Title: The human fetus preferentially engages with face-like visual stimuli

Short title: The human fetus prefers face-like stimuli

Vincent M. Reid[1], Kirsty Dunn[1], Robert J. Young[2], Johnson Amu[3], Tim Donovan[4], Nadja Reissland[5]

[1] Department of Psychology, Lancaster University, United Kingdom, LA1 4YF.

[2] Department of Physics, Lancaster University, United Kingdom, LA1 4YB

[3] Department of Obstetrics and Gynaecology, Blackpool NHS Trust, United Kingdom, FY3 8NR

[4] Medical and Sports Sciences, University of Cumbria, United Kingdom, LA1 3JD

[5] Department of Psychology, Durham University, United Kingdom, DH1 3LE

Lead Contact: Vincent Reid Correspondence and requests for materials should be addressed to Vincent Reid (Email: v.reid@lancaster.ac.uk), telephone: +44 (0)1524 592915

Summary

In the third trimester of pregnancy the human fetus has the capacity to process perceptual information [1,2,3]. With advances in 4D ultrasound technology, detailed assessment of fetal behavior [4] is now possible. Furthermore, modelling of intrauterine conditions has indicated a substantially greater luminance within the uterus than previously thought [5]. Consequently, light conveying perceptual content could be projected through the uterine wall and perceived by the fetus, dependent on how light interfaces with maternal tissue. We do know that human infants at birth show a preference to engage with a top-heavy, face-like stimulus when contrasted with all other forms of stimuli [6,7]. However, the viability of performing such an experiment based on visual stimuli projected through the uterine wall with fetal participants is not currently known. We examined fetal head turns to visually presented upright and inverted face-like stimuli. Here we show that the fetus in the third trimester of pregnancy is more likely to engage with upright configural stimuli when contrasted to inverted visual stimuli, in a manner similar to results with newborn participants. The current study suggests that postnatal experience is not required for this preference. In addition, we describe a new method whereby it is possible to deliver specific visual stimuli to the fetus. This new technique provides an important new pathway for the assessment of prenatal visual perceptual capacities.

Key words: Fetus, Face preference, Visual perception

Results and Discussion

In the present study, we examined how the human fetus would respond to upright and inverted face-like stimuli in a paradigm modified from newborn research [6]. Based on a prior computational model of the fetal visual system during the third trimester [8], we propose that the fetus will produce more head turning to the upright contrasted with the inverted stimuli, in a manner consistent with postnatal studies.

Behavioral responses to stimuli were assessed in 39 fetuses by an ultrasonographer and an experimenter, utilising 4d ultrasound. Once comfortable, a set of 2D scans were taken comprising the fetal head position, maternal tissue thickness, fetal biparietal diameter, occipitofrontal diameter, head circumference, abdominal circumference, femur length, and fetal estimated weight. Fetal biometry measurements demonstrated normal fetal growth without fetal anomalies. All participants were then asked not to talk during the study and to remain as still as possible in order to optimise image quality. The initial 2D scan also informed the experimenter of the precise location of the fetal head prior to the presentation of the stimuli.

The stimuli were projected in two orientations (“upright” and “inverted”) on the maternal abdomen (Fig. 1.). Both images were presented to the side of the fetal face, such that the stimuli were presented to the fetal retinal visual areas (left, N = 19, right, N = 20). The light was then moved across the maternal abdomen in a horizontal direction away from the fetal central visual location, for approximately 5 seconds at an average of 1 cm per second. This is

consistent with speeds reported in newborn studies [6] taking into account constraints specific to this population, i.e. the width of maternal abdomen that was accessible in order to present stimuli and the space within the womb available for the fetus to move. Timing was controlled via a stopwatch in view of the experimenter who was delivering the stimuli. This process was repeated a total of 5 times, with the procedure then immediately repeated with the alternate stimulus orientation. The presentation order for upright and inverted orientations of the stimuli was counterbalanced across the sample.

