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Overfeeding in infancy may lead to overweight and obesity in later childhood. Mothers are advised to “tune in” to their infant’s hunger, appetite and satiation cues to prevent overfeeding. The present study aimed to 1) assess stability and change in infant hunger and satiety cues (first two years of life) taken at six monthly intervals; 2) track the expression of appetite cues during the course of a meal (beginning, middle and end). Thirty-eight women (mean age 35.3+ 3.7 years) participated in the study. Mothers were within a normal weight range (BMI=22 + 3.3 kg/m^2), most were married (N= 35; 95%) and for most this was not their first child. After an initial investigation (T1) follow-up visits took place every six months with filmed meals involving solid foods. A typical meal contained foods high in protein and carbohydrate plus cooked vegetables. Films were viewed and communication cues (engagement indicating appetite and disengagement indicating satiation) identified and recorded by appearance using the NCAST (Nursing Child Assessment Satellite Training). Coding included the frequency and time at which each cue appeared. Results showed that infants were more likely to communicate potent engagement cues such as babbling, mutual gaze and looking at mother with age. None of the disengagement cues showed any significant main effects of time of follow up. Most, not all, feeding cues were stable across the segment of the feed and did not show a simple linear change across the meal, rather this appeared to develop with age. Raising awareness of these cues with mothers may encourage more responsive and positive mealtime interactions.

Infant hunger and satiety cues during the first two years of life: developmental changes of within meal signalling.

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Introduction

Childhood obesity is a major public health problem (de Onis et al, 2010; WHO, 2016) with approximately 41 million children under the age of 5 classified as overweight or obese (WHO, 2015). Research has identified a number of factors in pregnancy and early infancy that are associated with an increased risk of overweight in childhood (Weng et al., 2012; Baidal et al., 2016). The identification of these factors and the development of childhood overweight and obesity risk prediction tools provide a tangible opportunity for early intervention (Dahly and Rudolf. 2010*;* Druet et al., 2012).

In the UK approximately 31.2% of children aged 2 to 15 years are overweight or obese (England's Health Survey; 2014). Infants who breastfeed are less likely to become overweight or to develop chronic diseases of childhood and adolescence (Gunderson. 2007). The World Health Organization (WHO) suggests that mothers should breastfeed exclusively for the first six months of life, to continue to breastfeed beyond this time and to introduce appropriate complementary foods (WHO, 2002). A meta-analysis of 17 studies showed a strong relationship between increasing duration of months of breastfeeding (BF) and a reduced risk of overweight, with each month of BF producing a 4% reduction in risk (Harder et al., 2005). It has been demonstrated that formula feeding and early introduction to solid foods together promote excess weight gain in early life (Ong, 2006).

Although the WHO recommends exclusive breastfeeding until six months, and countries such as the United Kingdom, Australia and Canada have adopted this recommendation, adherence varies by country. For example, according to the UK Infant Feeding Survey conducted in 2010, 7 years after adoption of the current WHO recommendation, only 1% of mothers were exclusively breastfeeding at 6 months, around 30% of mothers had already introduced complementary foods by age 4 months, and 75% had done so by age 5 months (McAndrew et al., 2012). Around half of mothers reported introducing solid foods earlier than the recommended age of 6m since they perceived that their baby was no longer satisfied with milk feeds. A study by Modrek et al. (2017) examined the effect of breastfeeding practices on child weight outcomes at age 2 years. Findings from this study suggested that for every extra week that the child was breastfed, the risk of being obese at age 2 years was reduced by around 1% and although modest, this was statistically significant.

The consistency of these associations suggests that breastfeeding may have lasting protective effects independent of dietary and physical activity patterns later in life (Horta et al., 2013; Owen et al., 2005).

Furthermore, a systematic research review by Vail et al. (2015) explored whether age of complementary feeding (age 3-6 months) promoted rapid infant weight gain, taking account of baby weight before solid food introduction. Findings from the review revealed an inverse association between age at weaning and infant growth with some evidence of reverse causality. This suggests that mothers are responding to the needs of heavier babies, and are offering solid foods early to meet the higher energy requirements of their babies.

If breastfeeding does contribute to a lower risk of later overweight and obesity, it may be in part achieved through psychological mechanisms such as responsive feeding where mothers are “tuned in” to their infant’s satiety cues allowing the infant to control the feed rather than exerting control themselves. In support of this contention, longer duration of breastfeeding is linked to lower levels of parental control (Taveras et al, 2004; 2006). Parents who relinquish control and depend on their child to signal hunger, appetite and satiety appear to facilitate healthy self-regulation. A recent study by Shloim, Vereijken, Blundell & Hetherington (2017) reported higher levels of engagement (hunger) and disengagement (satiety) cues signalled by breastfed compared to formula fed infants. Interestingly, in an earlier study involving the same cohort of mothers it was found that BF was also associated with a more positive mealtime experience than other types of feeding in 2- 6 months infants (Shloim, Rudolf, Feltbower, Mohebati & Hetherington, 2015). These findings reveal behavioural benefits of breastfeeding beyond the well characterised nutrient, flavour and antibody content of milk, suggesting that BF promotes communication between mother and infant; providing a beneficial experience compared with other modes of feeding. Thus, “breast is best” includes behavioural components associated with breastfeeding, which may influence self-regulation and healthy eating patterns later in life.

