An investigation into the effects of prenatal flavour exposure through maternal diet on fetal and infant behaviours

BEYZA NUR USTUN

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Beyza Nur Ustun, BSc (Hons), MSc

Abstract

The research presented in this thesis is an examination of the effects of prenatal flavour exposure on fetal and infant behaviours. Throughout pregnancy, a fetus is exposed to a range of flavours in the amniotic fluid which contains maternal dietary aromas. There is a body of research investigating the effects of prenatal flavour exposure on infant behaviours, however, direct investigation of fetal reactions has to date been lacking. To address this, Chapter 3 examined the fetal facial reactions, via 4D ultrasound scans, to prenatally exposed flavours, namely kale and carrot. The findings indicate the first direct evidence of fetal facial reactions to flavours in the amniotic fluid. Chapter 4 is a systematic review and meta-analysis that indicated that this prenatal flavour experience provides continuous chemosensory information from prenatal to the first year of postnatal life. Chapter 5 indicated that neonates at around one month of age "prefer" the odour of flavours they experienced in the last three weeks of pregnancy. Additionally, a longitudinal analysis that was reported in Chapter 5 shows that fetal reactions to flavours can be modified by repeated exposure in utero, resulting in increased preferences for the exposed flavour irrespective of the type of flavour. This PhD thesis makes an important and novel contribution to the research literature by providing a window into the chemosensory world of the human fetus and showing a potential way to shape postnatal food preferences.

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Beyza Nur Ustun, BSc (Hons), MSc

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Abbreviations

AF	Amniotic fluid
BMI	Body mass index
SCoR / BMUS	Society and College of Radiographers / British Medical Ultrasound Society
2D / 4D	Two-dimensional / four-dimensional
FACS	Facial Action Coding System
FOMS	Fetal Observable Movement System
FM	Facial Movement
FM1	Inner-brow raiser
FM2	Outer-brow raiser
FM4	Brow lowerer
FM6	Cheek raiser
FM9	Nose wrinkle
FM10	Upper-lip raiser
FM11	Nasolabial furrow
FM12	Lip-corner puller
FM14	Dimpler
FM15	Lip-corner depressor
FM16	Lower-lip depressor
FM17	Chin raiser
FM18	Lip pucker
FM19	Tongue show
FM20	Lip stretch

FM24	Lip presser
FM25	Lips parting
FM26	Jaw drop
FM27	Mouth stretch
FM28	Lip suck

Declaration

I, Beyza Nur Ustun, confirm that none of the material presented in this thesis has been submitted elsewhere for any other qualification, and all the work presented in this thesis is my own, unless referenced. This work was funded by the Turkish Ministry of National Education.

Statement of Copyright

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Dedication

To my parents and my husband. I will be forever grateful for your presence in my life.

Publication and Dissemination Note

Chapter 3:

- The entirety of Chapter 3 is published in Psychological Science: **Ustun, B.**, Reissland, N., Covey, J., Schaal, B., & Blissett, J. (2022). Flavor Sensing in Utero and Emerging Discriminative Behaviors in the Human Fetus. *Psychological Science, 33*(10), 1651–1663. https://doi.org/10.1177/09567976221105460

  Author Contributions: B. Ustun designed the experiment, provided funding, collected data, coded ultrasound scans, analysed the data, and wrote the manuscript. N. Reissland, J. Covey, B. Schaal, and J. Blissett designed the experiment, provided guidance for data analysis, and reviewed and edited the manuscript. All the authors approved the final manuscript for submission.

- Part of the prenatal study reported in Chapter 3 was presented (poster) at the European Chemoreception Research Organisation ECRO XXXI, Cascais, Portugal, 2021 (see appendix 1).

- Chapter 3 has extensive media coverage & public engagement and was awarded for best publication for a postgraduate student by the Health in Preconception & Pregnancy, Early and Mid-Career Researcher Collective International, 2022 (see appendix 2 and 3).

Chapter 4:

- The entirety of Chapter 4 is under review for publication in PLOSOne: **Ustun, B.**, Covey, J. & Reissland, N. (2022). "Chemosensory Continuity from prenatal to postnatal life: A systematic review and meta-analysis."

  Author Contributions: All authors contributed to the conceptualization and methodology. BU conducted the formal analysis, literature search, data extraction and management, and prepared the original draft. JC contributed to the analysis

(guidance). JC & NR contributed to the data extraction as a second reviewer, reviewed and edited drafts and supervised the process. All authors approved the final version.

- Chapter 4 was also presented (oral) at the UK Ireland & Marcé Society for perinatal mental health (UKIMS) Annual Meeting (Virtual), London, UK, 2021.

Chapter 5:

- The entirety of Chapter 5 was prepared to be submitted for publication.
- The findings reported in Chapter 5 were presented (oral) at the Fifth Annual ECR Conference (virtual), Wolfson Research Institute, Durham, UK, 2022.
- The longitudinal findings from the prenatal to the postnatal period reported in Chapter 5 were also presented (oral) at the 9th International Conference on Nutrition & Growth (virtual), Kenes Group, Geneva, Switzerland, 2022.

In 2022, this thesis was awarded first place in the institutional Three-Minutes-Thesis (3MT) competition held at Durham University, second place in the regional 3MT competition held in the Northeast of England and was nominated as a finalist at the international 3MT competition held by the Matariki Network of Universities (see appendix 3).

For consistency across the thesis, table and figure numbers, formatting and references have been modified.

Chapter 1

General Introduction

The fetal environment, which is noisy and varying in light intensity and movement, is where a fetus begins to learn about its surroundings. Through their developing sensory abilities, a fetus can hear, see, and sense touch (Lagercrantz & Changeus, 2009; Marx & Nagy, 2017; Nagy et al., 2021; Reissland et al., 2019; 2020b). The fetus can also respond to the flavours of the food consumed by their mothers (Schaal, 2016). During a certain time of maximum neuroplasticity, these experiences provide important opportunities to learn about the outside world, including the food environment. Despite the importance of prenatal experiences, we know very little about the development of human fetal sensory responses to prenatal flavour exposure and what it means for postnatal food-related behaviours.

This thesis addresses the following questions to provide much-needed direct longitudinal evidence of the effects of prenatal flavour exposure on fetal and infant behaviours. What types of behavioural reactions to flavour cues from the maternal diet can be observed in the fetus? How do these reactions develop during pregnancy? How do these behavioural reactions differ depending on the type of flavour profile? Can a human fetus practice and learn the flavours experienced in the amniotic fluid environment, and can these flavours experienced in the womb be recalled after birth? Does prenatal exposure to certain flavours modify the initial reactions for those flavours?