The number of head turns made in response to the stimuli was assessed using condition-blind coding of the 4D scans. All groups presented normally distributed data with similar levels of variation. On average, Figure 2 shows that more head turns were made in the direction of the upright (M = 1.14, SD = 1.09) than in the direction of the inverted stimuli (M = 0.52, SD = 0.62). There were slightly more head turns in the opposite direction to the inverted (0.44, SD = 0.62) than the upright (M = 0.33, SD = 0.55) stimuli. A Wilcoxon Signed-Ranks test indicated that more head turns were directed towards than away from the upright stimuli (Z = 3.117, p = 0.002). Further, significantly more head turns were directed towards the upright than the inverted stimuli (Z = 2.380, p = 0.017). No further comparisons were found to be significant.

In addition, a paired-samples t-test compared difference scores (looks towards minus looks away) showing a significant difference between upright (*M* = 0.77, *SD* = 1.06) and inverted (*M* = 0.08, *SD* = 0.13) stimuli, t(38) = 2.924, *p* = 0.005.

These results indicate that the fetus in the third trimester is more likely to engage with stimuli featuring an upright face-like configuration when contrasted with an inverted configuration. We therefore conclude that postnatal experience is not necessary for the emergence of a preferential visual system for face-like stimuli. This finding rules out rapid postnatal learning, such as filial imprinting, as a mechanism for this visual proclivity. These mechanisms may be innate, or possibly the perceptual bias is triggered by exposure to patterned light in the womb during prenatal visual experiences.

Prenatal Visual Experience

In the third trimester of pregnancy the human fetus has the capacity to process perceptual information [1-3]. Despite this, newborn visual preferences are often attributed to innate mechanisms or to rapid imprinting. Postmortem analysis of the human eye has shown that there is substantial biological development from mid-gestation through to term, with many of the essential components for visual function present starting around 25 weeks gestational age (GA) [9,10]. This research also indicated more advanced development in peripheral visual regions. Before postnatal development, peripheral vision is therefore likely to be more sensitive than foveal vision for detecting environmental change. Work on prenatal visual development suggests that visual perceptual capacities are analogous to newborn functionality well before term. Evidence also derives from reports of visual function in low-risk pre-term infants. Studies have shown newborns perform fixing and tracking from 32 weeks GA [11-14]. Further, comparing visual evoked potentials in full-

term neonates to conception age-matched pre-term infants, no difference was found in neural response to visual stimuli [15]. Postnatal visual experience therefore did not affect the neural correlates of visual processing.

Recent modelling work has indicated a substantially greater luminance within the uterus than previously thought [5]. Animal models have demonstrated not only that light penetrates into the uterus but also that light penetration is critical in mice for preparing the eye and light response pathway for postnatal vision [16]. Together, these studies indicate that visual experience starts prenatally. Prenatal light levels are not only essential for the development of visual pathways but also allow for the innovative methodology used in the current study, with perceptual content projected through the uterine wall, taking into account how light interfaces with maternal tissue.

From Prenatal to Postnatal Visual Development

Control of the eyes by neonates is relatively advanced when contrasted with other motor abilities [17]. It is for this reason that visual paradigms are a key aspect of postnatal research. Research on fetal visual perception, however, is limited when compared with our current understanding of fetal abilities in other modalities [18]. During avian development, as a consequence of embryo orientation in the egg, differential exposure to light for the left or right eye due to the location of the wing results in brain lateralization in chicks [19]. Multiple studies have investigated the response of the human fetal brain to light [20], although none have delivered stimuli that have contained the percept of an

image. This absence has been driven by the complexity of delivering visual stimuli to the fetus.

One key, well replicated finding in newborn research is the preference to engage with a face-like stimulus when contrasted with other forms of stimuli, including the same stimulus presented in an inverted configuration [6,7]. There has been much debate on how and why this preference is present in the emerging visual system [21,22]. A comprehensive review of two decades of research offers an extension to the original theoretical model put forward in explanation of newborn face preference [23]. The underlying assumptions in much of the newborn visual literature are (1) that no visual experience has taken place prior to birth and (2) that the examination of fetal visual capacities is not possible. The present study illustrates that fetal visual perception can be indexed during the third trimester, given the technical advances in 4D ultrasound that can provide access to fetal fine grained behavior [24-26]. With appropriate modifications, other aspects of newborn infant perception could also be assessed in the third trimester, including biological motion processing [27]. An exploration of capacities at this stage of development could greatly inform our understanding of visual preferences as models of development feature different assumptions related to the underlying development of visual systems. For example, even though the results of the present study are compatible with superior colliculus activity [8], the same cannot be said for a proposed “gravity bias” for visual stimuli, which has been previously proposed [28].

