultrasound measurements: outcome variables

Post-natal cranial ultrasound was performed with a commercially available multifrequency electronic transducer (3.7–9.3 MHz) with a scan angle of 146°, usable for three-dimensional volume acquisition (Voluson 730 Expert, GE Healthcare, Waukesha, WI, USA). The probe was positioned on the anterior fontanel and a volume box was placed at the level of the foramen of Monro in a symmetrical coronal section. We scanned a pyramid-shaped volume of the brain tissue and measured the diameter of the gangliothalamic ovoid, the length of the corpus callosum and the volume of the lateral ventricular system offline. This

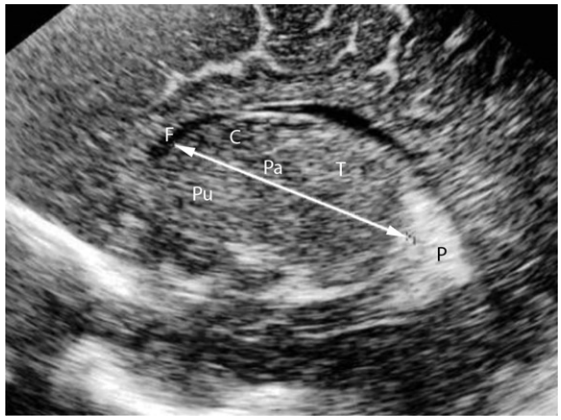

Fig. 1. Measurement of gangliothalamic ovoid diameter. The largest diameter was measured from the frontal horn (F) to approximately the middle of the choroid plexus (P). The following structures are identified: frontal horn (F), caudate head (C), putamen (Pu), pallidum (Pa), thalamus (T) and plexus (P).

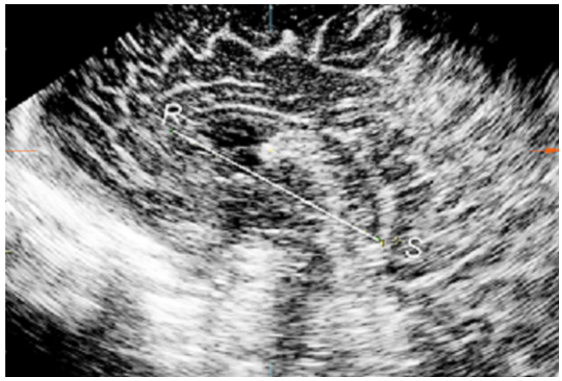

Fig. 2. Measurement of corpus callosum length. The largest diameter was measured from the rostrum to the splenium.

general post-natal ultrasound procedure has been published (Roza *et al*. 2008a,b), and previously analysed data on the gangliothalamic ovoid and the ventricular system indicate very good reliability (see below).

For the purposes of the current investigation, we measured the diameter of the gangliothalamic ovoid, encompassing the basal ganglia, i.e. striatum: caudate head, putamen, globus pallidus and thalamus, as described by Naidich *et al*. (1986) (see Fig. 1). Further details on the boundaries (Naidich *et al*. 1986; Govaert & De Vries 1995) and measurement (Herba *et al*. 2010) of this structure are available. Two raters, trained by an experienced neonatologist (P.G.), independently measured every image, and reliability was assessed. Raters also coded the quality of the ultrasound image, and those images with a quality rating of zero by both raters (left egg, $n = 3$; right egg, $n = 6$) were excluded from further analyses. The right and left gangliothalamic ovoids were measured separately, and the mean score (across right and left) was calculated for every image for each of the two raters. Reliability between the two raters was good [average measures Cronbach's $\alpha = 0.83$, intraclass correlation coefficient (ICC) = 0.83, 95% confidence interval (CI) = 0.80, 0.85], and the average gangliothalamic ovoid diameter across the two raters was used in the analyses.

In the best mid-sagittal view, we defined the corpus callosum length as the largest diameter from rostrum to splenium. With ultrasound techniques, variations in the thickness of corpus callosum cannot be reliably measured (Anderson *et al*. 2004). Thus, we used two-dimensional measurement along the entire body of the corpus callosum in the present study (see Fig. 2). The corpus callosum length was measured by two independent raters (C.H. and J.v.R.), and the average of these two measurements was used in our analyses, as previously published on the same participants (Ghassabian *et al*. 2012). The reliability of corpus callosum measurement between the two raters was good (Cronbach's $\alpha = 0.85$, ICC = 0.85 and 95% CI: 0.83, 0.87). Raters also coded the quality of the ultrasound image, and images of the corpus callosum with a quality rating of zero by both raters ($n = 3$) were excluded from the analysis.

Measurement of the ventricular system was conducted such that the volume of the ventricular frontal horns, ventricular body and trigone on both sides was quantified in millilitres. The atrial width of the lateral ventricle is the widest diameter of the atrium of one of the lateral ventricles that can be measured in an axial plane. Four raters manually traced the left and right ventricles. For reliability analysis, the raters each segmented 20 images twice and images of another 20 children were rated by all four raters. The intra- and inter-observer reliability of the volumetric measurements was very high, partly due to the high variance in ventricular volume within our population. Intra-

observer ICCs varied from 0.989 to 0.993 for the right ventricle and from 0.992 to 0.997 for the left ventricle. Inter-observer ICCs were 0.950 for the right ventricle and 0.981 for the left ventricle. This has been described previously in detail (Roza *et al*. 2008b,a).