The current introduction chapter starts by outlining the literature on fetal chemosensory and facial muscular abilities. From there the origin of flavour preferences is discussed, focusing on bitter and sweet tastes. Next, the concept of flavour exposure in utero

including the discussion of flavour transfer from mother to fetus is discussed. After that, the postnatal reactions to prenatal flavour exposure are discussed. Following this, the gaps in the literature, that are addressed in this thesis were described. Finally, the aims and hypothesis of the thesis were defined, followed by a thesis outline.

Development of fetal chemosensory abilities and facial movement profile

Human flavour perception is a dynamic and complex process that integrates olfactory, gustatory, and trigeminal systems (International Organization for Standardization, 2016). Flavour perception is largely elicited by the smell of food, which is detected by the nasal and/or oral cavity and reaches the olfactory epithelium (Duchamp-Viret et al., 2016; Seo & Hummel, 2012; Small & Prescott, 2005). Taste buds in the oral cavity, primarily on the tongue, detect basic tastes such as bitter, sweet, sour, and salty as well as umami and kokumi compounds (Briand & Salles, 2016; Seo & Hummel, 2012; Small & Prescott, 2005). The trigeminal sensation is mediated by the fifth cranial nerve and is responsible for the irritating and/or burning sensation, for example, while consuming spicy food (Briand & Salles, 2016; Seo & Hummel, 2012). Although little is known about fetal trigeminal systems, animal models have shown that fetal trigeminal nerves respond to flavour compounds such as ethanol (Glendinning et al., 2017; Smit et al., 2022).

After somesthesis, gustatory and olfactory systems are some of the earliest developing systems in human fetuses (André et al., 2018; Firestein & Beauchamp, 2020; Mellor, 2019). Human taste buds and gustative receptors emerge between seven and 11 weeks, fetuses started to swallow at around 12 weeks and the receptors are becoming functional by around 14 weeks' gestation (Bradley & Stern, 1967; Hersch & Ganchrow, 1980; Pritchard, 1965; Witt & Reuter, 1997; 1998). Adult-like olfactory bulbs emerge at 18.5 weeks and are

developed by 22 weeks' gestation, but do not start to function until the last trimester (Nishimura, 1993; Malnic et al., 2010; Moore et al., 2012). The nasal cavity is open at 24 weeks (Witt, 2000) and receptors are becoming functional by 28 weeks' gestation (Chuah & Zheng, 1987, Moore et al., 2012). Thus, fetal chemosensory systems are functioning in utero from the sixth month of pregnancy.

Much like sensory progress, the motor competence of facial muscles starts before birth. Simple fetal facial movements, based on the activity of facial muscles such as "smiling" can be observed from 20 weeks' gestation whereas complex facial movements such as "mouthing" and "grimacing" can be observed from 24 weeks' gestation (Hata et al., 2012; Kurjak et al., 2008; Reissland et al., 2011, 2013, 2016; Salihagic-Kadić et al., 2016; Sato et al., 2014; Yigiter & Kavak, 2006). From 26 weeks' gestation, these movements can also be coordinated to generate facial configurations or facial gestalts, namely "cry-face gestalt", and "laughter-face gestalt" (Reissland et al., 2011, 2013). These gestalts resemble postnatal "emotional expressions" or "hedonic reactions" (Reissland et al., 2011; Oster, 2006). For instance, the laughter-face gestalt may reflect appetite, preference, or attraction, in other words, a positive reaction, whereas the cry-face gestalt may suggest aversion, or dislike, in other words, a negative response (Berridge, 2000; Mennella et al., 2001; Schaal et al., 2000; Steiner et al., 2001).

Studies investigating the chemosensory reactivity and discriminative abilities for flavours of preterm infants supported that fetal chemosensory and facial muscular abilities are functional by extrapolating the findings to same-aged fetuses (Goubet et al., 2002; Maone et al., 1990; Sarnat, 1978; Schaal et al., 2004; Tatzer et al., 1985). Preterm infants born at 32 weeks showed increased sucking and global activity after a presentation of mint odour

stimuli. In contrast, younger preterm infants born at 28 weeks hardly reacted to the same stimuli (Sarnat, 1978). Moreover, Schaal et al. (2004) reported that preterm infants delivered between 28- and 33 weeks' gestation showed discriminative reactions to different odours, by increased respiration rates and appetitive responses to vanilla odour and increased aversive reactions and decreased respiration rates to butyric acid odour. The findings from premature new-borns suggest that fetuses can detect odours and taste compounds from 29 weeks' gestation.

Human fetuses perceive flavours by mostly swallowing but also inhaling the amniotic fluid, which is flavoured by the mother's food consumption (McLean & Shipley, 1992; Mistretta & Bradley, 1975; Schaal, 2016). The amniotic fluid pool does not block a fetus's sense of taste or smell rather it accelerates the flow of certain taste and odour compounds to receptors whenever a fetus swallows or breathes (McLean & Shipley, 1992). Given that it is not possible to dissociate taste, odour and trigeminal chemesthesis from each other in the amniotic fluid environment, reference is made throughout the thesis to '*flavour*' acknowledging that the effects of prenatal flavour exposure may be mediated by one or several of these chemosensory stimuli.

Origin of flavour preferences

Flavour preferences are significantly determined by innate characteristics (Bartoshuk & Beauchamp, 1994; Beauchamp & Mennella, 2009; 2011; Jackson et al., 2020; Mennella, 2014; Seo & Hummel, 2012; Steiner, 1974,1979). Because of their caloric sugar content, humans are biologically predisposed to prefer sweet foods, and this preference ensures an adequate intake of carbohydrates, which are rich in energy (Bartoshuk & Beauchamp, 1994; Beauchamp & Mennella, 2009; 2011). This innate preference is stronger during infancy and

childhood because it is assumed to develop to fulfil the caloric intake necessary for human growth and development (Beauchamp & Mennella, 2009; Drewnowski et al., 2012; Forestell, 2017; Schwartz et al., 2017). Bitter-tasting foods, on the other hand, are instinctively avoided because they may contain toxic substances (Bartoshuk & Beauchamp, 1994; Beauchamp & Mennella, 2009; 2011; Forestell, 2017; Glendenning, 1994; Siega-Riz et al., 2010). Despite the necessity of innate flavour preferences in avoiding poisons in the past, these biological predispositions no longer have an adaptive benefit to humans (Forestell, 2017). For example, many bitter-tasting vegetables such as kale, broccoli, and spinach are known to be safe and healthy.

Before ultrasonography was available, fetal reactions to flavours were tested indirectly (de Snoo, 1937; Liley, 1986) by measuring the changes in the waist size of pregnant women. In 1986 (Liley), it was demonstrated that injecting a bitter-tasting poppy seed oil solution into the amniotic fluid resulted in an increase in participants' waist circumference, which was attributed to the excess of amniotic fluid (polyhydramnios) due to decreased fetal swallowing. However, their waist circumference decreased when a saccharin solution was injected into the amniotic fluid, which was associated with increased fetal swallowing. This research provides indirect evidence of innate flavour preferences, by demonstrating that 34-week-old fetuses can detect flavours and might even “prefer” sweet taste compounds they sense in their amniotic fluid surroundings.