Even though the results of the current study are analogous to postnatal behaviors, due to the properties of the fetal environment, the paradigm and stimuli are not exactly the same between the current study and postnatal research. For example, only light from the red (or long wave) end of the spectrum penetrates maternal tissue. Despite this, the results are consistent with a model of fetal visual preferences [8], whereby the largest differential response was for a negative polarity stimulus set with white dots on a black background when contrasted with other stimuli, including black dots on a white background. It should also be noted that the results of the present study do not imply that the fetus can respond to faces presented externally under everyday circumstances. The behavior that has been demonstrated in the current study derives from the specific conditions of the experiment.

The capacity to (1) present visual stimuli through projected light and (2) precisely measure fetal behavior using ultrasound recordings, as demonstrated in the present study, allows for the execution of studies with the human fetus that closely resemble postnatal methodologies with infant populations. Such an approach will have implications for further understanding of the fetus [29], and developmental processes in general. Fetal research can consequently employ similar visual methodologies and control procedures as those seen in the infancy domain [e.g. 27,30]. Currently it is unknown how effective these methods would be in terms of producing responses earlier in gestation or if infant-derived paradigms, such as fixation time measurements, will be as likely to produce meaningful results with the fetus in the third trimester. Such work will undoubtedly provide more

information about the development of the visual system in addition to current animal models [16] and with respect to the transition from fetus to infant.

Author Contributions

Conceptualization: VR; Methodology: KD, RY, NR, VR; Formal Analysis, KD, VR; Resources: JA, TD; Data Curation, KD, VR; Writing – Original Draft: VR, KD; Writing – Review and Editing: VR, KD, RY, JA, TD, NR; Visualization: VR; KD, RY; Supervision: VR; Project Administration: VR; Funding Acquisition: VR, NR, RY

Acknowledgments

This work was supported by an Economic and Social Research Council Transformative Research Grant (grant number ES/L003155/1). VR is a Professor in the International Centre for Language and Communicative Development (LuCiD) at Lancaster University. The support of the Economic and Social Research Council [ES/L008955/1] is gratefully acknowledged. RY acknowledges support by the Royal Society through a University Research Fellowship (UF110555). We thank Janette Keit and Linda Walshaw for acting as sonographers, Jane Brooks for Research Midwife assistance and Dr. Steve Milan for input throughout the project.

References

1. DeCasper, AJ., and Fifer, WP. (1980) Of human bonding: Newborns prefer their mothers' voices. *Science* 208:1174-1176.

2. Zoia, S., Blason, L., D'Ottavio, G., Bulgheroni, M., Pezetta, E., Scabar, A., and Castiello, U. (2007) Evidence of early development of action planning in the human foetus: A kinematic study. Exp. Brain. Res. 176:217–226.

3. Witt, M., and Reutter, K. (1998) Embryonic and early fetal development of human taste buds: a transmission electron microscopical study. *Anatom. Rec.* 246(4):507-523.

4. Lopez-Teijon, M., Garcia-Faura, A., and Prats-Galino, A. (2015) Fetal facial expression in response to intravaginal music emission. *Ultrasound* 23:216-223.

5. Del Guidice, M. (2011) Alone in the dark? Modeling the conditions for visual experience in human fetuses. *Dev. Psychobi.* 53:214-219.

6. Johnson, MH., and Morton, J. (1991) *Biology and cognitive development: the case of face recognition* (Basil Blackwell, Oxford UK).

7. Fantz, R. (1963) Pattern vision in newborn infants. *Science* 140:296-297.

8. Pitti, A., Kuniyoshi, Y., Quoy, M., and Gaussier, P. (2013). Modeling the minimal newborn's intersubjective mind: the visuotopic-somatotopic alignment hypothesis in the superior colliculus. *PLoS ONE* 8(7):e69474.