The infant fronto-occipital head circumference (cm) was assessed during the 6-week visit, when the ultrasounds were also conducted. Head circumference represents the outer perimeter of the skull, measured in an axial plane. High intra- and inter-observer reliability has been reported for head circumference and atrial width of the lateral ventricle (Cardoza *et al*. 1988; Perni *et al*. 2004).

Covariates

Analyses were adjusted for factors, which, for theoretical reasons, might be associated with brain measures and/or a mother's choice or ability to breastfeed such as infant sex, age at the time of ultrasound, mode of delivery, maternal education, maternal mental health, maternal diet during pregnancy and head circumference. Other potential confounders such as family income, maternal age at enrolment in the study, smoking during pregnancy, alcohol use, health during pregnancy (hypertension, pre-eclampsia and gestational diabetes) and neonatal variables (baby's gestational age at birth, birthweight, Apgar score at 5 min and parity) were examined using correlational analyses and were only included in the models if they were significantly associated with our main determinant and/or outcome ($P \leq 0.05$). Date of birth, birthweight and sex of the infant were obtained from community midwife and hospital registries at birth. Gestational age at birth (measured in weeks), established through fetal ultrasound examinations within the Generation R study, was considered an important covariate as this is indicative of the maturity of the newborn. Maternal age and maternal education (highest level completed; a proxy for socio-economic status) were determined at enrolment. Maternal smoking and drinking alcohol during pregnancy was also assessed using self-report. Maternal psychopathology was assessed at 2 months of post-natal age using the Brief Symptom Inventory (de Beurs 2004; Derogatis & Melisaratos 1983). The mean score for maternal depression and anxiety subscales was calculated. Mode of delivery included three levels: spontaneous vaginal, vaginal instrumental and Caesarean section. This variable was considered as Caesarean delivery may be associated with a less mature baby at birth, and also with a lower tendency to breastfeed. We considered maternal nutrition during pregnancy as a confounder as DHA breast milk content is highly dependent on mother's diet (Michaelsen *et al*. 2011), and diet during pregnancy has been associated with perinatal outcome (Barger 2010). More specifically, we used a score for Mediterranean dietary pattern, comprising high intake of vegetables, vegetable oils, pasta, fish and legumes, calculated using factor analysis within this cohort as previously described (Timmermans *et al*. 2011). All analyses examining gangliothalamic ovoid diameter, corpus callosum length and ventricular volume were adjusted for head circumference (assessed at the time of the ultrasound) to ensure that the effects were specific to the size of the brain structure of interest and not simply reflective of general head size. Head circumference has been noted to be a possible proxy for total brain volume, and particularly for younger children, can be used as a quick and accurate measure of brain growth and normalcy of brain size (Bartholomeusz *et al*. 2002). Our final models included the following covariates: sex of baby, age at the time of ultrasound, gestational age at birth, birthweight, mode of delivery, maternal education, family income, maternal age at enrolment, maternal depression and anxiety (mean score) at 2 months of post-natal age, maternal smoking during pregnancy, maternal drinking during pregnancy, maternal Mediterranean diet during pregnancy, pre-eclampsia and head circumference.

Statistical analyses

Data were analysed within SPSS for windows (version 17.0; SPSS, Inc., Chicago, IL, USA). A series of univariate analyses of covariance (ANCOVAs) was conducted, examining the associations between current breastfeeding practices and (1) diameter of gangliothalamic ovoid; (2) length of corpus callosum; and (3) total volume of the lateral ventricles. These models included breastfeeding and child's sex as fixed

group variables, and the relevant covariates listed above. To investigate the effects of breastfeeding on total head size, we conducted a similar ANCOVA as described earlier. The interaction between breastfeeding and child's sex was also included to examine whether boys or girls might be differentially responsive to the effects of breastfeeding. Where this interaction was not significant, it was removed from our final model.

Missing data for continuous covariates [age at the time of ultrasound, gestational age at birth, birthweight, maternal education, family income, maternal age at enrolment, maternal depression and anxiety (mean score) at 2 months of post-natal age, maternal Mediterranean diet during pregnancy and head circumference] were imputed using the maximum likelihood estimation method within SPSS. Where main effects of breastfeeding are significant, differences in means for standardised brain measurements along with confidence intervals are presented, for which the Bonferroni correction was applied to test for significance. Differences among means for all comparisons (including non-significant comparisons) are presented in Table 2, along with adjusted R^2 values for each model of interest. Secondary analyses were repeated for the breastfeeding history variable to examine whether results were replicated following investigation of this categorisation, which examines more specifically those babies who were never breastfed.

Results

Descriptive data

Boys had larger head circumferences compared with girls ($P < 0.001$). Boys also had slightly larger gangliothalamic ovoid diameters [mean (SD) for boys = 4.34 (0.18); for girls = 4.30 (0.18); $P = 0.007$] and ventricular volumes [mean (SD) for boys = 1.06 (0.81) vs. girls = 0.93 (0.67); $P = 0.03$] compared with girls, although these sex differences disappeared once analyses were adjusted for head circumference. Girls had longer corpus callosum length compared with boys [mean (SD) for boys = 4.57 (0.35) vs. girls = 4.66 (0.34); $P = 0.001$]; this sex difference remained significant after covarying for head circumference. Descriptive data are presented in Table 1. Results of analyses for current breastfeeding practices in relation to brain structures of interest (as described below) for both adjusted and unadjusted models are presented in Table 2.