Another indirect evidence of an innate ‘preference’ for sweet taste comes from premature infants. For example, the frequency of sucking behaviour of preterm infants (the mean

postmenstrual age[1]of 35.5 weeks at testing) was higher when presented with a sweetened solution in the oral cavity compared to the presentation of a water solution (Tatzer et al., 1985). Similarly, 36 weeks postmenstrual age preterm infants sucked at a higher frequency when presented with a sucrose-flavoured nipple than an unsweetened gelatine-based nipple (Maone et al. 1990).

These innate taste preferences can be modified with experience (de Cosmi et al., 2017; Ventura et al., 2021; Ventura & Worobey, 2013). Repeated exposure is a simple but effective method to practice and learn about novel flavours (Ahern et al., 2014; Caton et al., 2013; de Wild et al., 2013). Exposure to certain flavours for an adequate period can heighten preferences for the target flavour, even for accommodating bitter-tasting compounds (Forestell & Mennella, 2007; Mennella et al., 2009). The breastfeeding period is one of the earliest periods to introduce novel flavours through breast milk which contains dietary aromas of the mother's food (Hausner et al., 2010). However, babies can be introduced to novel flavours even before they are born, and flavour exposure in utero would affect subsequent food-related behaviours (Beauchamp & Mennella, 2009).

Flavour exposure in utero

The first place where fetuses begin to sense different flavours is the amniotic fluid that surrounds them in the womb (Brumley & Robinson, 2010). In this intrauterine surrounding, a fetus is exposed to flavours that are passed from the foods eaten by their mother, and this experience might provide continuous sensory information from fetal to neonatal life

[1] "Postmenstrual age is the time elapsed between the first day of the last menstrual period and birth (gestational age) plus the time elapsed after birth (chronological age)" (Engle & American Academy of Pediatrics Committee on Fetus and Newborn, 2004).

(Forestell & Mennella, 2015; Mellor, 2019; Schaal, 2016). This section discusses, how these flavours are transferred from mother to fetus and how to measure the effects of prenatal flavour exposure.

Throughout pregnancy, maternal intake of certain flavours affects both the smell and the taste of the amniotic fluid (Mennella et al., 2001; Schaal et al., 2000; Schaal, 2016). The potential trajectory of flavours from the mother's diet to the fetal environment is as follows: When the mother eats food, all flavours first pass into the mother's bloodstream through the gut and systemic circulation. From here, taste compounds pass through the umbilical cord and placenta into the fetal blood; and from fetal blood, it is excreted into the amniotic fluid by renal and pulmonary pathways.

Several direct or indirect measurements have so far been used to test the effects of flavour transmission from mother to fetus including identifying the smell of amniotic fluid by adult judges (Mennella et al., 1995), chemical analyses of amniotic fluid (Fotiou et al., 2018), and postnatal studies examining reactions to flavour in both human and nonhuman samples (Faas et al., 2000; 2015; Figueroa et al., 2013; Hepper et al., 1995; Hepper & Wells, 2006; Mennella et al., 2001; Lévy et al., 2020; Schaal et al., 2000; Simitzis et al., 2008; Smotherman & Robinson, 1987; for a review, see Spahn et al., 2019). Nonhuman studies also visualized the spontaneous, direct responses to intraoral or intranasal infusion of flavours from surgically prepared fetuses. This strong approach, characterizing stimulus-related fetal responses to experimental flavours, has been applied in many non-human models (e.g., rats, sheep), but not yet in humans (Robinson et al., 1995; Schaal et al., 1991; Smotherman & Robinson, 1987). The prenatal research in this thesis (Chapter 3) addresses this gap.

As an example of adult sensory analysis of flavour transmission, a randomized experiment conducted by Mennella et al. (1995) collected amniotic fluid samples from five

pregnant mothers who consumed a capsule containing garlic extract and five pregnant women who consumed a placebo capsule (lactose) approximately 45 minutes before amniocentesis in the second trimester. Samples of the amniotic fluid (one from the garlic-exposed group, one from the placebo group) were presented in pairs to 13 blinded adult judges. The odour of the amniotic fluid was rated to be “stronger or more like garlic” odour in four of the five amniotic fluid samples collected from mothers in the garlic group in comparison to samples collected from the placebo group. This study provides evidence that garlic flavour is transmitted from the mother to the fetal environment, by showing that maternal garlic intake changes the odour of amniotic fluid. Similarly, Hauser et al. (1985) reported that the body odour of new-borns varied depending on what the mother had eaten preceding delivery. For example, the new-born baby whose mothers ate a Yemeni dish called "Khilba", smelled like fenugreek, shortly before giving birth. This sensory analysis study also supports the hypothesis that flavours are transmitted to the amniotic fluid from maternal diet, by showing that new-born babies have a distinctive odour due to this transmission.

A recent study by Fotiou et al. (2018) analysed the chemical composition of amniotic fluid samples collected from 65 pregnant women through amniocentesis in their second trimester of pregnancy. Through dietary assessment of the pregnant women, they identified two clusters of dietary patterns (“cluster 1: greater yellow cheese, poultry, red meat, refined cereals and ready meals; cluster 2: vegetables, fruits, whole cereals, nuts and pulses”) depending on the energy contribution of the food group. Relevant metabolites of these two clusters were determined in the amniotic fluid by chemical analysis and differed between the two groups depending on dietary patterns. This study provides strong evidence that the composition of amniotic fluid is affected by the mother’s dietary habits.

Despite its importance and providing direct evidence, sensory or chemical analysis of amniotic fluid in human samples is not always possible due to ethical considerations. Therefore, the researchers conducted postnatal studies to understand not only the flavour transmission mechanism but also fetal memory for flavours which leads to flavour continuity from fetal to neonatal life.

Postnatal responses to prenatal flavour exposure

Starting from prenatal life, olfactory memory plays an important role to recall chemosensory information, and therefore affects subsequent responses to flavours in humans and nonhumans (Hepper, 1996; Kawai et al., 2004; Schaal, 2015; Schaal et al., 2020; White et al., 2006). A fetus memorizes flavours through exposure learning and can access the embedded flavour information to benefit from it in their postnatal food environment (Mellor, 2019; Nicklaus, 2006; Schaal, 2006; Schaal et al., 2004). Exposure learning - the most effective learning method - is repeatedly exposing a fetus to a target stimulus and then comparing the response to either the "unfamiliar" stimulus or the reaction of unexposed fetuses to the target stimulus (Hepper, 1996). For example, Schaal et al. (1998) found that 3-day-old new-borns recognised the odour of familiar amniotic fluid (collected from their own mothers) by orienting their heads longer, compared to an unfamiliar amniotic fluid odour (collected from another mother) or to a control odour (i.e., distilled water). This study shows the existence of fetal memory and the ability to recall odour cues in the amniotic fluid postnatally.