9. Hendrickson, A., Possin, D., Vajzovic, L., and Toth, CA. (2012) Histologic development of the human fovea from midgestation to maturity. *Am. J. Opthalm* 154(5):767-777.

10. Hendrickson, A., and Drucker, D. (1992) The development of parafoveal and mid-peripheral human retina. *Beh. Brain Res.* 49(1):21-31.

11. Dubowitz, LMS., Dubowitz, V., and Morante, A. (1980a) Visual function in the newborn: A study of preterm and full-term infants. *Br. Dev.* 2:15-29.

12. Dubowitz, LMS., Dubowitz, V., and Morante, A. (1980b) Visual function in the preterm and fullterm newborn infant. *Dev. Med. Ch. Neurol.* 22:465-475.

13. Morante, A., Dubowitz, LMS., Levene, M., and Dubowitz, V. (1982) The development of visual function in normal and neurologically abnormal preterm and fullterm infants. *Dev. Med. Ch. Neurol.* 24:771-784.

14. Romeo, DM., Ricci, D., Serrao, F., Gallini, F., Olivieri, G., Cota, F., Romagnoli, C., and Mercuri, E. (2012) Visual Function assessment in late-preterm newborns. *Ear. Hum. Dev.* 88:301-305.

15. Baraldi, P., Ferrari, F., Fonda, S., and Penne, A. (1981) Vision in the neonate (full-term and premature): Preliminary result of the application of some testing methods. *Doc. Opthal.* 51:101-112.

16. Rao, S., Chun, C., Fan, J., Kofron, JM., Yang, MB., Hedge, RS., Ferrara, N., Copenhagen, DR., and Lang, RA. (2013) A direct and melanopsin-dependent fetal light response regulates mouse eye development. Nature 497:243-246.

17. Stanojevic, M., and Kurjak, A. (2008) Continuity between fetal and neonatal neurobehavior. *J. Ultra Obst.Gyne.* 2:64-75.

18. DiPietro, JA., Costigan, KA., and Voegtline, KM. (2015) Studies in fetal behavior: revisited, renewed and reimagined. *Mono. Soc.r Res. Ch. Dev.* 80(3):1-94.

19. Rogers, LJ. (1990) Light input and the reversal of functional lateralization in the chicken brain. *Beh. Brain. Res*. 38(3) 211-221.

20. Dunn, K., Reissland, NN., and Reid, VM. (2015) The functional foetal brain: a systematic preview of methodological factors in reporting foetal visual and auditory capacity. *Dev. Cog. Neuro.* 13:43-52.

21. Simion, F., Valenza, E., Umilta, C., and Barba, B. (1998) Preferential orienting to faces in newborns: a temporal nasal asymmetry. *J. Ex. Psych: HPP* 24(5):1399–1405.

22. Wilkinson, N., Paikan, A., Gredebäck, G., Rea, F., and Metta, G. (2014) Staring us in the face? An embodied theory of innate face preference. *Dev. Sci.* 17(6):809-825.

23. Johnson, MH., Senju, A., and Tomalski, P. (2015) The two-process theory of face processing: modifications based on two decades of data from infants and adults. *Neurosci. & Biobeh. Rev* 50:169-179.

24. Reissland, N., Francis, B., Mason, J., and Lincoln, K. (2011) Do facial expressions develop before birth? *PLoS ONE* 6(8):e24081.

25. Myowa-Yamakoshi, M., and Takeshita, H. (2006) Do human fetuses anticipate self-directed actions? A study by four-dimensional (4D) ultrasonography. *Infancy* 10(3):289-301.

26. Reissland, N., Francis, B., Aydin, E., Mason, J., and Schaal, B. (2014) The development of anticipation in the fetus: a longitudinal account of human fetal mouth movements in reaction to and anticipation of touch. *Dev.l Psychobiol.* 56:955–963.

27. Simion, F., Regolin, L., and Bulf, HA. (2008) Predisposition for biological motion in the newborn baby. *PNAS* 105(2):809-813.

28. Vallortigara, G., and Regolin, L. (2006). Gravity bias in the interpretation of biological motion by inexperienced chicks. *Current Biology*, 16(8), 279-280.

