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Development of prenatal lateralization: evidence from fetal mouth movements

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Development of prenatal lateralization: evidence from fetal mouth movements**Running title**

Lateralization of fetal mouth movements

Abstract

Background: Human lateralized behaviors relate to the asymmetric development of the brain. Research of the prenatal origins of laterality is equivocal with some studies suggesting that fetuses exhibit lateralized behavior and other not finding such laterality. Given that by around 22 weeks gestation the left cerebral hemisphere compared to the right is significantly larger in both male and female fetuses we expected that the right side of the fetal face would show more movement with increased gestation. This longitudinal study investigated whether fetuses from 24 to 36 weeks gestation showed increasing lateralized behaviours during mouth opening and whether lateralized mouth movements are related to fetal age, gender and maternal self-reported prenatal stress.

Participants: Following ethical approval, fifteen healthy fetuses (8 girls) of primagravid mothers were scanned four times from 24-36-weeks gestation. Two types of mouth opening movements - upper lip raiser and mouth stretch - were coded in 60 scans for 10 minutes.

Results: We modelled the proportion of right mouth opening for each fetal scan using a generalised linear mixed model, which takes account of the repeated measures design. There was a significant increase in the proportion of lateralized mouth openings over the period increasing by 11% for each week of gestational age (LRT change in deviance= 10.92, 1 df; $p < 0.001$). No gender differences were found nor was there any effect of maternally reported stress on fetal lateralized mouth movements. There was also evidence of left lateralization preference in mouth movement, although no evidence of changes in lateralization bias over time. This longitudinal study provides important new insights into the development of lateralized mouth movements from 24-36 weeks gestation.

Key words: human fetal development, lateralized fetal mouth movements, maternal stress, 4-D scans

INTRODUCTION

There has been long-standing interest in the development of lateralized behavior in the fetus (e.g. Goodwin & Michel, 1981; Previc, 1991). Examination of lateralized development is important because it is thought to relate to the asymmetric development of the brain (e.g. Michel, Nelson, Babik, Campbell, & Marcinowski, 2013). Toga and Thomson (2003), reviewing functional brain asymmetries, report that experience-dependent plasticity might induce neuronal changes in the hemispheres such that, for example, mice with dominant right whisker pads have a left paw preference. The functional differentiation of right and left hemispheres has been reported not only for animals but also in humans (Wallez & Vauclair, 2012). Research seems to agree that prenatal brain development proceeds differentially, with the left cerebral hemisphere compared with the right hemisphere being significantly larger in both male and female fetuses by around 22 weeks gestation (Hering-Hanit, Archiron, Lipitz & Archiron, 2001). Hence one would expect that the right side of the fetal face would show more movement.

The majority of studies examining prenatal lateral behaviors have concentrated on limb movement of the fetus, with a minority of studies focusing on head movement. Research in humans examining the prenatal origins of laterality, such as handedness is equivocal, with some studies suggesting that fetuses exhibit lateralized behavior (Hepper, McCartney, & Shannon, 1998; Kurjak, Veccek, Hafner, Bozek, Funduk-Kurjak, & Ujevic, 2002) and others not finding lateralized movements in utero (De Vries, Wimmer, Ververs, Hopkins, Savelsbergh, & van Geijn, 2001; Myowa-Yamakoshi, & Takeshita, 2006). Lateralization in infants has been observed not only in limb movements but also in the face and specifically in mouth opening movements. The lower facial area has largely contralateral neural projections, with each side of the face connected most directly to the opposite

side of the brain. According to Gazzaniga & Smylie (1990), the left half face is innervated primarily by the right hemisphere and it is postulated that because the right hemisphere is specialized in emotional responses, this is reflected in a greater left facial expressiveness in infants and adults. There are therefore two possibilities for mouth movement lateralization in the fetus. Given the importance of expressive movement in the immediate post-natal period, we might expect greater left-sided mouth movements. Alternatively, an anatomical approach would expect that fetuses might show greater right sided mouth openings, reflecting the faster left brain development. Furthermore, there is evidence for differential nerve innervations of the jaw and the facial muscles controlling facial expressions. According to Avivi-Arber, Martin, Lee, & Sessle (2011), two cranial nerve motor nuclei supply innervations of jaw and facial muscles, namely the trigeminal (Vth) motor nucleus supplies innervations of most jaw and mouth muscles, and the facial (VIIth) nucleus provides the motor innervation to the muscles of facial expression.