Breastfeeding and gangliothalamic ovoid

After adjusting for relevant covariates, the main effect of current breastfeeding practices was significant [$F_{(2,\ 642)} = 3.925$, $P = 0.02$]. This significant effect is explained by the fact that compared with bottle-fed babies, a larger gangliothalamic ovoid diameter was evident in babies who were exclusively breastfed (difference in means = 0.21, 95% CI = 0.02, 0.39, $P = 0.02$). Gangliothalamic ovoid diameter did not significantly differ between babies who were exclusively breastfed and those who were breast- and bottle-fed ($P = 1.00$) or between those who were bottle-fed compared with those who were breast- and bottle-fed ($P = 0.14$). Results are illustrated in Fig. 3. The interaction between sex and breastfeeding was not significant in the prediction of gangliothalamic ovoid diameter (nor for any other brain indices outlined below) and thus was not included in the final models.

Breastfeeding and corpus callosum

The main effect of current breastfeeding practices was not significant for corpus callosum length [$F_{(2,\ 648)} = 1.384$, $P = 0.25$]. Thus, the corpus callosum length did not differ between babies who were exclusively breastfed, breast- and bottle-fed, or bottle-fed (see Table 2 for difference between means).

Breastfeeding and ventricular volume

Following natural log-transformation, the total ventricular volume met the assumptions necessary for parametric analyses and the log-transformed variable was used in subsequent analyses. The main effect of current breastfeeding practice on ventricular volume was just significant [$F_{(2,\ 624)} = 3.016$, $P = 0.05$]. This result may be explained by the fact that compared with babies who were exclusively breastfed, babies

Table 1. Descriptive data: current breastfeeding practices

	Current breastfeeding practices*			Group differences
	Exclusive breastfeeding ($n = 318$)	Breast + bottle ($n = 119$)	Bottle-feeding ($n = 243$)	
Infant characteristics				
Sex (% boys)	50.6%	47.1%	51.4%	$P = 0.73$
Mode of delivery (% spontaneous vaginal; % vaginal instrumental; % C-section)	71.0%; 18.8%; 10.2%	67.3%; 19.8%; 12.6%	58.4%; 21.0%; 20.6%	$P = 0.01$
Post-natal age at time of ultrasound, weeks [mean (SD)]	6.79 (1.78)	6.88 (2.10)	6.83 (1.72)	$P = 0.90$
Gestational age at birth, weeks [mean (SD)]	40.16 (1.51)	40.17 (1.65)	39.83 (1.81)	$P = 0.04$
Prematurity (% <37 weeks gestation)	3.1	2.5	4.9	$P = 0.41$
Birthweight, g [mean (SD)]	3551.92 (492.27)	3509.64(524.33)	3486.98 (543.15)	$P = 0.33$
Low birthweight (% <2500 g birthweight)	2.2	2.5	4.1	$P = 0.40$
Maternal characteristics				
Age of mother at enrolment [mean (SD)]	32.10 (3.57)	32.66 (3.72)	31.34 (3.89)	$P = 0.004$
Maternal education (median, 25%, 75% CI)	4.00 (4.00, 5.00)	4.00 (3.00, 5.00)	3.00 (3.00, 5.00)	$P < 0.001$
Family income (% low income; i.e. less than 2000 euros per month)	9.7%	8.4%	14.8%	$P = 0.09$
Maternal depression and anxiety (2 months post-natal age) [mean (SD)]	0.11 (0.17)	0.15 (0.25)	0.17 (0.34)	$P = 0.02$
Maternal smoking during pregnancy (% continued smoking)	6.6%	10.1%	19.4%	$P < 0.001$
Maternal drinking during pregnancy (% continued drinking)	61.5%	63.7%	51.3%	$P = 0.002$
Mediterranean dietary pattern (factor score) [mean (SD)]	0.05 (0.91)	0.09 (0.77)	–0.20 (0.87)	$P = 0.001$
Pre-eclampsia (% yes)	1.3%	2.6%	2.1%	$P = 0.61$

SD, standard deviation. *The number of participants in this table represents those children for whom there is at least one ultrasound brain image available and data on breastfeeding.

who were bottle-fed (difference in means = –0.18, 95% CI = –0.40, 0.40, $P = 0.15$) or breast- and bottle-fed (difference in means = –0.21, 95% CI = –0.47, 0.05, $P = 0.15$) had larger ventricular volumes, although these differences failed to reach significance after correcting for multiple comparisons. Ventricular volume was not significantly different for babies who were both breast- and bottle-fed compared with bottle-fed ($P = 1.00$). Associations are illustrated in Fig. 4.

Breastfeeding and head circumference

There was a trend towards significance for the main effect of current breastfeeding practices on head circumference [$F_{(2, 704)} = 2.474$, $P = 0.09$].

Secondary analyses: associations between breastfeeding history and brain indices

The above analyses investigating associations between breastfeeding and brain structures were repeated for the breastfeeding history variable. This was performed to examine whether results were replicated with this variable that includes the category of babies who had never been breastfed ($n = 81$). Consistent with the results obtained for current breastfeeding practices, a similar pattern of results emerged between breastfeeding history and gangliothalamic ovoid diameter. The main effect of breastfeeding history was near-significant [$F_{(2, 642)} = 2.891$, $P = 0.06$]. Although this main effect just failed to reach significance, our further comparisons suggested that exclusively breastfed babies had larger gangliothalamic

Table 2. Current breastfeeding practices and breastfeeding history in relation to brain measures and head circumference