Similarly, the repeated exposure method has been used to test the effects of prenatal flavour exposure via manipulating the maternal diet, has been tested by measuring the postnatal reactions. Such repeated exposure resulted in a greater preference for the target

flavour in the exposed group compared to the control groups whose mothers did not ingest the same flavours (human studies: Faas et al., 2000; 2015; Hepper, 1995; Hepper et al., 2013; Mennella et al., 2001; Schaal et al., 2000, nonhuman studies: Figueroa et al., 2013; Youngentob & Glendinning, 2009). This continuity from prenatal to postnatal life, namely *"*transnatal chemosensory continuity*"*, can be observed days, weeks, months and possibly years after birth (Hepper, 1995; Hepper et al., 2013; Mellor, 2019; Mennella et al., 2001; Schaal, 2005; Schaal et al., 2000).

As an example of studies investigating the short-term effects of fetal experience by presenting olfactory stimuli, Hepper et al. (1995) found that new-borns aged 15-28 hours did not show aversive reactions to garlic odour if their mothers had eaten at least four garlic-containing meals a week during the last month of pregnancy. Supporting this finding, another study (Schaal et al., 2000) showed that neonates up to four days of age, whose mothers consumed anise-flavoured sweets in the last two weeks of pregnancy preferred the anise odorant by showing fewer frequency of negative facial reactions, more mouthing reactions and orienting their heads to the anise odour for longer compared to a nonexposed group. It is suggested that oro-facial reactions by infants indicate stimulus-bound hedonic reactions (Lipsitt, 1977; Soussignan et al., 1997, 1999; Wagner et al., 2013), and hence such reactions can be considered an accurate method of evaluating reactions to prenatal flavour exposure.

Furthermore, Mennella et al. (2001) tested the relatively long-term retention of the effects of maternal carrot juice consumption in the last trimester of pregnancy by assessing infant facial responses to carrot flavour at around 6 months of age. They found that infants exposed to carrot flavour in the womb displayed fewer negative facial expressions towards carrot-flavoured cereal than plain cereal. This finding adds to the hypothesis that there is evidence of stable, long-term recalling of fetal experiences of flavour that affects food acceptance postnatally. Finally, a study argued an even longer retention of the prenatal garlic flavour

experience (Hepper et al., 2013). They found that children exposed to garlic during pregnancy preferred to eat garlic-flavoured meals at ages 8-9. Although they attempted to control garlic consumption during childhood, the result of this study should be interpreted with caution, due to the lack of garlic consumption control in the intervening years (i.e., breastfeeding, weaning, early childhood).

An in-depth discussion of the literature on postnatal behaviours to prenatal flavour exposure is provided in Chapter 4 (a systematic review and meta-analysis).

Filling the gaps

Although studies mentioned in the previous sections allow us to better understand flavour transfer from mother to fetus, and the effects of prenatal flavour exposure on chemosensory development and continuity, none of the research to date has shown direct evidence of human fetal reactions to flavours in the amniotic fluid. Chapter 3 provides the first direct evidence - using 4D (four-dimensional) ultrasound scans - that fetuses sense and discriminate certain vegetable flavours which were transferred experimentally into the amniotic fluid via maternal consumption. Chapter 4 evaluates, for the first time, chemosensory continuity from prenatal to the first postnatal year through a systematic review and meta-analysis of existing evidence on prenatal flavour exposure (including flavour cues from maternal diet and the odour of amniotic fluid) on infant behaviours. Despite its importance, postnatal human research addressing the effects of prenatal flavour exposure through maternal diet are scarce, and only one type of flavour exposure was used in previous investigations. The postnatal study presented in Chapter 5 fills this gap by comparing two flavours that do not share the same flavour profile. Furthermore, Chapter 5 provides the first longitudinal comparison between initial (prenatal) facial responses and subsequent (postnatal)

facial responses to test whether we can change initial responses to flavours by repeated exposure. This thesis contributes significantly to the literature by filling these gaps.

Thesis aims and hypotheses

The principal aim of this project is to examine the effects of prenatal flavour exposure on fetal and infant behaviours by employing a variety of methodological assessments to gain an understanding of fetal chemosensory abilities, flavour transfer mechanism from mother to fetus, prenatal flavour learning, and chemosensory continuity. The thesis aims were achieved with the following objectives and research questions.

Prenatal phase - Fetal reactions to flavours in utero

Objective. To investigate the effects of flavours in the amniotic fluid transferred via maternal ingestion of the flavour on fetal facial reactions.

Research Question 1: Do fetuses respond to vegetable flavours transmitted into the amniotic fluid through the maternal diet during the last trimester of pregnancy?

Research Question 2*:* Is there a difference in fetal facial movements in utero for specific vegetable flavours in amniotic fluid?

Research Question 3: How do human fetal facial movements in-utero in response to vegetable flavours in amniotic fluid develop between 32 to 36 weeks' gestation?

Hypotheses. i) Fetuses will respond to certain flavours, kale, and carrot, ingested by their mothers in the last trimester of pregnancy (in comparison to unexposed fetuses). ii) Fetuses will react to their mothers ingesting carrot flavour with more laughter-face reactions and to kale flavour with more cry-face reactions in comparison to each other and to the control group of fetuses whose mothers did not ingest any flavours. iii) Fetuses will react to flavours with an increasing complexity of facial gestalt from 32 to 36 weeks.

Chemosensory continuity from prenatal to postnatal life: a systematic review and meta-analysis

Objective. To investigate the chemosensory continuity from prenatal to postnatal life through two distinct but related bodies of literature.

Research Question 1: Is there a chemosensory continuity from prenatal to the first year of postnatal life?

Research Question 2: Can infants show different behavioural profiles to the flavours transferred via the maternal diet experienced in utero?

Research Question 3: Can infants show different behavioural profiles to the odour of their own amniotic fluid?

Hypothesis. There is a chemosensory continuity from prenatal to postnatal life. Infants will have a different behavioural profile when presented with the flavour and odours experienced in utero compared to unfamiliar flavour / odour presentation or compared to unexposed groups.

Postnatal phase – Neonatal reactions to flavours in utero

Objective. To investigate the effects of flavour exposure in the amniotic fluid transferred via maternal ingestion of the flavour on neonatal facial reactions with a comparison to fetal reactions.

Research Question 1: Do facial responses to odours which they experienced in utero differ from the responses to unfamiliar odours which they did not experience in utero?

Research Question 2*:* How do facial reactions to prenatally exposed flavours change from prenatal to postnatal life? Can we increase preference for a certain odour with repeated exposure in utero?