Differences in mouth opening, depending on whether infants expressed an emotion or babbled, have been reported by Holowka & Petito (2002). They found that from 5 months of age, infants show differential lateralized behaviors depending on whether they babble or smile, indicating that there is cerebral specialization for language. They tested 10 babies between 5 and 12 month of age, when babbling, making non-babbling sounds or smiling, and found that all babies had right mouth asymmetry while babbling, equal mouth opening while non-babbles were produced, and left mouth asymmetry while smiling. In contrast to the video analysis of movements, Nagy (2012) analyzed still photographs of smiling faces from birth to 8 years of age and did not find a left mouth asymmetry. Given that cerebral asymmetries develop prenatally with the left hemisphere being larger than the right in both males and females by around 22 weeks gestation (Hering-Hanit et al, 2001), a difference in

asymmetries of videotaped mouth movements might be expected. Specifically, a dynamic analysis of fetal facial movements might show an increase in the proportion of lateralized mouth movements as fetuses develop from 24 to 36 weeks gestation. Based on the literature we might expect either proportionately more right sided mouth movements (reflecting faster left hemisphere maturation), or left sided movements (reflecting greater fetal expressiveness). Additionally, given that some research suggests that lateralized behavior may be experience dependent (e.g. Michel et al. 2013; Toga & Thomson, 2003) maternal stress during pregnancy might affect fetal mouth opening differentially for boys and girls.

METHODS

Participants

Fifteen healthy fetuses, 8 girls and 7 boys, were scanned in the second and third trimester. The fetuses were observed four times in the mornings in the radiography department of the James Cook Hospital in the north-east of England, where mothers had previously undergone their routine 12 and 20 week medical scans. The scans took place with the mothers lying in a darkened room on their back or on their side depending on the position of the fetus and how comfortable mothers were. The first research scan was performed at a mean age of 24.2 weeks (range 23.9-24.5 weeks); the second at 28.0 weeks (range 27.8-28.2 weeks); the third at 32.1 weeks (range 31.8-32.4 weeks); the fourth at 36.1 weeks (range 36.0- 36.4 weeks). All participants were first time mothers with mean age 27 years (range 19 - 40 years), specifically recruited through the midwives of the antenatal unit, and following established ethical procedures. All fetuses were assessed to be healthy after birth (mean: 40 weeks with range 37 -42 weeks gestational age) by a pediatrician, with mean weight 3283 grams (std. dev. 489

grams). Apgar scores were measured at 1 minute (mean 9.06, range 9-10) and 5 minutes (mean: 9.33, range: 9-10).

Ethics

Ethical permission for the study was granted by the County Durham and Tees Valley 2 Research Ethics Committee (REC Ref: 08/H0908/31) and the research and development department of James Cook University Hospital, as well as the Durham University (Department of Psychology ethics committee). All mothers gave informed written consent both for participation in the study and for the use of fetal images in publication.

Procedure

Mothers, who had completed normal 20-week anomaly scans, were invited to participate in this study. All participating mothers received four additional scans at 24, 28, 32 and 36 weeks gestational age, with fetuses being scanned in the morning for approximately 15-20 minutes. During consent, and before each procedure, mothers were made aware that these additional scans were for research purposes and not routine medical scans. Mothers were provided with a DVD copy of their scans. The fetal face and upper torso were visualized both by means of 4-D color full frontal or facial profile ultrasound recordings, as well as sequences of traditional monochrome images. Both were recorded for off line analysis with a GE Voluson E8 Expert Ultrasound System using a GE RAB4-8L Macro 4D Convex Array Transducer. Laterality was verified by the radiographer (K.E.). Fetuses did not change intra-uterine position during the length of the scan, but some fetuses changed intra-uterine position from one gestational age to the next. Mothers were asked to fill out a questionnaire on their stress levels at each scan. The Perceived Stress Scale (PSS) is a widely used valid and reliable 10 item five-point Likert-based scale (ranging from 0='no stress' experienced during the last month to 4='very often' stressed (Cohen, Kamarck & Mermelstein, 1983), which measures

the degree to which mothers perceive dimensions of their life as stressful. The theoretical maximum of the scale is 40.

Method of Coding

Following other research in this area, we coded two types of mouth movement, namely upper lip raiser and mouth stretch, which could be observed in fetuses. These can be reliably coded from fetal 4 D scans., using an adaptation of the Facial Action Coding System (Ekman & Friesen, 1978) used in previous studies (Reissland, Francis, Mason, & Lincoln, 2011; Reissland, Francis & Mason, 2012; Reissland, Francis & Mason, 2013). Upper lip raiser is the movement where the upper lip is raised by pulling the upper lip in a straight line towards the cheek, whereas mouth stretch is defined as the opening of the mouth with the jaw dropping. We coded mouth lateralization for upper lip raiser as left or right, and mouth stretch as left, right or neutral. Lateralization was only recorded when the two corners of the mouth were visible as well as the upper and lower lip. Figure 1 shows examples of left, right and neutral mouth stretch movements.