	Brain measures			Head circumference (cm)
	Gangliothalamic ovoid diameter (cm)	Corpus callosum length (cm)	Total ventricular volume (mL)	
Current breastfeeding practices				
Mean (SD) (raw scores)				
Exclusively breastfed (BF)	4.34 (0.17); $n = 307$	4.66 (0.34); $n = 310$	0.94 (0.71); $n = 298$	38.83 (1.51); $n = 330$
Breast- and bottle-fed (BF + B)	4.33 (0.16); $n = 117$	4.60 (0.34); $n = 117$	1.08 (0.86); $n = 112$	38.76 (1.59); $n = 129$
Bottle-fed (Bot)	4.28 (0.18); $n = 235$	4.57 (0.36); $n = 238$	1.03 (0.75); $n = 231$	38.47 (1.38); $n = 261$
Unadjusted analyses	$F_{(2, 656)} = 9.25$, $P < 0.001$ Adjusted $R^2 = 0.024$	$F_{(2, 662)} = 4.29$, $P = 0.01$ Adjusted $R^2 = 0.010$	$F_{(2, 638)} = 1.87$, $P = 0.16$ Adjusted $R^2 = 0.003$	$F_{(2, 717)} = 4.58$, $P = 0.01$ Adjusted $R^2 = 0.010$
*Difference in means (95% CI):				
BF vs. Bot	0.37 (0.16, 0.58), $P < 0.001$)	0.26 (0.04, 0.47), $P = 0.01$	–0.12 (–0.34, 0.09), $P = 0.48$	0.24 (0.05, 0.44), $P = 0.01$
BF vs. BF + B	0.05 (–0.22,0.31), $P = 1.00$	0.16 (–0.11, 0.42), $P = 0.47$	–0.20 (–0.46, 0.07), $P = 0.24$	0.05 (–0.20, 0.29), $P = 1.00$
BF + B vs. Bot	0.32 (0.04, 0.59), $P = 0.02$	0.10 (–0.18, 0.38), $P = 1.00$	0.07 (–0.21, 0.35), $P = 1.00$	0.19 (–0.06, 0.45), $P = 0.21$
Analyses adjusted only for head circumference	$F(2, 655) = 5.50$, $P = 0.004$ Adjusted $R^2 = 0.310$	$F(2, 661) = 2.12$, $P = 0.12$ Adjusted $R^2 = 0.114$	$F(2, 637) = 3.447$, $P = 0.032$ Adjusted $R^2 = 0.084$	NA
*Difference in means (95% CI):				
BF vs. Bot	0.23 (0.05, 0.41), $P = 0.01$	0.17 (–0.03, 0.37), $P = 0.13$	–0.18 (–0.39, 0.02), $P = 0.10$	
BF vs. BF + B	–0.01 (–0.23, 0.21), $P = 1.00$	0.11 (–0.14, 0.37), $P = 0.83$	–0.22 (–0.48, 0.04), $P = 0.13$	
BF + B vs. Bot	0.24 (0.01, 0.47), $P = 0.04$	0.06 (–0.21, 0.32), $P = 1.00$	0.04 (–0.23, 0.30), $P = 1.00$	
†Fully adjusted analyses	$F_{(2, 642)} = 3.93$, p = 0.02 Adjusted $R^2 = 0.327$	$F_{(2, 648)} = 1.38$, p = 0.25 Adjusted $R^2 = 0.172$	$F_{(2, 624)} = 3.02$, p = 0.05 Adjusted $R^2 = 0.084$	$F_{(2, 704)} = 2.47$, p = 0.09 Adjusted $R^2 = 0.470$
*Difference in means (95% CI):				
BF vs. Bot	0.21 (0.02, 0.39), $P = 0.02$	0.12 (–0.08, 0.33), $P = 0.45$	–0.18 (–0.40, 0.04), $P = 0.15$	0.14 (–0.01, 0.29), $P = 0.08$
BF vs. BF + B	0.01 (–0.21, 0.23), $P = 1.00$	0.13 (–0.12, 0.38), $P = 0.62$	–0.21 (–0.47, 0.05), $P = 0.15$	0.07 (–0.11, 0.25), $P = 0.99$
BF + B vs. Bot	0.20 (–0.04, 0.43), $P = 0.14$	–0.01 (–0.27, 0.26), $P = 1.00$	0.03 (–0.24, 0.31), $P = 1.00$	0.07 (–0.12, 0.26), $P = 1.00$