Responsive feeding promotes optimal communication between an infant and its mother. The infant communicates hunger and satiety to the caregiver, who in turn recognises, interprets and responds appropriately to these cues (Di Santis, Hodges, Johnson & Fisher, 2011). Barnard and Eyres (1979) suggest that the interaction between parent, child and the environment is paramount. The infant signals their hunger/satiety state to the mother who in turn responds to this. This concept fits well with responsive feeding and provides a useful framework to code infant communication during a meal. Research suggests that parental responsiveness to their child’s hunger, appetite and satiety signals is critical for the development of healthy eating habits and may affect offspring weight status (underweight or overweight; Disantis et al., 2012; McNally et al., 2016). A non-responsive feeding style could impact both the frequency of feeds and the quantities eaten (Hardon et al., 2013). It is therefore important to support parents to identify, interpret and respond to these cues (feeding in response to hunger cues, ending feeding in response to satiety cues).

As children progress from milk feeding to solid foods, their developmental maturity ensures that they display diverse means of communicating hunger and satiation. Hodges et al. (2008) explored maternal initiation and termination of feeding in a sample of 71 ethnically diverse mothers. They noted variations in the extent to which infant cues were evident in maternal approaches to feeding. They found some mothers focused on amount consumed whereas others focused on external cues such as eating schedule. As infants develop, they use language to signal enjoyment and dislike of a specific food (Hetherington, 2017). However, before language develops infants communicate hunger, appetite and satiety but there are few validated tools available to identify these cues (McNally et al., 2015). In order to encourage responsive feeding it is important to identify the ways infants communicate their needs as well as how mothers "tune in" to these cues. For example, a first step is to characterise how infants develop their capacity to signal hunger and satiety over time and whether these signals change in type and frequency according to need state during a meal and as they mature.

The present study was designed to: 1) assess stability and change in occurrences of hunger and satiety cues expressed during a meal in the first two years of life; 2) track the expression of appetite cues across the meal (beginning, middle and end). To achieve these aims, mealtime interactions were filmed on four occasions (every six months) from the time solid foods were introduced. Findings from the first follow up (infants age range from 2-6 months) addressed milk feeding cues and are reported elsewhere (Shloim et al., 2017). The present study went on to characterise feeding cues from 6-24 months from the beginning of complementary feeding to independent feeding. Cues were systematically recorded as engagement (interest in eating; hunger) and disengagement (disinterest in eating; fullness). It was hypothesized that as infants develop they shift towards greater intentionality in their communications using gesture, vocalisation (Hodges et al., 2017) and so communication cues will be more frequent and prominent with age; and b) engagement cues would be observed more frequently early in the meal and disengagement cues would be seen more frequently at the end of a meal (Shloim et al., 2017) to signal change in nutritional status from hunger and readiness to eat to satiation, repletion and meal termination.

Methods

Participants

One hundred and fifty-six women from Israel (N=67) and the UK (N=89) were recruited for a study exploring well-being and eating behaviours during pregnancy (Shloim et al., 2013). Women were recruited through distribution of posters and flyers in community centres and the University of Leeds, and emails were sent through the University circulation lists. Most of the women were in their first trimester (Shloim et al., 2015). Participants were asked to complete a five-part questionnaire, exploring eating behaviours and maternal well-being. Completed questionnaires were returned to the lead researcher via email or post. Inclusion characteristics to participate in the study were being pregnant (first trimester) with no comorbidities. Thus, women with diabetes for example or any other disease were excluded from the study, as well as those previously diagnosed with an eating disorder. The study protocol was ethically approved by the Joint School of Medicine Research Ethics Committee (reference number HSLTLM/10/021). Mothers were interviewed to investigate eating behaviours following pregnancy, their infant's daily schedule and eating habits (Shloim et al., 2014). From the original sample, 73 women (N=42 from Israel; N=31 from the UK) continued to take part in a follow up study (exploring eating behaviours and well-being in the postpartum period). The women who continued to take part in our study were older, more educated and more likely to be married than those who declined the follow up visits. The majority of women who did not participate in the follow up were from the UK (66%). This is more fully addressed elsewhere (Shloim et al., 2014). A sub-sample (N=38; N=20 from Israel; N=18 from the UK) continued to take part in the present study exploring mealtime feeding communication cues. Mothers provided written, informed consent to the filmed mealtime sessions. Maternal age, body mass index, and level of education achieved did not vary between participants who only took part in the questionnaire study and those who agreed to be filmed and interviewed as well (Shloim et al., 2015). Sessions took place every six months and mothers were filmed at their homes while feeding their infants. For the purpose of this study, the first author (NS) used one camera and manually recorded the feeding interaction from a distance of about 1m away from the infant and the mother. This home based method is more ecologically valid than filming mother-infant dyads in the laboratory. Milk feeding meals were recorded at ages 2-6 months (Shloim et al., 2017). Then for the analysis in the present paper the impact of mode of feeding (breastfed vs. formula fed) was then followed up from 6 to 24m but in a smaller cohort (10 breastfed and 14 formula fed).Thirteen were mixed fed and were removed from the milk feeding analysis.