Hypotheses. i) Neonates will show more laughter-face reactions to flavours experienced in utero and more cry-face reactions to flavours not experienced in utero. ii) From prenatal to

postnatal life, there will be an increase in the frequency of laughter-face reactions to odours after repeated exposure, while there will be a decrease in the frequency of cry-face reactions.

Thesis outline

This introductory chapter has elaborated on the background of the thesis and highlighted the gaps in the literature. The following chapter provides an overview of the methodological approach of the prenatal and postnatal stages of this thesis, including an in-depth discussion of the behavioural coding scheme used in this research. Chapter 3 reports the prenatal phase of this thesis, Chapter 4 is the detailed report of a systematic review and meta-analysis, and Chapter 5 reports the postnatal phase of this thesis. In the final chapter, the key findings from this thesis are discussed including contributions to the literature, limitations, implications, and future directions.

Chapter 2

General Methodology

The experimental studies reported in this thesis have a longitudinal design which evaluated fetal and neonatal reactions to flavours transferred from the maternal diet to the amniotic fluid during the last trimester of pregnancy. The current chapter presents information on the pilot study and a general methodology for prenatal (Chapter 3) and postnatal (Chapter 5) studies.

Ethical permission for this research was obtained from the Department of Psychology ethics committee at the University of Durham (ref: PSYCH-2019-03-12T15_59_32-wvgf27). The thesis is funded by the Turkish Ministry of National Education. The funder has not had any role in the study protocol, recruitment, analysis, or preparation of the article. The research took place in the Northeast of England. All mothers gave informed written consent. During consent and prior to each procedure, mothers were informed that the scans were for research purposes and not medical screening (see appendix 4).

Pilot study

To establish proof of principle and reduce the potential risks of the main study, an adult sensory analysis of the flavours was performed, and four pilot ultrasound scans were collected and analysed.

Methods and materials of the pilot study

Flavour stimuli. In this thesis, the effects of two vegetable flavour profiles in utero were examined. *Kale*, which like other dark green vegetables, has a bitter taste and is a relatively "disliked" flavour profile by infants and children (Johnson et al., 2021). Bitter polyphenols and alkaloids, present in plant-based products such as dark green vegetables, deliver a bitter

taste sensation, show ready transfer to the amniotic fluid in animals and humans (Arola-Arnal et al., 2013; Lambers & Clark, 1996; Soares et al., 2013; Welker et al., 2018). *Carrot* has previously been used in this type of research, and the effect of prenatal carrot exposure on infant carrot flavour acceptance has been reported (Mennella et al., 2001). Carrot flavour, which is likely to elicit relatively positive responses, is often described as "sweet" by adults, but also sometimes described as "fruity" or "woody" flavours, because of terpenoids (e.g., Beta-carotene; Alasalvar et al., 2001). Therefore, "non-bitter" will be used to refer to carrot flavour throughout the thesis.

The pilot study initially intended to use kale and carrot juice, following the previous study procedure by Mennella et al., (2001). However, mothers (n = 4) were reluctant to participate in the pilot study because they did not want to drink kale juice due to a strong aversion to the flavour and texture. Therefore, mothers were asked to take vegetable capsules containing either kale or carrot powder instead, and they reported that the method of taking the capsules was both acceptable and feasible. Flavour capsules have been previously used to test the flavour transfer from the mother's mouth to the fetal environment (Mennella et al., 1995). As a result, it was decided to use the kale and carrot capsules in both the pilot and main studies.

The capsules contained ~ 400 mg of pure, organic carrot or kale powder. The shell of the capsules consists of Hydroxypropyl Methylcellulose (HPMC), which is suitable for vegans and vegetarians (Marzorati et al., 2015). The amount of vegetable capsules was chosen considering adult vegetable serving sizes as well as previous research (Mennella et al., 2001). One capsule contains approximately the amount of kale or carrot powder that would be equivalent to one serving size for adults. It is equivalent to around one medium carrot or 100 g of chopped kale.

Sensory analysis. Adult sensory analysis was performed prior to the use of the capsules in the pilot scans to ensure that the capsules were odourless and tasteless. The smell of the vegetable capsules was rated by four healthy pregnant women (aged between 25 and 35 years old), blinded to the flavours. None of the mothers identified themselves as supertasters or non-tasters, meaning that they all had average taste perception. During the sensory analysis, each panellist was asked to consume the kale and carrot capsules with a mouthful of water at one-hour intervals to test whether the capsule itself was tasteless and odourless. All panel members stated that both capsules were tasteless and odourless.

Recruitment and procedure. Following the sensory analysis, the four healthy pregnant women (participants in the sensory analysis) between the ages of 18 and 40 who were non-smokers, with pre-pregnancy body mass index of 18.5 to 30 and no diagnosed medical or mental health condition, were recruited to determine:

- the optimal waiting time following the mother's flavour ingestion,
- the optimal duration of the 4D ultrasound scans following the waiting time,
- the effects of kale and carrot capsule exposure on fetal facial movements.

Each participant received one ultrasound scan at 32 weeks' gestation at around 3 p.m. on the day of the scan. Mothers were asked not to eat any foods containing kale or carrot on the day of the scans and were asked not to eat or drink any foods or beverages one hour prior to the scans. Two participants received a single dose of kale capsule and the other two received a single dose of carrot capsule. All mothers gave written consent and received a copy of their scans.

The capsule shell dissolves in the small intestine around 10-15 mins after ingestion (Marzorati et al., 2015). Based on this evidence, the waiting period between the ingestion of the kale or carrot capsule (either 10 mins or 15 mins) and the time of the start of 4D ultrasonography was randomised between the conditions and between the participants. The duration of the 4D scan (either around 30 mins or around 35 mins) depended on the waiting period after ingestion following the safety guidelines (Society of Radiographers & British Medical Ultrasound Society, 2021). The total time from capsule ingestion to the end of the scan was approximately 45 mins for each participant (see Table 2.1).

Table 2.1. Details about the pilot scans

Participant ID	Waiting time after ingestion (mm:ss:ss)	Scan duration (mm:ss:ss)	Total window after ingestion (mm:ss:ss)	First movement after ingestion (mm:ss:ss)	Last movement after ingestion (mm:ss:ss)
KALE 1	15:00:00	30:29:43	45:29:43	24:54:00	41:55:00
KALE 2	10:00:00	35:07:34	45:07:34	26:46:00	41:50:00
CARROT 1	10:00:00	34:40:43	44:40:43	23:39:00	41:31:00
CARROT 2	15:00:00	30:12:27	45:12:27	25:15:00	43:16:00

Behavioural coding scheme. Neonatal studies suggested that facial reactions indicate hedonic reactions linked to flavour ingestion, and therefore would be more sensitive to show plasticity in comparison to the movements indicating seeking or detection of stimuli such as head orientation (Marlier et al., 1998a; Schaal et al., 2000; Wagner et al., 2013; 2019). Therefore, a coding scheme based on muscular activation in the face would be suitable for analysing responses to flavours in amniotic fluid.