Table 2. Continued

Breastfeeding history	Gangliothalamic ovoid diameter (cm)	Corpus callosum length (cm)	Total ventricular volume (mL)	Head circumference (cm)
Mean (SD) (unadjusted for covariates)				
Exclusively breastfed (BF)	4.34 (0.17)	4.66 (0.34)	0.94 (0.71)	38.83 (1.51)
Breast- and bottle-fed (BF + B)	4.31 (0.17)	4.60 (0.36)	1.06 (0.76)	38.63 (1.44)
Never breastfed (NB)	4.25 (0.19)	4.52 (0.33)	1.00 (0.89)	38.32 (1.50)
Unadjusted analyses	$F(2, 656) = 8.51$, $P < 0.001$	$F(2, 662) = 5.70$, $P = 0.004$	$F(2, 638) = 2.59$, $P = 0.08$	$F(2, 717) = 4.22$, $P = 0.01$
	Adjusted $R^2 = 0.022$	Adjusted $R^2 = 0.014$	Adjusted $R^2 = 0.005$	Adjusted $R^2 = 0.009$
*Difference in means (95% CI):				
BF vs. NB	0.51 (0.20, 0.82), $P < 0.001$	0.41 (0.10, 0.72), $P = 0.004$	–0.08 (–0.39, 0.23), $P = 1.00$	0.39 (0.05, 0.62), $P = 0.01$
BF vs. BF + B	0.19 (–0.01, 0.39), $P = 0.08$	0.17 (–0.03, 0.37), $P = 0.14$	–0.17 (–0.37, 0.04), $P = 0.15$	0.13 (–0.05, 0.32), $P = 0.27$
BF + B vs. NB	0.32 (0.01, 0.64), $P = 0.04$	0.24 (–0.07, 0.56), $P = 0.18$	0.08 (–0.23, 0.40), $P = 1.00$	0.21 (–0.08, 0.49), $P = 0.26$
Analyses adjusted only for head circumference	$F_{(2, 655)} = 3.78$, $P = 0.02$	$F_{(2, 661)} = 2.77$, $P = 0.06$	$F_{(2, 637)} = 3.67$, $P = 0.03$	NA
	Adjusted $R^2 = 0.307$	Adjusted $R^2 = 0.115$	Adjusted $R^2 = 0.084$	
*Difference in means (95% CI):				
BF vs. NB	0.289 (0.03, 0.55), $P = 0.02$	0.27 (–0.02, 0.57), $P = 0.08$	–0.19 (–0.49, 0.11), $P = 0.40$	
BF vs. BF + B	0.11 (–0.06, 0.28), $P = 0.39$	0.12 (–0.08, 0.31), $P = 0.43$	–0.20 (–0.39, 0.00), $P = 0.05$	
BF + B vs. N	0.18 (–0.08, 0.44), $P = 0.30$	0.15 (–0.14, 0.45), $P = 0.65$	0.01 (–0.30, 0.31), $P = 1.00$	
†Fully adjusted analyses	$F_{(2, 642)} = 2.89$, $P = 0.06$	$F_{(2, 648)} = 1.81$, $P = 0.16$	$F_{(2, 624)} = 3.27$, $P = 0.04$	$F_{(2, 704)} = 3.17$, $P = 0.04$
	Adjusted $R^2 = 0.325$	Adjusted $R^2 = 0.173$	Adjusted $R^2 = 0.084$	Adjusted $R^2 = 0.471$
*Difference in means (95% CI):				
BF vs. NB	0.26 (–0.01, 0.53), $P = 0.07$	0.22 (–0.08, 0.52), $P = 0.24$	–0.18 (–0.49, 0.14), $P = 0.53$	0.22 (0.00, 0.44), $P = 0.05$
BF vs. BF + B	0.10 (–0.07, 0.28), $P = 0.44$	0.11 (–0.09, 0.30), $P = 0.56$	–0.19 (–0.39, 0.01), $P = 0.06$	0.09 (–0.05, 0.23), $P = 0.37$
BF + B vs. N	0.16 (–0.11, 0.42), $P = 0.48$	0.11 (–0.18, 0.41), $P = 1.00$	0.02 (–0.29, 0.33), $P = 1.00$	0.13 (–0.09, 0.34), $P = 0.45$

CI, confidence interval; NA, not applicable; SD, standard deviation. *The difference in means is presented for standardised scores of brain measures. In the case of ventricular volume, analyses were conducted on transformed standardised scores. For ease of interpretation, differences in non-transformed standardised means are presented for ventricular volume. †Adjusted analyses include the following covariates: sex of baby, age at the time of ultrasound, gestational age at birth, birthweight, mode of delivery, maternal education, family income, maternal age at enrolment, maternal depression and anxiety (mean score) at 2 months of post-natal age, maternal smoking during pregnancy, maternal drinking during pregnancy, maternal Mediterranean diet during pregnancy, pre-eclampsia and head circumference (except for the model predicting head circumference).

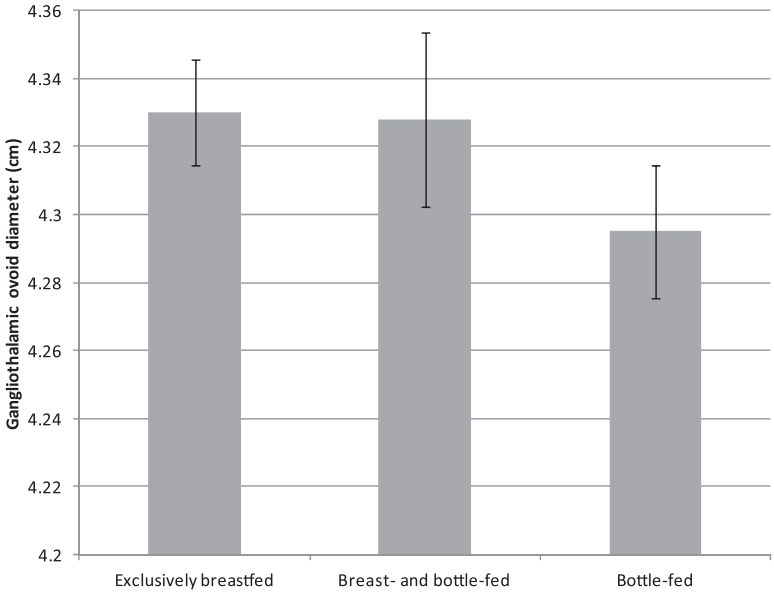

Fig. 3. Current breastfeeding practices and gangliothalamic ovoid diameter. Estimated marginal means of gangliothalamic ovoid diameter [adjusted for sex of baby, gestational age at birth, weight at birth, mode of delivery, age at time of ultrasound, maternal education, family income, maternal depression and anxiety (mean score) at two months of post-natal age, maternal age at enrolment, maternal smoking during pregnancy, pre-eclampsia, maternal drinking during pregnancy, maternal Mediterranean diet during pregnancy and head circumference] are presented along the *y*-axis, and categorisation for current breastfeeding practices (i.e. exclusively breastfed, breast- and bottle-fed, or bottle-fed) is shown on the *x*-axis. Error bars represent the 95% confidence intervals.

