Highlights

- Maternal depression and anxiety affect fetal reactions to visual stimuli

- Fetuses react differently to light projected in a striped configuration compared with face like configurations.

- Fetal growth factors affect fetal reactivity to face-like compared to non-face-like control light stimuli

Effects of maternal mental health on fetal visual preference for face-like compared to non-face like light stimulation

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Abstract

The question of whether humans react differentially to face-like versus non face-like light stimulation in the prenatal period has been much discussed, but to date has remained unresolved. In this feasibility study we have come closer to understanding fetal vision. In contrast to other studies examining fetal reactions to prenatal light stimulation, we controlled maternal factors known to affect fetal neurodevelopment; including maternal mental health and attachment. We found that, for fetuses at 33 weeks gestation, maternal mental health (anxiety and depression), and fetal growth factors (femur length) all had a significant effect on fetal reactivity to face-like compared to a non-face-like control light stimulus. This calls into question some previously published results. We discuss implications of these findings in terms of the development of fetal visual perception.

Key words: fetus; maternal mental health; vision

Introduction

Human faces provide vital information starting at birth [1]. However, the question of when humans develop specific mechanisms for face processing remains unresolved [2]. Relatively new to the debate on face processing is research in the fetal period, which offers a paradigm to explore prenatal abilities to react to specific configurations of light stimulation including face like versus non-face like stimuli. However, missing from research of prenatal abilities to process light stimuli are data on the influence of maternal prenatal mood [3, 4, 5]. This crucial factor is known to affect both fetal neurodevelopment and postnatal reactions to facial expressions [6].

Arguably, although testing the visual preference of fetuses to face-like stimuli is more technically demanding than testing neonates, recent studies have overcome some of the difficulties by projecting images constructed of red LED lights through maternal tissues into the womb environment. Fetal reactions to different configurations of light stimuli can be observed by simultaneously recording fetal behaviour using ultrasound technology for offline analysis [5, 7]. Such a protocol bridges the gap between the fetal and neonatal periods and permits a test of prenatal preference for specific configurations of light stimuli, taking into account maternal factors including prenatal stress, depression, anxiety and attachment.

Research with neonates has already found that there is an innate preference for *highly schematised face-like configurations* (e.g., simple designs with two squares representing eyes and one square representing the mouth that resemble a basic face shape) over other visual stimuli, as shown by increased orientation towards such stimuli [8]. Cashon & Holt [8] reviewed the effects of face inversion, by comparing various stimuli such as three dots versus a face in terms of the infants

following the stimulus with their eyes. Infants preferred the face-like compared with the dot design, and, when summarizing their results, they suggested that infants preferred a top-heavy compared with bottom-heavy configuration. Such findings indicate that even at birth there may be a domain-specific mechanism, which acts as an early face detector that guides later increasingly complex face learning. However, there have been some significant challenge to the notion of a domain-specific innate mechanism that is specifically tuned to faces [9, 10]. Macchi et al [9] suggested that top-heavy stimuli do not necessarily attract visual attention of neonates; rather they conclude that neonates are not biased towards a face geometry. Overall several studies have suggested that early face preference may be an example of a non-specific, domain-general, and gradually narrowing perceptual preference for up-down asymmetry, which could be best explained by an innate structural bias towards top-heavy configurations across a range of stimuli, including designs with a low resemblance to a basic face shape.

If it is possible to determine that a preference for top-heavy configurations exists during the fetal period; then one might argue that this representational bias is independent of experiential input. One study examined this question using a 3-dot design and concluded that fetuses preferentially engage with top-heavy arrangement of visual stimuli, namely 2- dots in the upper part of the image and one in the lower part of the image [7]. Such findings suggest that even prenatally there may a domain-general mechanism, acting as an early-generalized "face" detector, which guides later more specific face expertise. In support of this conclusion, Murty et al [10] report, in their fMRI study comparing sighted and congenitally blind participants that having the experience of seeing faces is not driving the development of face selectivity in the lateral fusiform gyrus. Results of

this research suggest that the bias is evident regardless of visual input, thereby challenging the idea of an early, generalized "face" detector. It is clear that further fetal research regarding reactivity to light stimulation, specifically investigating the distribution of light stimuli, taking account of fetal maturity, maternal mood and prenatal attachment must be carried out in order to draw further conclusions about a potential inherent preference for top-heavy face like compared to a nonface like control stimulation.