Reliability

Using Cohen's Kappa, reliability was established for these movements, on 22% of scans which were assessed independently by a new coder trained in the coding system. This resulted in reliability estimates for mouth stretch and upper lip raiser (mean= 0.91, mean range 0.87–1.00).

Statistical methods

For each of the sixty scans, we counted the number of left, right and neutral mouth movements. To assess whether the proportion of right mouth movements differed from 0.5, we used a two-sided exact binomial probability test provided by the `bitesti` command in

STATA, which calculates exact p-values based on the binomial distribution. To assess change over time, we examined two measures- the changing proportion of neutral movements out of all mouth movements by gestational age, and the changing proportion of right mouth movements out of all lateralized (left and right) mouth movements. For both analyses we also assessed whether there was evidence that the covariates gender and maternal stress was associated with lateralized mouth movement. There are two sources of variability in this observational study - within fetus and between fetus, and a binomial mixed effects model was therefore used to analyse the proportions (Pinheiro & Bates, 2000). For the first analysis, the response variable was taken to be the number of neutral events (N_{it} for fetus i and gestational age t) out of the total number of mouth movements events T_{it} in the scan period. For the second analysis, the response variable was the number of right mouth movements (R_{it}) out of the total number of lateralized mouth movements ($L_{it} + R_{it}$), where $L_{it} + R_{it} + N_{it} = T_{it}$. The main effects binomial logistic mixed effects model for the first analysis can be written as

$$\text{logit}(p_{it}) = \beta_0 + u_i + \beta_1 \text{age}_{it} + \beta_2 \text{gender}_i + \beta_3 \text{stress}_{it} \quad N_{it} \sim \text{Binomial}(p_{it}, T_{it})$$

where p_{it} is the proportion of neutral mouth movements out of all codable mouth movements for fetus i at age t , u_i is the random effects term for each fetus, which are assumed to be normally distributed with mean 0 and variance σ^2 , and $\beta_0, \beta_1, \beta_2$ and β_3 are unknown parameters representing the intercept, gestational age slope, gender effect, and stress effect respectively. The model for the second analysis can be written similarly. The use of a binomial model rather than a normal model is more appropriate where proportions are based on small denominators; and the random effects term accounts for the inter-fetus variability. The models were fitted using the `glmer` function version 1.0.5 in the `lme4` package in the statistical software R (Bates, Maechler, Bolker & Walker, 2013). Significance of the effect

of individual covariates was assessed by fitting two models – one with the covariate and the second without the covariate, and testing the difference between the two models using a likelihood ratio test (LRT), which is then compared to a chi-squared distribution on one degree of freedom.

RESULTS

Table 1 shows the overall number of neutral mouth movements and the number of mouth movements. Out of 126 total mouth stretch movements observed over the 60 scans, exactly half (63) were neutral and half were lateralized. No neutral upper lip raiser movements were observed.

The proportion of right lateralized movements out of the total number of lateralized movements was 0.317 for mouth stretch movements and 0.420 for upper lip raiser movements, giving an overall proportion of 0.363 for both types of mouth movement. There is evidence that there are significant differences from 0.50 (equality of left and right) for mouth stretch ($p=0.005$) and all mouth movements ($p=0.005$), but not for upper lip raiser movements alone ($p=0.32$).

In analysing maturational changes in the fetus, we examined both the changing proportion of neutral mouth movements, and the changing proportion of right lateralized movements over gestational age. Table 2 shows the results of fitting a binomial mixed effects model to the proportion of neutral mouth movements for both types of mouth movement. The results indicate that the proportion of neutral mouth movements decline with gestational age ($\beta_1 = -0.116$, $\exp(\beta_1) = 0.890$; $p < 0.001$). The decline in the odds of neutral mouth movements is thus around 11% for each week of gestational age. There were no gender differences in the overall proportion of neutral movements ($\beta_2 = -0.343$; $p = 0.28$), nor

did maternal stress influence the proportion of neutral mouth movements ($\beta_3=-0.025$; $p=0.21$).

Table 3 shows the results of fitting a binomial mixed effects model to the proportion of right lateralized mouth movements. There was no evidence of any maturational change in the proportion of right lateralized movements ($\beta_1=-0.011$; $p=0.88$). There was also no significant gender difference in the overall proportion of right lateralized movements ($\beta_2=1.422$; $p=0.07$), nor did maternal stress influence the proportion of right lateralized mouth movements ($\beta_3=0.034$; $p=0.48$).