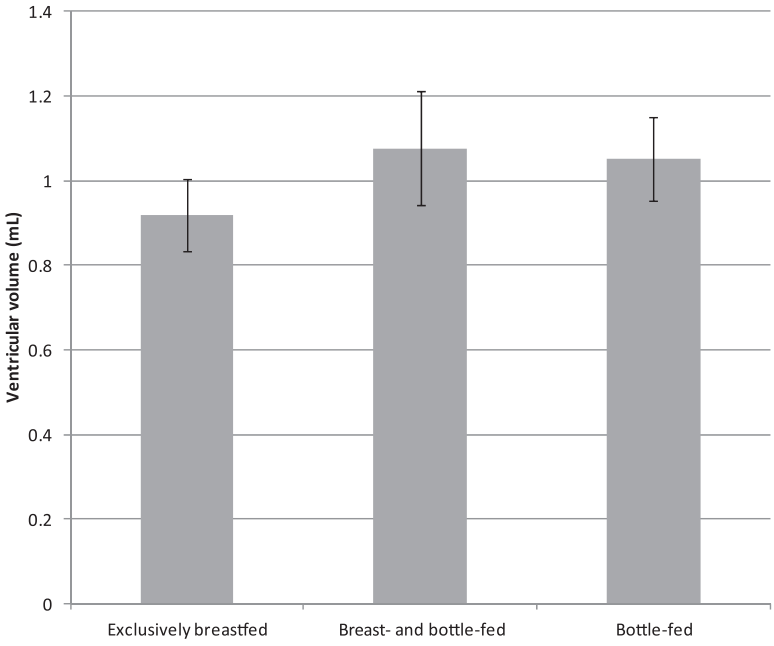

Fig. 4. Current breastfeeding practices and ventricular volume. Estimated marginal means of ventricular volume [adjusted for sex of baby, gestational age at birth, weight at birth, mode of delivery, age at time of ultrasound, maternal education, family income, maternal depression and anxiety (mean score) at two months of post-natal age, maternal age at enrolment, maternal smoking during pregnancy, pre-eclampsia, maternal drinking during pregnancy, maternal Mediterranean diet during pregnancy and head circumference] are presented along the *y*-axis, and categorisation for current breastfeeding practices (i.e. exclusively breastfed, breast- and bottle-fed, or bottle-fed) is shown on the *x*-axis. Error bars represent the 95% confidence intervals.

ovoid diameters compared with babies who were never breastfed (difference in means = 0.26, 95% CI = –0.01, 0.53, $P = 0.07$). Exclusively breastfed babies did not significantly differ from babies who were breast- and bottle-fed ($P = 0.44$), nor did breast- and bottle-fed babies differ from never breastfed babies ($P = 0.48$). Results of analyses for breastfeeding history in relation to brain structures of interest for both adjusted and unadjusted models are presented in Table 2. Similar to the results obtained for current breastfeeding practices and corpus callosum, the main effect of breastfeeding history on corpus callosum length also failed to reach significance [$F_{(2, 648)} = 1.81$, $P = 0.16$].

Consistent with the results obtained for associations between current breastfeeding practices and ventricular volume, the main effect of breastfeeding history on ventricular volume was significant [$F_{(2, 624)} = 3.27$, $P = 0.04$]. Pairwise comparisons indicated that exclusively breastfed babies did not significantly differ from babies who had never been breastfed ($P = 1.00$). Babies who were never breastfed did not significantly differ from babies who were breast- and bottle-fed ($P = 1.00$). Hence, the overall effect was explained by the observation that babies who were exclusively breastfed had smaller ventricular volumes compared with babies who were breast- and bottle-fed [$P = 0.032$; difference in means (untransformed standardised scores) = –0.19, 95% CI = –0.39, 0.01].

Examination of links between breastfeeding history and head circumference was similar yet more pronounced than the results obtained for current breastfeeding practices and head circumference. The main effect of breastfeeding history was significant for head circumference [$F_{(2, 704)} = 3.17$, $P = 0.04$). Pairwise comparisons indicated that babies who were exclusively breastfed had larger head circumferences compared with babies who were never breastfed (difference in means = 0.22, 95% CI = 0.00, 0.44, $P = 0.05$). However, this fell to trend level following adjustment for multiple comparisons. Exclusively breastfed babies did not significantly differ from babies who were breast- and bottle-fed ($P = 0.37$), and breast- and bottle-fed babies did not significantly differ from babies who had never been breastfed ($P = 0.45$) (see Table 2).

Discussion

Using data from a large-scale longitudinal study, we found that maternal reports of breastfeeding are associated with more mature neural development, assessed using cranial ultrasound at approximately 7 weeks post-natal age. Following adjustment for general head size, findings were most specific to the gangliothalamic ovoid diameter. There was also some support for breastfeeding to be associated with differences in ventricular volume and head circumference, indicative of more general brain development. This is the first study, to our knowledge, to examine the associations between neural structures and breastfeeding in a large number of healthy infants, the vast majority born at term.

We hypothesised that more mature brain development would be associated with maternal reports of breastfeeding in infancy (i.e. at approximately 7 weeks of age). Our findings support this hypothesis; proxy measures of neural development, such as the diameter of the gangliothalamic ovoid (encompassing the basal ganglia, thalamus), and the more general measure of head circumference were larger among exclusively breastfed babies compared with bottle-fed or never breastfed babies assessed around 2 months of age. The significant effects were only seen between exclusive breastfeeding and exclusive bottle-feeding or never breastfed reported at 2 months of age (and neither group significantly differed from babies who were both bottle-fed and breastfed).