29. Abbott, A. (2015) Neuroscience: The brain, interrupted. *Nature* 518:24–26.

30. Rochat, P. (2011) What is it like to be a newborn? *Oxford Handbook of the Self*, eds Gallagher S (Oxford University Press: Oxford).

31. Peters, VG., Wyman, DR., Patterson, MS., and Frank, GL. (1990) Optical Properties of normal and diseased human breast tissues in the visible and near infrared. *Phys Med Biol.* 35:1317-34.

32. Nijhuis, JG., Prechtl, HFR., Martin, CB Jr., and Bots, RSGM. (1982) Are there behavioral states in the human fetus? *Ear. Hum. Dev.* 6:177–195.

33. Stockman, A., Jägle, H., Pirzer, M., and Sharpe, LT. (2008) The dependence of luminous efficiency on chromatic adaptation. *J. Vision* 8(16):1–26.

34. Johnson, M.H., Dziurawiec, S., Ellis, H., and Morton, J. (1991). Newborns preferential tracking of face-like stimuli and its subsequent decline, *Cognition*, 49, 1-19.

35. Jacques, SL. (2013) Optical properties of biological tissues: a review. *Phys. Med. Biol.* 58:5007.

36. Landis, JR., and Koch, GG. (1977) The measurement of observer agreement for categorical data. *Biometrics 33*:159-174.

Figure Legends

Figure 1: A conceptual illustration of the stimuli utilized in the current study, depicting (A, B) upright and (C, D) inverted orientations. Panels (A) and (C) illustrate the stimuli prior to contact with maternal tissue. Panels (B) and (D) display the consequence of interaction with 30 mm of maternal tissue based on our equation. To calculate the expected projection size we used the simple equation for the anisotropy of scatter [35] along with a value for adipose tissue [31] from the corrected version of Figure 8 (expanded view in the Corrigendum, page 2): Projected diameter = tan(arccos(g)) x thickness of the tissue x 2. From the figure, g ~ 0.98 for adipose, giving a diameter after 30mm of tissue of ~12mm.

Figure 2: The mean number of head turns made towards (left two bars) and away (right two bars) for face-like (red) and non-face-like (gray) stimuli. Error bars represent standard errors. Stars indicate significant differences between conditions with the brackets representing the relevant comparisons.

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Vincent Reid (v.reid@lancaster.ac.uk).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The sample size was calculated based on attrition rates for newborn paradigms with the assumption of a 20% larger attrition rate for the current study due to the number of fetal head orientations that would allow for the delivery of stimuli. All pregnant women participating received written information prior to agreeing to take part in the study and gave informed written consent before participation. This study was approved by NHS Health Research Authority National Research Ethics Service, the Lancaster University Research Ethics Committee, the Durham Research Ethics

Committee and the Cumbria University Ethics Committee. Behavioral responses to stimuli were assessed in 83 fetuses by an ultrasonographer and an experimenter, utilising 4d ultrasound. Thirteen were excluded due to poor image resolution. All instances of exclusion in this sample due to fetal head position causing visual stimuli presentation to become unviable, co-occurred with poor image resolution. Twenty-nine were excluded at the coding stage as these fetuses indicated a lack of eye or body movements throughout the scanning period and appeared to be in a deep sleep state, otherwise referred to as behavioural state 1F [32].

The final sample consisted of 39 fetuses, gestational age between 231 and 252 days (*M* = 240.62 days). Maternal tissue thickness ranged from 13.1 mm to 69.7 mm (*M* = 27.47, *SD* = 10.32). Seventeen fetuses were female (44%) with the sample showing an average APGAR score of 9.64 (SD = 1.06), although birth records for 5 participants could not be obtained. Twenty-one scans were performed at Blackpool Victoria Hospital, the remainder at Cumbria University Medical Imaging Unit. All participants had singleton pregnancies with no known complications and a BMI of approximately 30 or lower at the start of pregnancy. A BMI of this level or lower was required in order to ensure a similar quality of presentation of the stimuli through varying amounts of maternal tissue.