A key element of the debate concerns the experimental effects of what is determined to be face-like and which aspect of the face-like stimuli elicit a response [1,2]. We argue that, not only a control stimulus, but also variations of what is considered face-like must be included in the experimental design. According to Chen & Chen[11], 'undoubtedly, the structure of a face is up-down asymmetrical' (e.g., two eyes on the upper and one mouth in the lower part), and thus a preference towards a simple top-heavy geometric structure is thought to map directly onto some type of face-specific representational bias. Eyes are an important focus of the face, and the top visual field of the face provides key non-verbal emotional information that is especially important in the early developmental stages, during which speech comprehension is less important for engagement. Nevertheless, one cannot underestimate the importance of the mouth in facial configuration representation. This is because the mouth elongates during speech and emotional expression, resulting in larger surface compared to that occupied by the eyes. Furthermore, the developmental importance of engaging with the mouth region already shown by neonates who imitate mouth movements during the first minutes after birth cannot be underestimated [12]. This raises a key question regarding representing schematic facial configurations as top- versus bottom-heavy designs. In fact, the iconic 'smiley face'

representation used in children's marketing is entirely bottom-heavy, with two small eyes in the upper part and a larger semi-circular smile in the lower part of the display [13]. Hence, creating simple schematic representations of a basic face shape may be a more complex story than originally believed. We argue that, to measure fetal reactions and analyse mechanisms underlying an early face-like preference, both top-heavy and bottom-heavy faces must be tested and compared to a non-face-like control configuration.

More importantly and missing from the debate so far on prenatal reactions to light stimulation is the fact that, for fetal research to be valid, one must control for a range of intrauterine conditions. These factors include maternal stress [14], depression [15, 16], anxiety [5, 17], and attachment [18], which all have been found to influence fetal neurodevelopment as expressed in their behavioural profile [5]. For example, Glover, et al [17], argue that there is evidence that the function of the placenta is altered if the mother is anxious or depressed, and this may control the exposure of the fetal brain to hormones including cortisol, neurotransmitters, and other factors such as brain derived neurotrophic factor that can affect brain development. Furthermore, it is essential for testing fetal development that pregnant women are not on any drug treatments for stress, depression or anxiety so as not to influence the fetal womb environment. Importantly, even women with nonclinical symptoms of depression affect the fetal environment. Specifically, Field, Diego, & Hernandez-Reif [19] compared prenatal dysthymia versus major depression effects and found that the dysthymic group, that is the group with persistent mild depression, had higher prenatal cortisol levels and lower fetal growth measures including estimated weight, femur length and abdominal circumference as measured at the first ultrasound at 22 weeks gestational age.

Research has consistently demonstrated that factors such as maternal mental health [14], fetal growth parameters [e.g. 20] and womb condition [e.g. 21] all affect fetal movement profiles. These factors need to be controlled in any design when looking at fetal reactivity to experimental stimuli such as light or sound [5]. In the present feasibility study, we therefore controlled for maternal anxiety, depression, stress, antenatal attachment, as well as gestational age, fetal femur length, head circumference, and the fetal position in the womb.

2. Material and methods

2.1 Participants

For the current feasibility study, we recruited nine women, with healthy fetuses (5 males, 4 females) as per their 20-week anomaly scan. All pregnant women were non-smokers, without diagnosed medical conditions and a healthy BMI of 18-25.

This study was conducted in accordance with the Declaration of Helsinki. Ethical permission was granted by Durham University PSYCH-2019-09-05T15_18_01-dps0nr.

2.2 Apparatus and stimuli

2.2.1. Light source

The light source consisted of twelve red 3mm LED bulbs (3W each) with a centre wavelength of 640nm. The output measured 12 x 0.6 =7.2 lm (lumen), on a total surface area of 0.001225 m^2, giving an external light illuminance on the participant's abdomen of 5877.55 lx. Light intensity received by the fetus

depends on the intervening thickness of abdominal muscle, placenta and fat measured during the scan and recorded on the scan. Maternal tissue thickness used in our measurement of internal illumination ranged between 12.9 mm to 42.1mm (mean=21.8, SD = 9.43).