Our hypothesis of more specific effects for the gangliothalamic ovoid was supported by our results. We had predicted, based on findings of non-human primates (Diau *et al*. 2005; Hsieh *et al*. 2007) and of studies on preterm babies (Isaacs *et al*. 2008), that the nutritional benefits of breast milk would be most pronounced for the subcortical structures such as the basal ganglia, also encompassing the caudate. Findings indicated that larger gangliothalamic ovoid diameters were seen among babies who were exclusively breastfed compared with those who were bottle-fed at 2 months of age. Results were replicated with our breastfeeding history variable that included the category of babies who were never breastfed. Although effect sizes were relatively small, significant differences consistently emerged, following adjustment for a range of relevant covariates including socio-demographic variables and maternal prenatal diet, as well as adjustment for general head size. Babies who were both breast- and bottle-fed did not significantly differ from those who were exclusively breastfed or from those who bottle-fed or never breastfed. It is also worth noting that we found no evidence for a moderating effect of sex. Isaacs *et al*. (2008) found that enhanced nutrition was associated with a larger caudate volume, but this was only evident among the boys in their sample of preterm babies. Yet, important differences between this study and our study could

account for these discrepancies: we focused on a large sample of healthy (mainly born at term) babies, and furthermore, Isaacs *et al.* examined the nutritional benefits of an infant formula specifically designed for a higher fat and protein content to meet the needs of their preterm participants; they did not examine the effects of breastfeeding per se.

We had predicted that a longer corpus callosum length would be evident in exclusively breastfed babies compared with babies who were currently bottle-fed. The main effect of breastfeeding on corpus callosum length failed to reach significance (following adjustment for relevant covariates and head size). Similar non-significant results were obtained for our secondary analyses with the breastfeeding history variable. Thus, our hypothesis that we would see differences in white matter associated with breastfeeding practices was not supported. Isaacs *et al.* (2010) reported that white matter volume was affected by breastfeeding to a greater extent than grey matter volume, particularly among boys born preterm. However, mean age at magnetic resonance imaging (MRI) was 15.9 years, while we focus on infancy. Furthermore, in the Isaacs *et al.* study, the authors examined cortical white and grey matter volumes but did not specifically examine the corpus callosum or the gangliothalamic ovoid. Thus, it is not possible to study whether certain grey matter regions (such as the basal ganglia and thalamus that are assessed in our measure of gangliothalamic ovoid diameter) were specifically affected. As noted earlier, we might expect these regions to be especially sensitive to the effects of breastfeeding given that they have been shown to be very rich in DHA, which is abundant in breast milk (Diau *et al.* 2005; Hsieh *et al.* 2007).

Findings for ventricular volume were less clear. Although the overall main effect of breastfeeding was significant, further comparisons between groups yielded mixed results. Following adjustment for multiple comparisons, there was only a trend towards significance for a smaller ventricular volume to be associated with exclusive breastfeeding compared with bottle-fed babies or breast- and bottle-fed babies. Moreover, this was not replicated when we examined our categorisation of breastfeeding history (i.e. contrasting against the group of never breastfed babies).

Finally, in addition to assessing ultrasound measurements of specific brain structures (adjusting for general head size), we also examined whether breastfeeding was associated with general head size. Head circumference may be considered as a possible proxy for total brain volume (Bartholomeusz *et al.* 2002) and has been noted to increase at a maximal rate of 1.1 mm per day (see Wang *et al.* 2003). Findings of previous research regarding the effect of breastfeeding on head circumference have been mixed. One study of Turkish babies demonstrated that despite having similar head circumference measurements at birth, larger head circumferences in the first month of life were seen among babies who were breastfed compared with those who were breast-and bottle-fed or those who were bottle-fed. Yet, these significant differences were no longer evident after 4 months. Other studies have not reported significant differences in head circumference in relation to breastfeeding (see Dewey 1998). In our study, associations between breastfeeding and head circumference was only significant for our categorisation of breastfeeding history, such that a larger head circumference was evident among those babies who were exclusively breastfed compared with those who were never breastfed. However, this difference between exclusively breastfed and never breastfed babies fell to trend level following adjustment for multiple comparisons. Babies who were both breast- and bottle-fed did not differ significantly from those who were exclusively breastfed or never breastfed. Thus, our findings are somewhat consistent with the Turkish study demonstrating larger head circumference in relation to breastfeeding in the early post-natal period. However, it is also possible that our significant effects of breastfeeding on head circumference dissipate as these babies grow; a finding reported in the Donma study, and this would also be consistent with other studies, which have not found significant differences in head circumference in relation to breastfeeding in slightly older babies (Donma & Donma 1997; Dewey 1998).

Previous studies of preterm babies or of healthy-developing babies point towards a dose–response relationship between breast milk (i.e. percentage of breast milk in the baby's diet or duration of breast-

feeding) and child outcome (Isaacs *et al*. 2010; Chiu *et al*. 2011; Guxens *et al*. 2011). We were not able to examine a dose–response relationship per se due to the non-continuous nature of our breastfeeding variables. However, we had predicted that the greatest advantage would be seen between exclusively breastfed babies and those who were bottle-fed or never breastfed, and to a lesser extent, there would be a benefit of breast- and bottle-feeding compared with those babies who were bottle-fed or never breastfed. We only found support for the difference between exclusively breastfed babies and bottle-fed or never breastfed babies.