Mothers determined the timing of solid food introduction then visits were organised to permit further filming. During these sessions the episode began only when the mother felt it was the right time to feed and the meal commenced after infants demonstrated hunger by crying or by being unsettled (Shloim et al., 2015). The starting time of the meal was defined as the time when the meal was placed in front of the child (Shloim et al., 2017). The ending was defined when food was removed by the mother or when the infant terminated the meal by pushing the plate away or leaving the table for example. The present study explored feeding cues from solid food introduction to the "post weaning" phases at T2 (6-12 months), T3 (12 - 18 months) and T4 (18-24 months). The average age for introduction of complementary feeding in the UK and in Israel is around 5 months (McAndrew et al., 2012; https://www.health.gov.il/Subjects/infants/feeding/Pages/feeding_infants_first_year.aspx). This was the case in the present sample.

The menu varied between participants but a typical meal contained foods high in protein (e.g. chicken, schnitzel), and items which are high in carbohydrate content (e.g. pasta, rice, potatoes, bread) and cooked vegetables (e.g. broccoli, carrots). Israeli mothers were unlikely to offer any dessert or dairy type products as part of the meal due to adherence to a "Kosher" lifestyle (not mixing meat and dairy in the same meal). Mothers from both countries did not offer any dessert. Since this is unusual for UK mothers, it is possible that as participants were aware of being recorded while feeding, dessert may have been excluded from the meal on these occasions. Most filmed feeds were done at lunchtime with very few recorded during evening meal.

Materials

NCAST (Nursing Child Assessment Satellite Training)

The NCAST was developed in the 1980s by Dr. Kathryn Barnard (Beel-Bates et al., 2012) who identified environmental factors critical to a child's well-being and demonstrated the importance of parent-child interaction as a predictor of later cognitive and language development. The NCAST team suggested that a child's physical, emotional, intellectual and social domains interact and impact on the child's overall health. Thus from a very young age children depend on adults to mediate experiences and create learning experiences for them. The Nursing Child Assessment Project (NCAP) team created a framework for the child's health assessment in which the infant produces clear communication cues and the mother responds to these cues. This process resonates with responsive feeding and provides a useful framework for coding infant communication during the meal.

For the purpose of this research we explored 83 feeding cues, divided into engagement; hunger (e.g. babbling and mutual gaze) and disengagement cues; satiety (e.g. crying and lateral head shake; Givens. 1978; NCAST Barnard, 1994). Films were viewed on average 2-3 times by the lead researcher (NS), a qualified psychotherapist with training in NCAST. An additional three researchers (IS, BM, JX) were trained by the lead researcher (NS) and viewed the films independently. All communication cues (hunger and satiety) were applied, identified and recorded by appearance. For each film, the time (in seconds) of cue was coded.

Data Analysis

The analysis was conducted using SPSS (IBM, version 22). Data were coded to calculate the time (in seconds) each cue appeared and feeds were divided into three equal segments from the beginning, middle and end of feed. These sections of the meal were simply determined by sampling each third of the meal duration, so first, second and final third of the recorded meal segment. The analysis always addressed the specific filmed feed. The number of behaviours were counted for each cue separately first for the total number of appearances and then for each segment (beginning, middle, end). Histograms were plotted and kurtosis was calculated to identify any skewness from normality.

An additional sample of films (N=50; 60%) was coded by the trained researchers and quality ratings were subjected to inter-rater reliability analysis. The lead researcher (NS) re-coded a random selection of 15 films to check reliability. The process was conducted separately for the engagement (hunger) and then for the disengagement (satiety) feeding cues. A high level of interrater agreement was found (single measures interclass correlations by use of a two way random effects model for the disengagement cues ($r= 0.77$, $p =0.03$) and for the engagement cues ($r= 0.71$, $p = 0.01$). Cronbach's alpha indicated that the disengagement and the engagement feeding cues had acceptable internal consistency ($\alpha= 0.81$; $\alpha = 0.85$ respectively).

To examine whether frequency of cues varied within a meal and over time of follow-up, repeated measures analyses of variance (ANOVA) were carried out with two within subjects factors (meal segment, follow-up). Where Mauchley's test indicated that the assumption of sphericity was violated, Greenhaus-Geisser corrections were applied. Where ANOVA revealed significant main effects, Bonferroni corrected pairwise comparisons were calculated to investigate significant differences between the levels. The effective retained α was < 0.017 for these comparisons. Mean differences and p values are presented.