METHOD DETAILS

Stimuli The stimuli were constructed from custom-made semiconductor laser 2mm dot diodes emitting at 650 nm. Three diodes were arranged in a triangular pattern, with 15 mm distance between each dot. The light source

was calibrated to output optical powers of 0.5 mW, 1 mW or 5 mW for maternal tissue thickness (t) below 15 mm, between 15 mm and 30 mm and above 30 mm respectively. This ensured that a consistent level of light was delivered to the fetus irrespective of variations in maternal tissue thickness. Intrauterine illuminance (L_I) was calculated using the equation:

$$L_I = L_E 10^{-(.0942+t\frac{.032+.058r}{1+r})}$$

modified from [5] to remove the clothing factor. L_E, the external illuminance was calculated using the output power of the light source, assuming a projected spot size diameter of 10 mm for a maternal tissue thickness of 30 mm, and correcting for the source wavelength of 650 nm, based on values in [33]. An approximate muscle to fat ratio (r) of 2 was used, as in [5]. Taking three scenarios as examples, with maternal tissue thicknesses of 15 mm, 20 mm and 30 mm, we calculate corresponding intrauterine illuminances of 36, 24 and 16 lx respectively. Figure 1 displays the projected input to maternal tissue and the approximate consequence of interaction with maternal tissue for the stimuli. The light levels of the stimuli are all within the range that the fetal visual system is thought to work best, and significantly lower than the illuminances that a fetus may be exposed to on a bright sunny day [5]. The estimated distance between the dots subtended 23.5 degrees of visual angle. The laser diodes were 15mm apart and 2mm in diameter. We estimated an average scatter of 5mm in either direction, resulting in 5mm between the dots. This degree of visual angle between the dots is similar to that seen in postnatal studies [e.g. 34].

The dispersion of the input configuration of the light source after travelling through 30 mm of maternal tissue, as illustrated in Fig. 1, was estimated using data from [35]. Using a value for the anisotropy of scatter at the source wavelength for adipose taken from [34] results in an expected broadening of a point source to a diameter of 12 mm after transmission through 30 mm of tissue. It should be noted that the minor dispersion, due to amniotic fluid between the fetus' eye and the uterine wall, has not been taken into account due to uncertainties in quantifying this measure.

Data Acquisition Maternal tissue was measured from maternal skin to the uterine wall using 2D ultrasound. The 4D live ultrasound technology operated on a bandwidth of 2-8MHz. Fetal behavior was recorded at Blackpool Victoria Hospital using a GE Healthcare Voluson E8 Expert BT13 advanced 4D HD live ultrasound scanner and 4D probe, model RM66. At Cumbria University Medical Imaging Unit, a GE Healthcare Voluson iBT07 4D live ultrasound scanner and 4D probe, model RAB4-8-RS was used. Recordings were saved to DVD for offline coding.

Procedure The ultrasonographer ensured the ultrasound recording occurred while the experimenter selected the correct strength stimulus, depending on maternal tissue depth, and conducted the procedure. Typically the participant was lying on her side. Timings were controlled via a stopwatch and noted on a clipboard, typically by a second experimenter. Once comfortable, an initial set of 2D scans were taken comprising the fetal head position, maternal tissue thickness, fetal biparietal diameter, occipitofrontal diameter, head circumference, abdominal circumference, femur length, fetal weight. Fetal

biometry measurements demonstrated normal fetal growth and no fetal anomalies. Note that depth scans were taken in the same maternal position as for when the stimulus was then presented. All participants were then asked not to talk during the study and to remain as still as possible in order to optimise image quality. The 2D scan also informed the experimenter of the precise location of the fetal head prior to the presentation of the stimuli.

The stimuli were delivered in two orientations ("upright" and "inverted") to the maternal abdomen (Fig. 1.) relative to the position of the fetus (breech presentation/head up, N = 6, cephalic presentation/head down, N = 33). Both were presented to the side of the fetal face, such that the stimuli were presented to the fetal peripheral visual areas (left, N = 19, right, N = 20). The experimenter was not blind to the condition that was presented. The ultrasound image, interpreted by the ultrasonographer, informed the initial placement of the light source on the maternal abdomen at the start of each trial. This gave the greatest likelihood that the experimenter could place the light in the peripheral visual field of the fetus. The experimenter utilised the ultrasound image for initial placement but attended to the movement of the stimulus across the maternal abdomen, precluding the viewing of the ultrasound image once the trial had started. The administration of the stimulus was standardised across conditions by ensuring equivalent timing between conditions. From the 39 scans, the mean distance of the fetal eye to the uterine wall was 12mm, SD 7.5mm, with range 2mm to 30mm.