Although some studies, particularly those in preterm babies, have highlighted a differential advantage of breastfeeding among baby boys, we did not find any significant sex by breastfeeding interactions for any of our brain structures measured or for general head size. It is possible that these sex-specific advantages may be particularly important for the preterm male, given that male babies have been shown to be more vulnerable in the perinatal period compared with female babies (McGregor *et al*. 1992; Sheiner *et al*. 2004; Di Renzo *et al*. 2007; Cuestas *et al*. 2009). The vast majority of our participants were born at term and, hence, this sex-specific vulnerability may not be relevant in our sample of participants.

Although our results are consistent with previous research and make sense from a biological perspective, we cannot dismiss other factors that might also influence associations between breastfeeding and brain development. Previous research investigating links between breastfeeding and IQ has shown that this association may be largely attributed to differences in the parenting quality and intellectual ability of mothers who choose to breastfeed vs. not (Jacobson *et al*. 1999; see also Jain *et al*. 2002). We adjusted for maternal education and other factors that might have an important impact on both breastfeeding and brain development (such as mode of delivery, gestational age at birth and birthweight). We also tested the contribution of factors such as maternal psychopathology or maternal health behaviours during pregnancy (smoking, drinking). Although our covariates did alter our results, they did not fully explain associations between breastfeeding and early structural markers of brain development. Furthermore, we attempted as far as possible to adjust for maternal prenatal diet reported by the mother during pregnancy. However, we were not able to adjust for parent–child interaction or parenting style that might differ between our breastfeeding groups and could potentially have influenced our results. Nevertheless, we might expect that the influence of parenting on outcome (such as IQ scores as measured in other studies) would likely exert a greater influence when breastfeeding and outcome are more widely spaced apart, and where parenting might have a greater influence on the continued development of the child. Breastfeeding has been associated with better maternal mood and more interactive behaviours, which also indirectly contribute to infant development (Tanaka *et al*. 2009). There is also evidence to suggest that the striatum (encompassing the putamen and caudate nucleus) activates in response to rewarding social experience, such as in the case of maternal love (Bartels & Zeki 2004), or in response to positive social interaction (Vrticka *et al*. 2008). It is very plausible that the act of breastfeeding is socially rewarding for a baby. Further work needs to be performed to try to tease apart the social interactive nature of breastfeeding and the nutritional component. This could perhaps be achieved by assessing the neural development of those infants who breastfed vs. infants who were bottle-fed expressed breast milk. Additional limitations include the fact that our data relied on maternal reports of breastfeeding. Furthermore, when a mother noted that her baby was bottle-fed, we did not have more specific information on the type of formula used, or whether it contained nutritional supplements, such as DHA. Similarly, we lack detailed information on the nutritional composition of the breast milk for our participants. Although as noted earlier, we did attempt to address this limitation by covarying for mothers' reported adherence to the Mediterranean dietary pattern, which may be associated with the content of the breast milk. Furthermore, while the ultrasound technology we used is a safe, non-invasive and cost-effective method to image the infant brain, this method does not provide fine-tuned measurement of brain substructures. Thus, future research might also benefit from more fine-tuned

measurement of these structures, such as structural MRI that might allow for a more detailed examination of each of the structures embedded within this larger gangliothalamic ovoid, or more detailed measurement of the corpus callosum (such as thickness, which could not be reliably measured using our ultrasound technology). Finally, we have a short follow-up period. Despite this short-term follow-up, we provide evidence that breastfeeding within the first 2 months of life is associated with the size of certain brain structures, more specifically the gangliothalamic ovoid, and more general brain development as measured using head circumference. Previous studies have reported that substantial DHA and AA levels accumulate in the infant brain in the first months after birth (Heird & Lapillonne 2005; Caspi *et al*. 2007), and that incorporation of DHA into the brain cortex increases with the duration of breastfeeding (Makrides *et al*. 1995). Thus, findings of detectable differences in brain structure or biochemistry following a limited breastfeeding duration highlight that breastfeeding in the early post-natal period can still influence early brain development. Similarly, animal studies have provided evidence that early nutrition in the first weeks after birth can have lasting effects on brain structure and function (Wang *et al*. 2003). Furthermore, after 4–6 months, with the introduction of solid foods, the examination of the effects of breastfeeding on child outcome becomes confounded with the nutritional quality of the supplemented foods. Although our study does provide important data on breastfeeding practices and early brain development, future work would benefit by assessing a longer-term follow-up of the brain development of these babies.

Conclusion

Using data from a longitudinal, prospective study of approximately 680 babies followed from fetal life, we have shown that maternal reports of breastfeeding are associated with neural development, and more specifically with a larger gangliothalamic ovoid diameter. Also noteworthy is that the greatest advantage for breastfeeding was seen among exclusively breastfed babies compared with those who were bottle-fed or those who had never been breastfed. To our knowledge, this is the first study to report such findings in a large sample of healthy babies, the vast majority of whom were born at term. Results indicate that indeed maternal reports of breastfeeding are associated with early neural development, and that results are most evident for the DHA-rich subcortical structure of the gangliothalamic ovoid, although there was still some evidence for a more general benefit as indicated by our findings for ventricular volume and head size.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Contributions

CMH analysed and interpreted the data and wrote the initial draft of the manuscript. PG and SR pro-

vided guidance as to the measurement and interpretation of ultrasound data. HT designed the study together with CMH. HT and SR assisted in the interpretation of results. All co-authors participated in the manuscript preparation and critically reviewed all sections of the text for important intellectual content. HT is the guarantor of this study.

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