The first anatomically based coding scheme for human facial movements was published in 1924 (Landis). Following this, the Facial Action Coding System (FACS) was created by Ekman & Friesen (1978) for analysing adult human faces. After around 30 years, Oster (2006) published the Facial Action Coding System for Infants and Young Children (BABYFACS) for analysing the facial reactions of neonates and infants up to 3 years old. Finally, based on the descriptive style of FACS, Reissland et al. (2011) published the Fetal Observable Movement System (FOMS) to examine fetal facial movements from 23 to 37 weeks of gestation using 4D ultrasound visualization. The FOMS was developed over time and the latest coding scheme was published in 2016 (Reissland et al.).

Although several researchers use 4D scans to analyse fetal facial movements, the definition of standardised movements is limited. Most studies report on the overall perception of fetal facial reactions without identifying distinct movements induced by muscular activity (Hata et al., 2012, 2013; Kurjak et al., 2003, 2007; Sato et al., 2014). For example, subjective terminology such as “smile” has been used to describe fetal facial reactions without giving a detailed description of the movements involved in the reaction. Although a "smile" is mainly caused by the activation of a zygomatic major muscle, this reaction usually contains two or three different muscles, and this leads to different codes such as lip pulling, lip stretching or lip parting when using the anatomical-based coding system (Cohn & Ekman, 2005; Reissland et al., 2016).

Coding of pilot scans. The pilot (and main study) scans were anonymised by a lab assistant who was unaware of the study hypotheses and the conditions, and therefore it was ensured that the main coder (Beyza Ustun) and the reliability coders were blinded to the conditions. Beyza Ustun is a qualified Fetal Observable Movement System coder (FOMS, Reissland et al., 2016) and a certified Facial Action Coding System scorer (FACS, Ekman & Friesen, 1978, see appendix 3).

Following FACS coding scheme (Ekman & Friesen, 1978), this thesis coded several facial movements frame-by-frame which can be occurred in fetal and neonatal faces. In the pilot study, twenty independent facial movements (FMs) were investigated before deciding on the final coding scheme (see Table 2.2). Of these twenty FMs, the ones which were detected while coding the pilot 4D ultrasound scans were moved to the final coding scheme that was used in the main study. FMs that were reported in reaction to a certain flavour in earlier studies but were not observed in the pilot analysis were added to the (initial) main study coding scheme for further investigation before deciding on the final coding scheme.

Table 2.2. Facial Movements (FMs) investigated in the pilot study

Facial movements (FMs)	FMs reported in previous research on postnatal flavour-elicited reactions	FMs detected in pilot scans
FM1: inner-brow raiser	√ (Rosenstein &Oster, 1998)	√
FM2: outer-brow raiser	√ (Rosenstein & Oster, 1998)	√
FM4: brow lowerer	√ (Mennella et al., 2001; Rosenstein & Oster, 1998; Schaal et al., 2000; Steiner et al., 2001)	√
FM6: cheek raiser	√ (Rosenstein & Oster, 1998; Steiner et al., 2001)	√
FM9: nose wrinkle	√ (Ganchrow et al., 1983; Mennella et al., 2001; Rosenstein & Oster, 1998; Schaal et al., 2000; Steiner et al., 2001)	√

FM10: upper-lip raiser	√ (Mennella et al., 2001; Rosenstein & Oster, 1998; Schaal et al., 2000; Steiner et al., 2001)	√
FM11: nasolabial furrow		√
FM12: lip-corner puller	√ (Ganchrow et al., 1983; Steiner et al., 2001)	√
FM14: dimpler[a]		
FM15: lip-corner depressor[b]	√ (Ganchrow et al., 1983; Steiner et al., 2001)	
FM16: lower-lip depressor	√ (Ganchrow et al., 1983)	√
FM17: chin raiser[a]		
FM18: lip pucker	√ (Ganchrow et al., 1983; Rosenstein & Oster, 1998; Steiner et al., 2001)	√
FM19: tongue show	√ (Ganchrow et al., 1983; Schaal et al., 2000; Steiner et al., 2001)	√
FM20: lip stretch	√ (Schaal et al., 2000)	√
FM24: lip presser		√
FM25: lips parting	√ (Ganchrow et al., 1983; Mennella et al., 2001; Rosenstein & Oster, 1998; Schaal et al., 2000; Steiner et al., 2001)	√

FM26: jaw drop	√ (Ganchrow et al., 1983; Mennella et al., 2001; Rosenstein & Oster, 1998; Schaal et al., 2000; Steiner et al., 2001)	√
FM27: mouth stretch	√ (Ganchrow et al., 1983; Mennella et al., 2001; Rosenstein & Oster, 1998; Schaal et al., 2000; Steiner et al., 2001)	√
FM28: lip suck	√ (Ganchrow et al., 1983; Rosenstein &Oster, 1998)	√

[a]Excluded from the final coding scheme in the main study. [b]Excluded from the final coding scheme after preliminary analysis of the scans in the main study

To test the required waiting period after maternal capsule ingestion and the duration of the 4D ultrasound scans following the waiting period, the mean time elapsed from maternal ingestion of kale or carrot capsule to the appearance of the first and the last fetal FMs were tested. To test the effects of kale and carrot flavour exposure on fetal FMs, the relative frequency of each FM per minute was calculated. Reliability was checked on 100% of the scans by two other blinded and trained FOMS coders, with an overall excellent agreement (Cohen's Kappa = .95, min = .89, max = .98).

Results of the pilot study

The pilot study indicated that fetuses begin to respond to flavours around 25 mins after maternal capsule ingestion. When the participant ingested a single dose of a kale capsule 15 mins before the scan, the first movement was detected around 10 mins after the scans began. However, when the participant waited 10 mins after the kale capsule ingestion, the fetus responded to the kale flavour around 15 mins after the 4D scan started. Flavour-elicited

movements could not be observed approximately 40 mins after the ingestion. Fig. 2.1 visualises the timing of fetal FMs for each participant during the scan.