We assessed the light intensity received by the fetus by basing our approach on work by Del Giudice [22]. His approach was as follows. First, given muscle thickness m and the thickness of adipose tissue a (both in mm), a transmission coefficient T gives the ratio between the internal illuminance received by the fetus and the external illuminance from the light source. Del Guidice's formula for the transmission coefficient is given by

$$\log_{10}(T) = -0.942 - 0.058\,m - 0.032a.$$

In our sample the mean thickness of muscle and fat were m=7.51mm and a=14.29mm, respectively, yielding the transmission coefficient T= 0.0146255. The resulting internal illumination is 85.96 lx.

When applying this procedure fetus by fetus, rather than on their overall mean, the transmission coefficients for the nine fetuses range from 0.0023594 to 0.0363915 and the resulting internal illuminations range from 13.87 to 213.89 lx, notably all above the 10lx threshold for fetal vision according to Del Guidice (2010).

2.2.2 Light stimulus configuration

Three types of light stimuli ("face-like" top- and bottom-heavy designs, and a non face-like control) were constructed using twelve LEDs arranged in three different configurations on an 8 x 8 grid (see Figure 1) with a total surface area of (3.5cm)^2= 0.001225 m^2.

Fig 1. Light stimuli projected through the maternal abdomen onto fetal eyes

Control non face-like   Bottom heavy face-like   Top heavy face-like
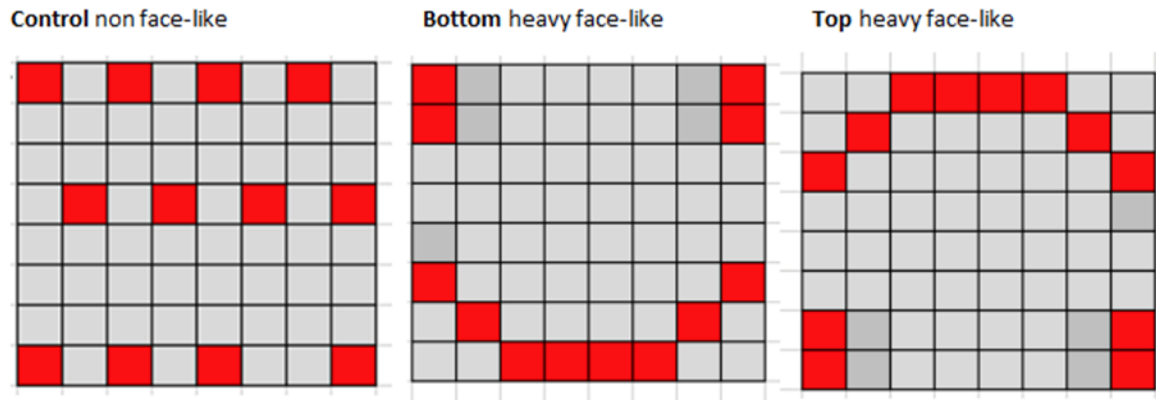
2.3 Procedure

Fetuses underwent ultrasound recordings at 33 weeks gestation (mean=33.29, SD=.49) using a Voluson E8 scanner with Wideband Convex Ultra-Light Volume probe. Mothers completed validated questionnaires assessing Anxiety, Depression, Attachment and Perceived Stress (see [5]). Measurements of fetal head circumference, femur length, and maternal tissue layers were recorded during the scan. Videos of the scans were coded offline frame by frame for fetal head turns.

2.4 Reliability

Each presentation was labelled by a code on the ultrasound scan. Given that 4D ultrasound quality varies from scan to scan, and since this variation can skew the results, the current study computed head turns per codable length of ultrasound scan to provide relative values of head turns in order to increase

accuracy when comparing fetal head turn frequency across the scans. We defined fetal reaction to light stimulation as any head turn observed during the presentation of the light stimuli, taking account of fetal position in the womb.

Reliability coding by a coder unaware of the hypotheses and labels of the scan was performed on 10% of the sample (Cohen's Kappa 0.87, range 0.83-0.92).

3. Analysis

3.1 Exploratory analysis

In this analysis, we recorded fetal head turns in the control and face-like stimulation conditions for each fetus (see Table 1) which indicate that fetuses showed fewer mean relative frequency head turns toward the control stimulus compared to fetal head turns toward both the face-like top- and bottom-heavy stimuli.