Bonferroni corrections were applied when multiple pairwise comparisons were carried out following a statistically significant effect – for example if there was a significant effect of time, this would be followed by comparing each follow up time period, which itself produces 3 ttests. This was to keep the familywise error rate at 5%. Where the ANOVA revealed statistically significant interactions, these were investigated by separate 1-way ANOVAs for each follow-up meal. Where appropriate these were again further investigated by Bonferroni corrected pairwise comparisons.

In order to assess differences in communication cues between infants who have been previously breastfed or formula fed (Shloim et al., 2017), the analyses further explored the subset of N=27 infants included in the previous paper. We carried out repeated measures ANOVA as above, on each communication cue, with breastfeeding status during the previous film as a between subjects factor.

Ethical Considerations

The study was approved by the School of Psychology Ethics Committee and the School of Healthcare at the University of Leeds, reference no. #11-0137.

Results

Thirty-eight infants (Israel; N=23; UK; N=15) were filmed during a feed. Three participants did not complete the study. Two families moved abroad and one did not reply to the researcher's attempts to arrange a filmed feed. No significant differences were identified between Israeli and UK infants for infants' age and gender. The analysis was therefore combined across sites. Most mothers were married (N= 35; 94.6%) and for more than half of the sample this was not the first child (see Shloim et al, 2017). Most mothers were in paid work at each stage of the follow ups (59% T2, 79%, T3 and 85% T4).

Maternal and infant age (Table 1) and duration of feed (in seconds) per session were calculated. Mean (SD) duration of feed (seconds) was 799 ± 379 (T2); 932 ±453 (T3) and 891.7 ± 497 (T4).

Table 1; Mother and infant characteristics during follow up visits.

	N (%)	Mean (SD)	Median	IQR	Missing data
2nd follow-up (T2)					
Maternal age (years)	41	35.3 (3.7)	37	27-41	
Maternal BMI kg/m2	39	22.6 (3.1)	22.5	16.1-30.3	5%
BMI < 25 kg/m2	31 (80%)				
BMI ≥ 25 kg/m2	8 (20%)				
Working Status:					
In paid work	24 (59%)				
Not in paid work	17 (41%)				0
Infant age at follow-up (weeks):	41	39.5 (6.8)	36	27-56	
Israel	23	42.0 (6.8)	42	32-56	
UK	18	36.5 (5.7)	36	27-45	
3rd follow-up (T3)					
Maternal BMI kg/m2	38	22.5 (3.1)		16.5-30.4	8%
BMI < 25 kg/m2	31 (82%)				
BMI ≥ 25 kg/m2	7 (18%)				
Working Status:					
In paid work	30 (79%)				0
Not in paid work	8 (21%)				
Infant age at follow-up (weeks):	38	64.0 (7.2)	61	51-80	2.5%
Israel	21	66.0 (7)	66	56-80	
UK	17	61.4 (6.8)	60	51-72	
4th follow-up (T4)					
Maternal BMI kg/m2	38	22.4 (3.1)		16.3-30.4	5%
BMI < 25 kg/m2	31(84%)				
BMI ≥ 25 kg/m2	6(16%)				
Working Status:					
In paid work	33(85%)				
Not in paid work	6(15%)				
Infant age at follow-up (weeks):	39	87.4 (8.8)	86	56-104	
Israel	22	90.4 (6.8)	90	80-104	
UK	17	83.4 (9.6)	84	56-96	

(T2:6-12 months; T3: 12- 18 months; T4: 18-24 months).

Feeding Cues

In total 28 feeding cues were identified. The frequency of feeding cues as a total and by part of feed was calculated and histograms were produced. The findings suggested that several feeding cues could be related as outliers and removal did not affect the analysis. Thus 10 cues were withdrawn from the list for coding since they appeared infrequently across the three sessions ($N \leq 40$; see table 2 for the numbers of each cue). There were more observed disengagement cues compared to engagement cues overall, and more disengagement than engagement cues recorded within the meal. This is with agreement with the NCAST grid.

Our findings are based on 18 cues consisting of 8 engagement (hunger) cues and 10 disengagement (satiety) cues. Tables 2a and 2b show the type of cue (engagement vs disengagement), the count by meal segment (beginning, middle and end) by time point (T2, T3, T4). Main effects of time of follow-up, segment of the feed and the interaction between time and segment for each specific feeding cue are highlighted.