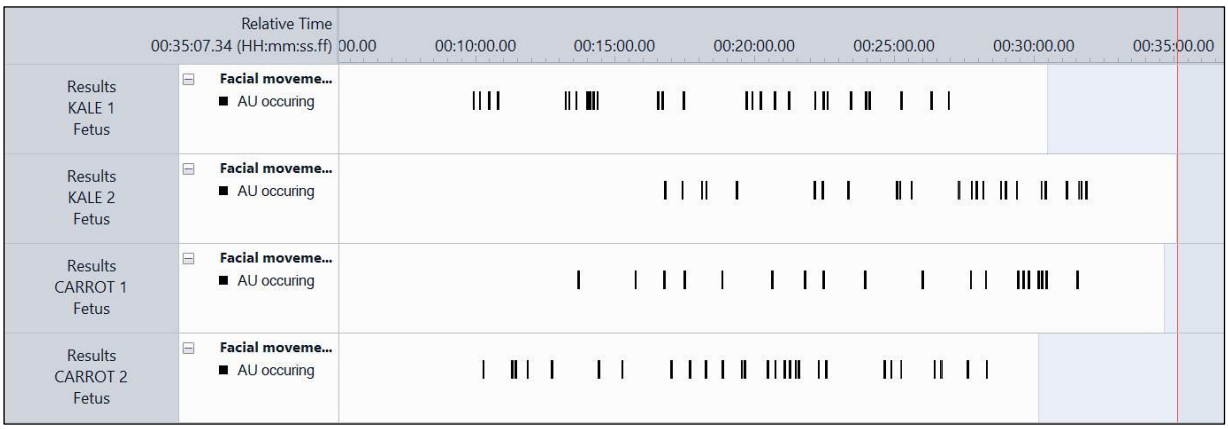


Fig. 2.1. Visualisation of fetal movements throughout the scan.

Based on the pilot scans and previous research studying flavour-elicited postnatal reactions (e.g., Schaal et al., 2000), 18 independent FMs were selected for inclusion in the preliminary analysis of the main study. All movements apart from dimpler (FM14), lip-corner depressor (FM15), and chin raiser (FM17) were detected in the pilot scan analysis. None of the previous research so far reported dimpler (FM14) and chin raiser (FM17) as a flavour-elicited movement. This result was in line with previous findings reporting that dimpler (FM14) was infrequently shown by fetuses (mean frequency rate when it was shown = .03 per 10 mins, Reissland et al., 2016). Therefore, dimpler (FM14) and chin raiser (FM17) were excluded in the final coding scheme for the main study. However, lip-corner depressor (FM15) was investigated further in the preliminary analysis of the main study to decide the final coding scheme because it was identified as an aversion reaction in the previous studies (Ganchrow et al., 1983; Steiner et al., 2001). Although nasolabial furrow (FM11) and lip presser (FM24) were not reported in previous research as a reaction to flavours, these

movements were included in the final coding scheme of the main study because they had been observed in the pilot scans (see Fig. 2.2).

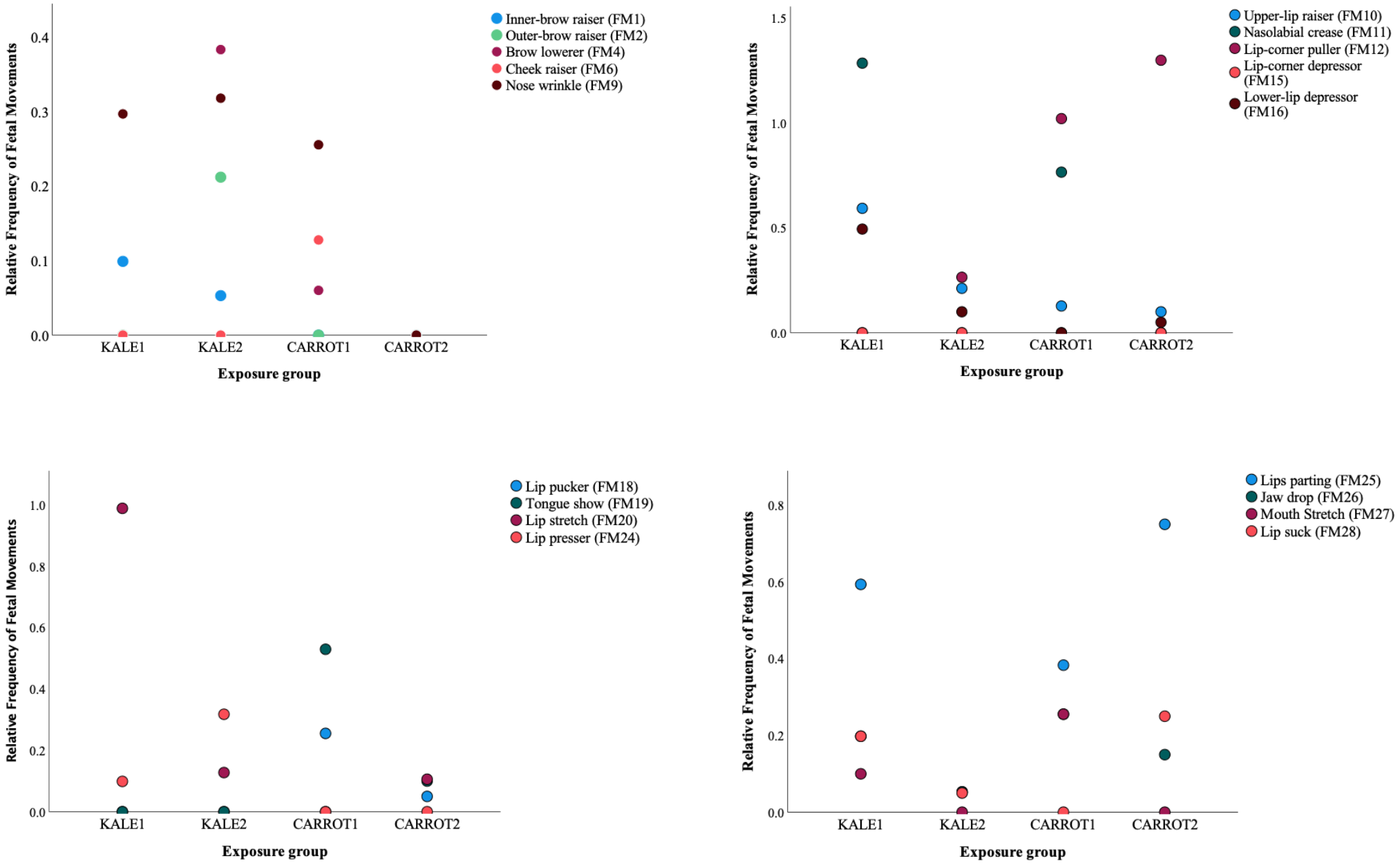


Fig. 2.2. Individual movements detected in the pilot scans.

In sum, a waiting period of 20-25 mins after ingestion of the capsule and a scan duration of 20-25 mins with a total time of 45-50 mins from ingestion to the end of the experiment, yielded key group differences in the pilot study of fetal behaviour, and therefore this timing was applied into the main study. 17 independent FMs (FM1, 2, 4, 6, 9, 10, 11, 12, 16, 18, 19, 20, 24, 25, 26, 27, 28) were included in the final coding scheme to be analysed in the main study.