Table 1

Fetal head turns per codable minute in relation to top-heavy versus bottom-heavy and control stimuli

	codable scans	mean relative head turn	std deviation	min relative head turn	max relative head turn
control	9	0.83	1.06	0	3.00
face-like (bottom-heavy)	9	1.65	2.67	0	8.48
face-like (top-	6	1.70	1.54	0	4.32

heavy)					
face-like (combined)	15	1.67	2.22	0	8.48

3.2 Modelling

In order to assess whether the visual, maternal, and fetal predictor variables affect significantly the relative frequency of head turns, we fitted Negative Binomial count data models with a random effect for fetuses, using the statistical programming language R [23].

For modelling, the light stimuli were represented by indicators `FaceLike' (taking the value 1 for the face-like stimuli, and 0 for control) and `TopHeavy` (taking the value 1 for top-heavy stimuli and 0 otherwise).  We control for the effect of maternal tissue through an additional variable `IllumInt' which represents the fetus-specific internal illuminations as explained in Section 2.2.1. The other variable names are self-explanatory.

The final model (M), on which the p-values presented in the Results section are based, can be written as

$$\log \lambda = \log(ScanLength) + \beta_0 + \beta_1 Anxiety + \beta_2 Depression + \beta_3 Attachment$$
$$+ \beta_4 FemurLength + \beta_5 IllumInt + \beta_6 FaceLike + \beta_7 TopHeavy + u$$

where the total number of movements Y follows a negative Binomial distribution $Y \sim$ NB(??, ??) and the fetus-specific random effect a Gaussian distribution $?? \sim$ Normal($0, \sigma_u^2$). It is noted that a Negative Binomial, rather than Poisson, model is

used since there is some overdispersion, even after inclusion of the random effect. The output of fitted model (M) is provided in Table 2.

Table 2: Fitted model (M)

Variable	Estimate	Standard error (S.E.)	p-value
Intercept	-23.759	5.485	<0.001
Anxiety	0.193	0.067	<0.004
Depression	-1.505	0.254	<0.001
Attachment	0.080	0.074	0.276
Femur length	0.313	0.124	0.011
IllumInt	-0.007	0.005	0.146
FaceLike	0.539	0.264	0.041
TopHeavy	-0.253	0.296	0.393

4. Results

4.1 Illumination

The effect of the internal illumination on the relative head movements was non-significant, with a value of p=0.146. Despite this, it is important to include this variable into the model to avoid confounding the illumination effects with other effects of interest.

4.2. A face-like preference

The difference in relative head turns when exposed to face-like stimuli (both top- and bottom-heavy) compared to the control stimulus was significant at the 5% level, with p=0.041. However, to note is that fetuses turned their heads equally often to top-heavy and bottom-heavy face-like configurations of the stimuli, p=0.393.

4.3 Maternal mental health and reactivity

The maternal depression score was significantly negatively correlated with the relative frequency of fetal head turns across all conditions (p<0.001). Fetuses who were exposed to higher levels of maternal depression were less reactive to the light stimuli (see Table 2).

Maternal anxiety also had a significant effect on relative head turns (p < 0.004), with higher levels of anxiety corresponding to higher reactivity.

While the perceived stress score, PSS, was highly correlated with both Depression (r=0.72) and Anxiety (r=0.73), it was statistically unrelated to relative frequency of head turns (p>0.7) and is therefore not considered in the presented model.

4.5 Maternal prenatal attachment and reactivity

Maternal attachment, which has been found in some studies to be affected by maternal anxiety [24] showed no significant effect on fetal head turns (p=0.276).

4.6 Fetal maturity and reactivity

Femur length, an indicator for fetal neurobehavioural maturity, was positively correlated with relative frequency of head turns across all conditions (p=0.011). Fetuses who had larger femur lengths were significantly more reactive to the light stimuli, compared with fetuses having smaller femur lengths (see Table 2).

Head circumference and gestational age showed no correlation with reactivity to light stimuli.