Main effect of time of follow-up

A significant main effect of follow-up was found, indicating a significant change in frequency of several feeding cues over time. For the engagement cues, higher levels of engagement cues were observed with time. As such, infants were more likely to use verbal cueing (babbling) as their age increased ($F(1.62, 60.02) = 5.79$, $p = 0.008$). This was due to infants babbling less in the first follow up compared with the last follow up (mean difference = 5.33, $p = 0.011$). There was also a significant effect of time of follow-up on the infants tendency to look at their mother's face (mutual gaze ($F(1.67, 61.73) = 3.24$, $p = 0.045$), as more of these cues occurred in T4 than T2 (mean difference = 1.68, $p = 0.04$)). Infants were also more likely to take their hand to their stomach or mouth as their age increased ($F(2, 74) = 6.66$, $p = 0.002$). In this case there was a significant difference between T2 and T3 (mean difference = 5.83, $p = 0.007$) and between T2 and T4 (mean difference = 3.94, $p = 0.029$). Table 2a provides a full description of the effect of time of follow-up on frequency of engagement cues.

None of the disengagement cues showed any significant main effects of time of follow up.

Table 2a: Mean (SD) frequency and distribution of engagement cues across follow-ups and meal segment

Type of Cue	Time of follow up	Meal segment mean (sd)			Main effect of time of follow up	Main effect of meal segment	Interaction between time of follow up and segment
		Beginning	Middle	End			
Babbling	**T2**	**1.9 (4.2)**	**2.0 (4.2)**	**2.9 (6.4)**	**F (1.62, 60.02) = 5.79 P=0.008***	**F (1.64,60.79)= 0.40 P=0.63**	**F (2.64,97.52) = 0.58 P=0.61**
	T3	**4.6 (8.8)**	**3.5 (6.3)**	**3.9 (6.3)**			
	T4	**7.3 (11.4)**	**7.5 (10.1)**	**7.8 (10.4)**			
Feeding Sounds	**T2**	**0.07 (0.3)**	**0.07(0.3)**	**0.2 (1.3)**	**F (1.26, 46.72) = 1.08 P=0.32**	**F (1.14, 42.25) = 1.42 P=0.24**	**F (1.33, 49.0) = 1.44 P=0.24**
	T3	**0.9 (4.06)**	**0.2 (0.6)**	**0.1 (0.5)**			
	T4	**0.2 (0.8)**	**0.1 (0.5)**	**0.07 (0.2)**			
Hand to stomach/mouth	**T2**	**2.7 (5.1)**	**2.5 (5.1)**	**2.2 (7.3)**	**F (2,74) = 6.66 P=0.002***	**F (1.57, 58.16) = 1.02 P=0.35**	**F (2.59, 95.92) = 0.88 P=0.44**
	T3	**10 (11.2)**	**7.3 (9.4)**	**7.5 (11.3)**			
	T4	**6.4 (8.4)**	**5.9 (7.3)**	**6.8 (8)**			
Mutual Gaze	**T2**	**0.8 (1.5)**	**0.9 (2.1)**	**0.8 (1.6)**	**F (1.67,61.73) = 3.24 P=0.045***	**F (2,74) = 4.07 P=0.02***	**F (2.64, 97.49) = 2.85 P=0.048***
	T3	**2.3 (4.5)**	**0.9 (2.5)**	**0.7 (2.4)**			
	T4	**2.6 (5.3)**	**2.8 (4.7)**	**2.2 (4.1)**			
Reaching towards caregiver	**T2**	**0.2 (1.1)**	**0.2 (0.8)**	**0.3 (1.06)**	**F (1.57, 58.10) = 4.83 P=0.02***	**F (1.42, 52.56) = 0.23 P=0.79**	**F (2.15, 79.57) = 0.44 P=0.66**
	T3	**1.2 (2.1)**	**0.9 (2.7)**	**1.5 (4.05)**			
	T4	**0.6 (1.3)**	**0.8 (1.9)**	**0.7 (1.4)**			
Reaching towards food	**T2**	**2.6 (6.2)**	**2.9 (6.8)**	**2.4 (6.5)**	**F (2,74) = 1.20 P=0.31**	**F (1.56, 57.68) = 0.93 P=0.37**	**F (2.69, 99.54) = 1.00 P=0.39**
	T3	**5.3 (7.4)**	**4.0 (6.5)**	**3.6 (8.3)**			
	T4	**4.7 (6.0)**	**3.7 (5.1)**	**4.6 (6.2)**			
Spoon/fork to mouth	**T2**	**5.5 (7.8)**	**5.0 (7.4)**	**4.0 (7.7)**	**F (2,74) = 0.08 P=0.92**	**F (1.43, 53.03) = 0.47 P=0.63**	**F (2.72,100.68) = 0.19 P=0.89**
	T3	**5.6 (9.4)**	**4.9 (7.5)**	**4.8 (9.5)**			
	T4	**5.1 (8.7)**	**4.1 (5)**	**4.5 (6)**			
Turning head to caregiver	**T2**	**0.6 (1.4)**	**0.3 (0.7)**	**0.2 (0.7)**	**F (1.44, 53.34) = 3.12 P=0.07**	**F (1.70, 62.99) = 5.42 P=0.01***	**F (2.58, 95.30) = 0.50 P=0.65**
	T3	**1.7 (2.6)**	**1.5 (2.4)**	**1.1 (2.4)**			
	T4	**2.2 (5.4)**	**1.6 (3.8)**	**1.1 (2.1)**			

(T2:6-12 months; T3: 12- 18 months; T4: 18-24 months).