DISCUSSION

In terms of maturational change, results indicated that as fetuses grew older they showed a decrease in neutral mouth movements. Neutral mouth movements declined by 11% for each gestational week. This decline in neutral mouth movements and increase in lateralized mouth movements indicates that fetal facial lateralized movements are established gradually over time. We did not find a relationship between maternal stress and fetal neutral mouth movements nor were there any sex differences in terms of the relative decline of neutral mouth movements. There was evidence that fetuses showed a bias towards leftward mouth movements when examining all lateralized mouth movements, suggesting that the “expressive development” hypothesis is more strongly supported in our study. Regarding maturational change, although lateralized mouth openings increased as the fetus ages, there was no evidence that fetuses showed *increasing* right mouth openings. Rather both right and left mouth openings could be observed to increase over gestational age.

Chi, Dooling, & Gilles (1977), measured the brains of 207 fetuses at a gestational age of 10–44 weeks. Although they reported that a left-right asymmetry of the transverse temporal gyri and the temporal plane were present at fetal brains older than 31 weeks gestation, this was not reflected in findings of fetal mouth movements in the present sample of fetuses observed longitudinally from 24-36 weeks gestation. These findings reflect reports by Fagard (2013), who argues when discussing differential development of lateralized behaviors that both cross-sectional observations of 14-month-old infants and longitudinal research on 8- to 20-month-old infants showed that infants left-handed for grasping objects were as likely to point with their right hand as right-handed infants. Fagard (2013) concluded that the emergence of pointing does not influence handedness for grasping objects. Lateralized mouth movements could point to the differentiation of brain lateralization generally. Our results suggest that some lateralization preferences may indicate expressive development, and others to anatomical hemispherical brain development.

Although, in infants, the presence of right asymmetry in mouth openings during verbal and non-verbal tasks compared with left mouth opening during emotional tasks has been widely used as a key measure of left hemisphere cerebral specialization for language driven tasks (Wolf & Goodale, 1987) it is unlikely that such inferences are warranted. These tasks have been used for example in the treatment of speech disorder in young children (Wilson, Green Yanusova & Moore, 2008). However, theories of the motor control of mouth movements have been found to be task specific and not transferrable (Wilson et al 2008). For example, sucking movements can be observed prenatally at around 12-14 weeks gestation (e.g. de Vries et al., 2008) and involves lips, jaw and tongue as well as hard and soft palate, but observation of such sucking movements cannot transfer to articulatory movements and hence cannot help speech training (Wilson et al., 2008). Hence, prenatally observed mouth

movements although helping in the mapping of neuronal connections cannot be seen as direct precursors of functional movements observed post-birth. Nevertheless, these movements, specifically asymmetric mouth movements, will undoubtedly help in demonstrating the maturation of pathways to the right and left hemispheres.

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Table 1. Total and neutral mouth movements observed over 60 fetal scans, and proportion q of right mouth movements (R) out of all lateralized (L+R) mouth movements (left and right) by type of movement.

	Total mouth movements	Neutral mouth movements N	Lateralized mouth movements L+R	Right lateralized mouth movements R	$q = R/(L+R)$	p-value testing for $q=0.5$ (two-sided exact test)
Mouth stretch	126	63	63	20	0.317	0.0052
Upper lip raiser	50	0	50	21	0.420	0.3222
TOTAL	176	63	113	41	0.363	0.0046

Table 2. Results of the binomial mixed effects model examining the changing proportion of *neutral* mouth movements in relation to all codable mouth movements by gestational age, gender and stress.

	Estimate β	s.e.	Exp(β)	LRT change in deviance
β_0 (intercept)	3.695	1.126		
β_1 (Age)	-0.116	0.037	0.890	10.92 on 1 df ; p<0.001
β_2 (Female)	-0.343	0.301	0.710	1.15 on 1 df ; p=0.28
β_3 (stress)	-0.025	0.021	0.975	1.53 on 1 df ; p= 0.21

Table 3. Results of the analyses of the changing proportion of right mouth movements in relation to all lateralized mouth movements by gestational age, gender and stress.

	Estimate β	s.e.	Exp(β)	LRT change in deviance in deleting term
β_0 (intercept)	-1.162	1.847		
β_1 (Age)	-0.011	0.056	0.989	0.02 on 1 df ; p=0.88
β_2 (Female)	1.422	0.736	4.145	3.35 on 1 df: p=0.07
β_3 (stress)	0.034	0.046	1.035	0.49 on 1 df ;p=0.48

Figure Caption

Fig 1. Examples of fetal scans showing (from left to right) a left, neutral and right mouth stretch. The left and neutral scans show a 35-week old fetus, the right shows a 28-week old fetus.

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Figure 1

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Highlights

- Prenatal lateralized facial behavior as a marker of brain development (24-36-weeks)
- Fetal lateralized mouth movements were examined in 4-D ultrasound scans
- Lateralized mouth- opening increased by 11% for each week of gestational age ($p < 0.001$)
- There was evidence of left lateralization preference in mouth movement

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