5. Discussion

Our results indicate that maternal mental health factors significantly affect fetal reactions to light stimulation. Furthermore, femur length, a measure of fetal maturity, is a significant variable in terms of fetal reactivity to face-like and control light stimulation. The light stimuli were strong enough to be in theory perceived by the fetus. Del Guidice [22: 215] cites a threshold of 10lx, as a conservative estimate of the threshold for fetal vision, which is enough light for an adult to read small printed text. With the light intensity in our study exceeding the amount of light emitted as a threshold for fetal vision, this feasibility study indicates that fetuses do not distinguish in their head turn reactions between top-heavy or bottom-heavy face-like stimulation. Though we found that fetuses reacted significantly less frequently to the control stimulus compared with the face-like stimuli; it is clear from our results that fetuses did not prefer top- heavy to bottom-heavy face-like stimuli. This suggests that it may be an oversimplification to say that there exists an inherent *top-heavy* bias [7], which also has been criticized by Scheel, Ritchie, Brown, & Jacques, [25] in fetal reactions to light stimulation. Instead, attentional systems set-up for face learning are sensitive to arguably primitive face-like over non face-like configurations, even if they are bottom-heavy. This interpretation of the data is in accord with Chien [26] who reported no consistent bias for the top-heavy configuration in 3 - 5.5 months old infants.

Furthermore, in the current feasibility study, we analysed relative counts of head turns and we introduced a control stimulus, calibrated to equal light intensity distributed over the same surface area as top- and bottom-heavy face-

like light stimulation. Additionally, we controlled for maternal mental health (stress, depression and anxiety) and prenatal attachment, all factors that have been reported to influence fetal reactivity to stimulation. Therefore, this study indicates that it is essential to consider maternal variables that, as we have demonstrated, can skew fetal research significantly. Suggestive results of this feasibility study show that fetuses react to general face-like stimuli, rather than just light stimulation of the same intensity. It seems clear that the story regarding an innate face preference is highly complex and requires further substantial research, which can be guided by the current feasibility study.

Importantly, results of the current study show that femur length, a measure of fetal maturity, was significantly positively correlated with increased fetal reactivity to light stimulation. As femur length can be considered a more precise indicator for fetal neurobehavioural development [27] than gestational age, it is essential to consider this finding in the context of how individual maturity factors and development trajectories may relate to differing reactivity levels in fetuses. This is a crucial finding for the field of fetal development research as it highlights the need to control for these variables in all fetal research. During the prenatal period, sensory and motor systems develop to become increasingly complex. Specifically Fagard et al [28] reviewing research on the fetal origins of sensory motor behavior talk about a development from "primitive motor babbling" to mature sensorimotor behavior. General movements occur at around 17 weeks gestational age [28;29] and further more specific maturation can be observed from around 24-36 weeks gestation where fetuses develop from general movements of touching mouth to anticipating touch by opening the mouth [30]. Hence the process can be characterized in terms of the first primitive movements which establish neuronal connections [28] to the development of intentional

reactions to stimuli [31]. Much like fetal growth trajectories, this process of maturing an increasingly functional distributed sensory-motor cortical network is likely to be slightly different in each fetus. By demonstrating that femur length was significantly correlated with fetal reactivity, we show that femur length, compared with gestational age, seems to be a more reliable indicator for individual neurobehavioural development progress in healthy fetuses. This is a crucial finding as it indicates the importance of controlling for growth factors in all fetal research to establish accurate prenatal time-points whereby certain skills are normally evident, with the possibility of being able to identify delays that might need further investigation.

Furthermore, maternal non-clinical depression was significantly negatively correlated with fetal head turns. Many studies have demonstrated that maternal mental health factors, including stress, depression, and anxiety significantly affect fetal development [15, 31, 33]. Our findings add to the wealth of research regarding the nature of these effects. In this case, fetal reactivity decreased with increasing maternal depression.

This finding seems to go against some research, which suggests that maternal depression is linked to increased fetal activity [33] and increased fetal heart rate [34]. Important to note is that in the present research study we did not examine *general* fetal activity, but rather reactive head turns to specific light stimuli. This process involves a higher level of cognition than general activity, and thus our finding suggests that maternal depression may disrupt the attentional mechanisms, which guide sensory-motor responses such as head-turns. Although the exact mechanism, which transmits maternal depression to the fetus, is unknown, the dysregulation of maternal hormones (e.g. cortisol) is thought to be involved [35]. Dysregulated maternal hormones can alter the corticolimbic

network involved with emotional regulation in depressive disorders. This can exert further disruption to areas of the prefrontal region including dorsal and ventral lateral prefrontal cortex, medial prefrontal cortex, and especially the orbitofrontal cortex [36]. This result may link to research by Sandman, Buss, Head, & Davis [37] who found a significant thinning of the precentral and postcentral cortex in the right hemisphere for fetuses exposed to maternal depression. The precentral and postcentral cortex is the primary motor cortex, and thus this cortical thinning could explain why fetuses in our study were less reactive to external stimuli when exposed to higher level of maternal depressive symptoms.