Table 2b: Mean (SD) frequency and distribution of disengagement cues across follow-ups and meal segment

Type of Cue	Time of follow up	Meal segment mean (sd)			Main effect of time of follow up	Main effect of meal segment	Interaction between time of follow up and meal segment
		Beginning	Middle	End			
Choking	T2	0.05 (0.2)	0.1 (0.5)	0.1 (0.6)	F (1.72, 63.46) = 0.59 P=0.56	F (1.50, 55.57) = 0.98 P=0.38	F (1.57, 57.90) = 0.69 P=0.49
	T3	0.07 (0.2)	0.4 (2.1)	0.3 (0.8)			
	T4	0.2 (0.7)	0.3 (0.7)	0.1 (0.3)			
Crying	T2	0.6 (2.6)	0.02 (0.1)	0.2 (0.8)	F (2,74) = 0.34 P=0.71	F (1.64,60.70) = 0.24 P=0.74	F (1.43, 53.07) = 1.26 P=0.28
	T3	0.1 (0.5)	0.1 (0.5)	0.2 (0.7)			
	T4	0.07 (0.3)	0.4 (2.4)	0.2 (0.9)			
Fussing	T2	0.5 (1.5)	0.4 (1.4)	1.4 (3.8)	F (1.23, 45.47) = 1.97 P=0.17	F (1.61,59.60) = 2.70 P=0.09	F (1.82,67.47) = 3.80 P=0.03*
	T3	0.2 (0.6)	0.2 (0.6)	0.2 (0.6)			
	T4	0.4 (1.1)	0.3 (0.9)	0.3 (0.9)			
Halt Hand	T2	0.2 (1.1)	0.2 (0.9)	0.2 (0.4)	F (2,74) = 0.48 P=0.62	F (2,74) = 0.45 P=0.64	F (2.97, 109.71) = 0.72 P=0.54
	T3	0.1 (0.6)	0.4 (1.3)	0.3 (0.8)			
	T4	0.2 (1.1)	0.1 (0.5)	0.1 (0.5)			
Hand behind head\ear\eye	T2	0.4 (1.1)	0.1 (0.4)	0.4 (1.1)	F (2,74) = 0.13 P=0.88	F (2,74) = 0.24 P=0.79	F (2.91, 107.63) = 3.22 P=0.03*
	T3	0.4 (1.1)	0.1 (0.4)	0.5 (1.6)			
	T4	0.2 (0.7)	0.7 (1.6)	0.2 (0.8)			
Lateral Head Shake	T2	0.2 (0.7)	0.1 (0.4)	0.05 (0.3)	F (1.44,53.32) = 3.00 P=0.074	F (1.45,53.80) = 0.39 P=0.61	F (2.56,94.61) = 1.28 P=0.28
	T3	1.0 (2.8)	0.8 (1.8)	0.8 (2.1)			
	T4	0.3 (0.8)	0.3 (1.1)	0.6 (1.9)			
Looking away	T2	3.2 (5.4)	2.6 (5.6)	2.1 (5.1)	F (2,74) = 0.91 P=0.41	F (2,74) = 3.85 P=0.03*	F (4,148) = 0.55 P=0.70
	T3	1.8 (3.8)	1.9 (4.1)	0.6 (1.1)			
	T4	2.2 (4.7)	2.7 (5.2)	1.8 (3.9)			
Overhand beating movements of arms	T2	0.1 (0.4)	0.2 (0.6)	0.1 (0.6)	F (2,74) = 0.29 P=0.75	F (1.52,56.39) = 0.29 P=0.69	F (2.51,92.84) = 0.86 P=0.49
	T3	0.2 (0.9)	0.1 (0.3)	0.2 (0.9)			
	T4	0.05 (0.2)	0.1 (0.6)	0.2 (0.6)			
Pulling away	T2	0.8 (2.8)	0.5 (1.9)	0.6 (1.7)	F (1.55,57.37) = 2.34 P=0.12	F (2,74) = 0.66 P=0.51	F (1.80,66.68) = 0.38 P=0.66
	T3	0.4 (1.2)	0.4 (1.1)	0.5 (1.5)			
	T4	0.1 (0.4)	0.07 (0.3)	0.07 (0.2)			
Tray Pounding	T2	0.1 (0.5)	0.02 (0.1)	0.2 (0.7)	F (1.37,50.56) = 1.36 P=0.26	F (1.58, 58.56) = 4.38 P=0.02*	F (2.70, 99.80) = 0.62 P=0.59
	T3	0.1 (0.9)	0.4 (1.2)	0.5 (1.6)			
	T4	0.07 (0.3)	0.1 (0.6)	0.4 (1.5)			

(T2:6-12 months; T3: 12- 18 months; T4: 18-24 months).