The stimulus was then moved across the maternal abdomen in a horizontal direction away from the fetal central visual location, for 5 seconds at an

average speed of 1 cm per second. This is consistent with speeds reported in newborn studies [6] taking into account constraints specific to this population, i.e. the width of maternal tissue that was accessible in order to present stimuli and the space available for the fetus to move. Movement and light offset was simultaneous and timing was controlled via a stopwatch in view of the experimenter who was delivering the stimuli. This process was repeated a total of 5 times, with the procedure then immediately repeated with the alternate stimulus orientation. The presentation order for upright and inverted orientations of the stimuli were counterbalanced across the sample. Participants were blind to condition.

Data coding During the scan, the position of the light in relation to the recorded image of the fetus was noted. The ultrasound image was rotated by the sonographer such that the fetal face appeared upright. This was done so that, at a later stage, coding consisted of simply “left” and “right” head movements in the coronal plane. Coding of recordings was conducted offline using Observer XT 12.5 software (Tracksys Ltd). The duration of head movements away from the individual fetus’ initial resting head position were measured, along with the direction of the movement for each trial. At the start of each trial, the resting position of the head on the ultrasound image was recorded and coded as “center”. At the onset of a head movement, a code denoted the start time and direction of movement. This code then remained active until a second code was given to indicate a return to the initial resting head position, a head movement in the opposite direction (an opposite head movement was then coded) or the end of a trial. The onset of any head

movement was not constrained to the onset of the stimulus but no movements were coded following stimulus offset.

Following blind coding, ‘left’ and ‘right’ head movements were transformed to indicate whether movements were made towards or away from the stimuli. An orientation towards the stimuli was a horizontal head movement in accordance with the position of the light recording during the scan. An orientation away from the stimuli was a horizontal head movement performed in the opposite direction to the recorded position of the light source.

Cohen’s kappa was performed to determine interrater agreement on fetal head movements. There was a substantial agreement between two coders’ judgements, κ = 0.797 (95% CI, 0.503 -1.00), p = <0.001. Behavioral measurement was thus deemed to be suitable for use in the hypothesis tests in the present study [36].

QUANTIFICATION AND STATISTICAL ANALYSIS

All groups presented normally distributed data with similar levels of variation. Using SPSS statistical data package, version 23, a comparison of the number of head turns across conditions was made using the Wilcoxon rank signed test. A comparison of difference scores was also made using a paired-samples t-test.

DATA AND SOFTWARE AVAILABILITY

Description: Data are available in the Dataserve Digital Repository: doi:10.7910/DVN/RLIBYV

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Bacterial and Virus Strains		
Biological Samples		
Chemicals, Peptides, and Recombinant Proteins		
Critical Commercial Assays		
Deposited Data		
Experimental Models: Cell Lines		
Experimental Models: Organisms/Strains		
Oligonucleotides		

Recombinant DNA		
Software and Algorithms		
IBM SPSS statistical package, version 23	https://www.ibm.com/analytics/us/en/technology/spss/	N/A
Observer XT Behavioural Coding and Analysis Software	http://www.noldus.com/human-behavior-research/products/the-observer-xt	N/A
Other		
GE Healthcare Voluson E8 Expert advanced 4D HD live Ultrasound Scanner (Location: Blackpool Victoria Hospital)	http://www3.gehealthcare.com/en/products/categories/ultrasound	BT13
GE Healthcare Voluson 4D live Ultrasound Scanner (Location: Cumbria University)	http://www3.gehealthcare.com/en/products/categories/ultrasound	iBT07
GE Healthcare 4D Transducer (Location: Blackpool Victoria Hospital)	http://www3.gehealthcare.com/en/products/categories/ultrasound/ultrasound_probes	RM66
GE Healthcare 4D Transducer (Location: Cumbria University)	http://www3.gehealthcare.com/en/products/categories/ultrasound/ultrasound_probes	RAB4-8-RS
Custom Built light source	N/A	N/A