Maternal anxiety was significantly related to relative head turn frequencies, with higher levels of maternal anxiety corresponding to higher fetal reactivity.   This result replicates findings of increased fetal eye-blink reactions to sound stimulation in fetuses of anxious mothers [5]. Additionally, research indicates that prenatal maternal anxiety has long-term effects on cognitive development [38], fetal development and birthweight [39].

This current study adds to the growing number of research findings indicating that maternal mental health is a vital factor for healthy fetal development. It follows that maternal prenatal care is not only essential for birth outcomes, such as gestational age and weight, but also has implications for the prenatal foundations of cognitive and socio emotional development.

Limitations of the Study

In this study, we were able to demonstrate that fetuses react more to facial configurations compared to a non-facial control stimulus. However, given the relatively small sample size of this feasibility study, this research needs to be

replicated with a larger sample of fetal participants. Furthermore, the experimenter moving the stimulus should be videotaped to control offline direction and speed of movement of the stimulus in relation to fetal position.

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"Conflict of interest statement"

All authors declare that they have no conflicts of interest.

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depression and anxiety and fetal-neonatal growth. Journal of Paediatrics.

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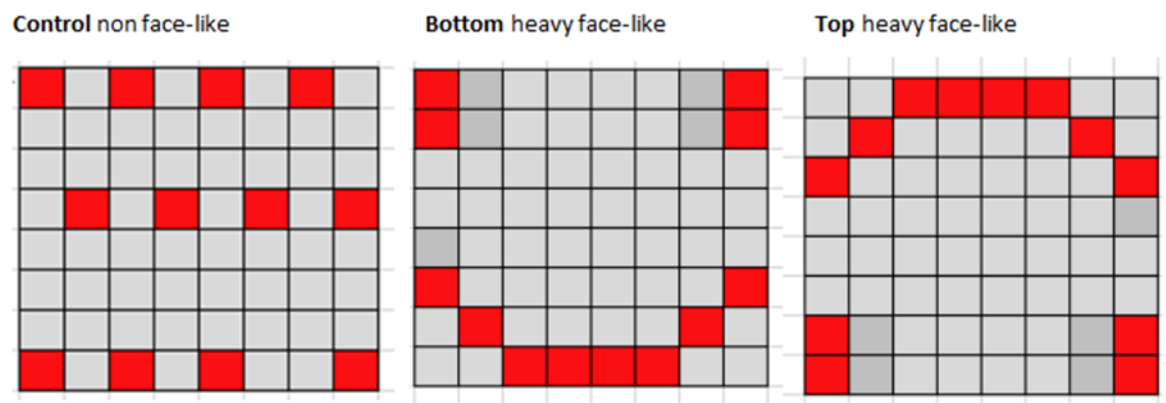# Fig 1. Light stimuli projected through the maternal abdomen onto fetal eyes

Control non face-like

Bottom heavy face-like

Top heavy face-like

Table 1

Fetal head turns per codable minute in relation to top-heavy versus bottom- heavy and control stimuli

	codable scans	mean relative head turn	std deviation	min relative head turn	max relative head turn
control	9	0.83	1.06	0	3.00
face-like (bottom-heavy)	9	1.65	2.67	0	8.48
face-like (top-heavy)	6	1.70	1.54	0	4.32
face-like (combined)	15	1.67	2.22	0	8.48

Table 2: Fitted model (M)

Variable	Estimate	Standard error (S.E.)	p-value
Intercept	-23.759	5.485	<0.001
Anxiety	0.193	0.067	<0.004
Depression	-1.505	0.254	<0.001
Attachment	0.080	0.074	0.276
Femurlength	0.313	0.124	0.011
IllumInt	-0.007	0.005	0.146
FaceLike	0.539	0.264	0.041
TopHeavy	-0.253	0.296	0.393

Author statement

Nadja Reissland: Conceptualization of the project; Development of methodolog; design of light stimuli; writing the initial draft; supervision of RA; redrafting the paper, reviewing the paper

Jochen Einbeck: creation of models; formal analysis; redrafting the paper; reviewing the paper

Alison Lane: Conceptualization of the project; design of light stimuli; reviewing the paper

Rebecca Wood: Design of methodology; performing the experiments, data collection; data coding; writing the initial draft; reviewing the paper