Main effect of meal segment

For the engagement cues, a significant difference was identified for mutual gaze (F (2, 74) =4.07, P=0.02). Infants tended to look at their mother's face more in the first segment of the meal compared to the end segment (mean difference = 0.67, p = 0.039). There was also a significant change in the amount the infant turned its head to the caregiver (F1.70, 62.99) = 5.42, p = 0.01). Again, this was due to a higher frequency in the initial segment than in the end segment (mean difference = 0.73, p = 0.017). For the disengagement feeding cues (table 2b), there was a significant change across the meal in "tray pounding" (F(1.58, 58.56) = 4.38, p = 0.02). There was more of this behaviour at the end of the feed compared to the beginning (mean difference = 0.28, p = 0.043). There was also a significant main effect of meal segment on the infant looking away (F(2, 74) = 3.85, p = 0.03), with more looking away during the beginning and the middle of the meal than the end. The only significant difference however was between the middle of the meal and the end of the meal (mean difference = 0.90, p = 0.046). This latter finding was against expectations and is discussed later.

For most engagement and disengagement feeding cues frequencies were similar across the beginning, middle and end of the meal. Table 2a and 2b summarizes the main effect of meal segment on the different feeding cues.

Interaction between time of follow-up and meal segment

For mutual gaze, there was a significant interaction between time of follow-up and meal segment (table 2a). Analysis of each follow-up separately revealed no significant effect of meal segment for T2 (F (1.47, 54.30) = 0.18, p = 0.77), or T4 (F (1.55, 57.27) = 0.76, p = 0.44). However, there was a significant effect of meal segment at T3 (1.29, 47.71) = 6.28, p = 0.01). Bonferroni corrected pairwise comparisons revealed this was due to children showing more mutual gaze at the beginning of the meal than at the end (mean difference = 1.55, p = 0.024).

Table 2b indicates the interaction between time of follow-up and meal segment for each of the disengagement feeding cues.

For fussing, there was a significant interaction between the time of follow-up and meal segment (table 2b). Analysis of each follow up separately revealed a significant effect of meal segment at T2 (F(1.18, 43.58) = 4.30, p = 0.038) but not at T3 (F(2, 74) = 0.68, p = 0.51) or T4 (F(1.53, 56.47) = 1.41, p = 0.25). However, there were no significant differences between meal segments in T2 when Bonferroni pairwise comparisons were examined, although there was a large numerical difference between the end of the meal and the other times.

There was also a significant interaction for hand behind their ear/eye. Examination of each follow-up separately showed no effect of meal segment at T2 ($F(1.60, 59.11) = 1.87$, $p = 0.16$), T3 ($F(1.71,63.36) = 1.79$, $p = 0.17$) or T4 ($F(1.53, 56.68) = 3.05$, $p = 0.07$). The interaction is a result of the larger effect at T4 compared to the other time periods. However, in all children these behaviours were relatively rare and so it is difficult assign anything meaningful to this observation.

Effect of mode of feeding (breastfeeding vs. formula feeding) on the development of communication cues (engagement and disengagement cues)

Repeated measures ANOVA were conducted on each of the feeding cues, with a between subjects factor of feeding mode (breastfed or formula fed). There was a significant effect of previous mode of feeding for 'Hand to stomach/mouth', $F(1, 22) = 5.63$, $p = 0.027$. The findings suggest that breastfed infants (mean = 7.72 $\pm$ 1.21); Shloim et al., 2017) were more likely to put their hand to mouth/stomach compared to previously formula fed infants (mean = 3.95 $\pm$1.02). All other main effects and interactions (between mode of feeding, by meal segment, and by follow-up were non-significant (largest $F = 3.55$); there was a trend ($p = 0.078$) for more engagement cues to be used by breastfed infants than formula fed (overall) but the sample was underpowered to test this effectively.

To conclude, as the infants developed, they were more likely to communicate potent engagement cues such as babbling, mutual gaze and looking at mother perhaps to indicate interest in eating. Most feeding cues were stable across the segment of the feed (apart from mutual gaze, looking away, tray pounding), and did not show a simple linear change with time within the meal, rather this appeared to develop with age. Interaction effects between age and meal segment demonstrated the predicted pattern of change within the meal, indicating more competence in signalling change in hunger state during the meal segment with time.

Discussion

The present study aimed to assess stability and change in hunger and satiety cues in the first two years of life and to track feeding cues by segment of the meal (beginning, middle and end). It was hypothesized that as infants get older they become more able to communicate appetite cues as intentionality is asserted and signalled to the caregiver through the meal. Findings partly support this hypothesis with an increase in frequency of some, but not all cues with development. Four of the eight engagement cues appeared to increase in frequency with age, however no disengagement cues increased with age. For example, babbling and hand to stomach/mouth were all very potent signals that were expressed more frequently over time. Interestingly, previously breastfed infants produced this engagement feeding cue (hand to stomach/mouth) more often than formula fed infants.

However, there was no simple, linear change in frequency of engagement and disengagement cues as a function of meal segment. This was surprising as it was thought that engagement cues signal interest (hunger/appetite) and disengagement cues signal disinterest (satiation/satiety) as the meal progresses (Hetherington 2017). With age and growing competence, changes in feeding cues within the meal were more clearly signalled. For example, mutual gaze was used more frequently at the beginning of the meal as children developed, reaching towards the caregiver was most frequent age 12-18m especially at the beginning of the meal; and fussing was most apparent at the end of the meal only in children aged 6-12m. This could indicate over time mothers learn to anticipate disengagement behaviours such as fussing and terminate the meal before this occurs.

It is possible that a larger sample would have resulted a clearer and stronger pattern in feeding cues by segment of the feed. The results highlight the complexity of communication behaviours and mealtime interactions which are not straightforward nor predictably linear. However, one explanation for this is that mothers anticipate and respond to cues before they become very obvious by the end of the meal.

One of the main challenges faced in a study of this kind is in coding such a large dataset and validating the coding process. To ensure good methodological practice, the study protocol, hypothesis and analysis, were agreed prior to data collection. This process aligns well with the report by Pesch and Lumberg (2017) summarizing the main methodological considerations for observational coding of eating and feeding behaviours in children and their families. The authors suggested that the coding of behaviours and specific methods followed are not widely shared in the literature resulting in challenges to replicate, validate and test the reliability of coding schemes across settings.

Changes in behaviour over time

With age infants communicated hunger and satiety cues more often and this is likely to influence mothers' ability to follow responsive feeding. Worobey et al. (2009) observed lower maternal sensitivity to feeding cues (hunger and satiety) at 6 months compared to 12 months, which predicted infant weight gain between 6 and 12 months. Most cues which were identified in the present study were potent (babbling, reaching towards caregiver etc.) and therefore clearly communicated by the infants. Our findings support previous research by

Hodges et al. (2016) suggesting that early receptiveness cues were relatively rare, whereas active receptiveness cues were much more common.

Infants communicate hunger through their interest or disinterest in food using a series of communication cues (Hetherington. 2017). Schwartz et al. (2011) suggest that preserving internal cues in early life might be more beneficial than treating obesity later on. Our findings suggest that infants were able to communicate hunger and satiation more strongly with age. Previous research by the authors (Shloim et al., 2015; 2017) noted the importance of positive mealtime interaction in terms of setting, positioning and not feeding while disengaging. Thus, when the infant communicates satiation and such feeding cues are ignored or misunderstood, it might lead to a shift towards using external rather than internal cues to determine food intake (Caballero et al., 2003). This is further supported by Worobey et al. (2009) who showed that mothers who were less sensitive to their infants' cues had infants who gained more weight by the age of 1 year.

In the present study mothers were asked to feed their infant as they normally do, as previously suggested by Barnard and Eyres (1979), understanding infant's communication cues (hunger or satiety) is a shared responsibility by the infant and its mother. It is possible that a more sensitive analysis, focusing on mother and infant simultaneously, would have permitted an insight into responsiveness, as has been achieved by Hodges et al (2016).

Limitations

There are several limitations to consider. Most women recruited for this study were of a relatively high socio-economic status and highly educated which could have influenced their feeding behaviours and interactions during feeds. While in the current study we examined whether breastfeeding at the time of the first film impacted on subsequent development of communication cues, it did not take into account the length of time for which infants had been breastfed. Breastfed infants are more likely to display higher levels of engagement cues and therefore future studies should consider using a larger set of participants, compare between breastfeeding and formula feeding and with varying social economic statuses as well as education levels

Another limitation to consider is that the feeding interaction filming was not limited to one set meal (for e.g. dinner time) therefore time of day could also affect the number of engagement or disengagement cues displayed.

Both mothers and infants were aware they were being filmed and may have been influenced by the presence of the researcher for e.g. infants have been more distracted during the filmed meal compared to normal.

Longitudinal studies looking into feeding cues beyond the first year of life are limited. Research investigating feeding interactions and communication during meals is important to enhance understanding of these interactions and their role in promoting self-regulation behaviour. Providing mothers with the knowledge to identify hunger and satiety cues may result in better mealtime interactions and responsive feeding, whilst providing a better experience of feeding between mothers and infants.

The important contribution that parents make to their children's eating behaviours is one of the main reasons why parents of young children are frequently targeted in public health interventions aimed at reducing the prevalence of childhood obesity. As eating behaviour traits are relatively stable throughout childhood and into adulthood, a focus on the early years should be maintained.

Future research is needed to further examine the unique and interactive contribution of parental and child factors, to better integrate knowledge and to provide further insight into the complex mechanisms involved in developmental of children's eating behaviour, appetite regulation and weight status.

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