The pilot scans were included in the analysis of the main study, but to be consistent in terms of waiting period after capsule ingestion, the last 25 mins of ultrasound scans were analysed. The four participants from the pilot study continued the study in the same flavour group they were initially assigned to.

Main Study

Recruitment

Recruitment commenced in June 2019 and concluded in April 2021. The last study scan was collected in August 2021 and the last postnatal testing was conducted in October 2021. Participants were recruited from the Facebook page of the Fetal and Neonatal Research Lab, Durham University, UK (https://www.facebook.com/FetalInfantResearchDurham), the private ultrasound clinics in the northeast of England, local parent and toddler groups in County Durham, and local internet support groups for a healthy pregnancy.

Mothers who met the following eligibility criteria were invited to participate in the study:

- 18 - 40 years of age
- Pre-pregnancy BMI[2] between 18.5 and 30 via self-report

[2] BMI: Body mass index.

- Non-smoker during pregnancy (including not using e-cigarettes and Nicotine Replacement Therapy)
- Not consuming alcohol during pregnancy
- Not using prescription medication or recreational drugs
- No known allergies, including to kale and carrot vegetables
- Not diagnosed with a mental or medical health condition
- Not diagnosed with gestational diabetes
- Not having Hyperemesis Gravidarum (HG)
- No complications of pregnancy
- Singleton pregnancy
- Ability to swallow a vitamin pill-sized capsule
- A routine 20-week anomaly scan confirming a healthy pregnancy

Design

The project is a longitudinal mixed design. In the prenatal stage, three groups of fetuses, kale exposure, carrot exposure and a control group with no flavour exposure were tested at 32 weeks' gestation and at 36 weeks' gestation. After recruitment and informed consent, participants were randomly allocated to either the kale or the carrot group using an online randomization programme (http://www.randomiser.org). Due to the limited research budget, control group participants with the same eligibility criteria were included in the study from archived data in the Fetal and Neonatal Research lab, Durham University. This allowed us to increase the number of participants in the experimental groups (kale and carrot). The same randomization tool was used to select control group participants based on 75 eligible participants.

After 36 weeks' gestation, kale and carrot group fetuses were exposed to either kale or carrot flavour repeatedly (at least four capsules per week for three consecutive weeks)

depending on their initial group assignment. In the postnatal stage, kale and carrot group fetuses were followed in the first postnatal month to test the effects of repeated prenatal flavour exposure on neonatal facial reactions (see Fig. 2.3). Details about the procedure in the prenatal and postnatal stages were provided in the upcoming sections of this chapter.

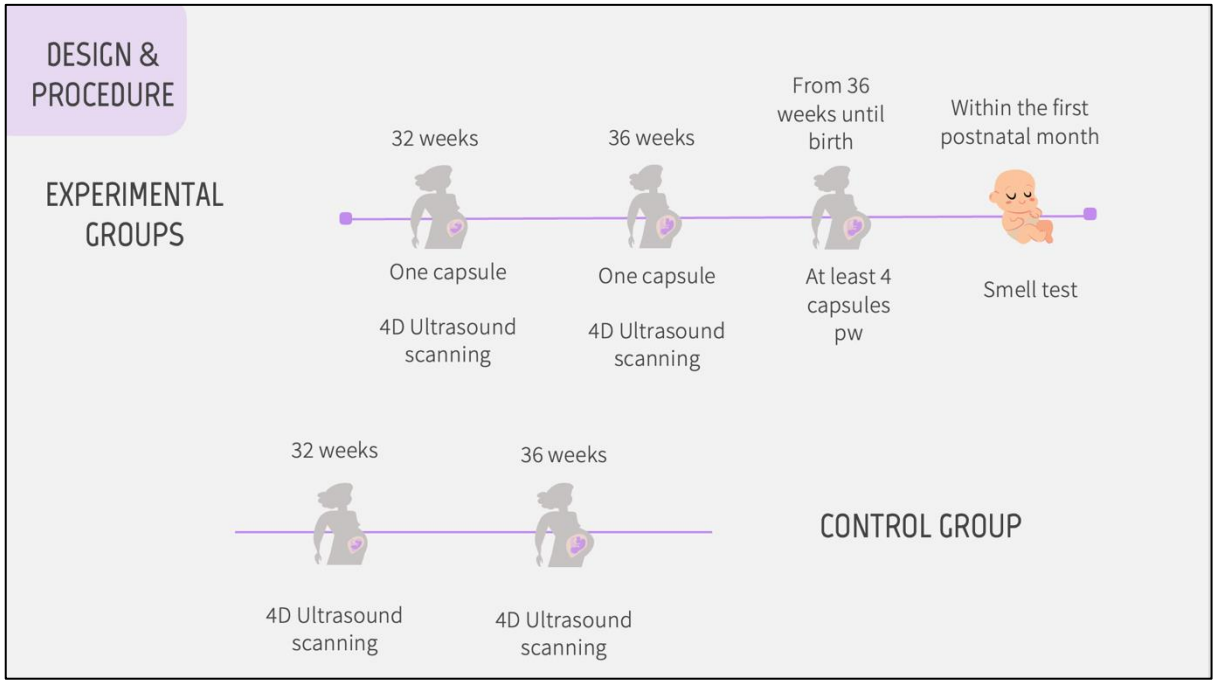


Fig. 2.3. Illustration of research design and procedure.

Study power

Study power (G*Power 3.1) was calculated to obtain 85% power (f = .25). In the prenatal stage, the power analysis revealed that - repeated measures of MANOVA (three groups at two-time points) - the between factors effect would need n = 93, the within factors effect would need n = 75, and ANOVA the within-between interaction would need n = 48. A total sample size of 100 participants was determined to allow for around a 10% dropout rate. The required sample size was reached in the prenatal stage.

In the postnatal stage, the power analysis revealed that - repeated measures of MANOVA (two groups at three-time points) - the between factors effect would need n = 50,

the within factors effect would need $n = 62$, and the within-between interaction (ANOVA) would need $n = 32$ to obtain 85% power ($f = .25$). Although the postnatal part of the study could not reach the aimed sample size due to Covid restrictions, sensitivity analysis indicated that 32 participants would be powerful to detect (at 85%) effect sizes of Cohen's $f = .32$ in between factors MANOVA, effect sizes of Cohen's $f = .36$ in within factors MANOVA and effect sizes of Cohen's $f = .25$ in within-between interaction ANOVAs. It is also notable that previous studies with a similar or lower sample size have found large to medium effect sizes when comparing infants' responses to prenatal flavour exposure (Hepper et al., 1995; Mennella et al., 2001; Schaal et al., 2000). Table 2.3 indicates the number of participants involved in each stage of the research.

Table 2.3. Number of participants who participated in each stage of the research

Group	Recruited	32 weeks	36 weeks	Postnatal stage
Kale	35	34	26	14
Carrot	35	33	27	18
Control	30	30	30	NA
Total	100	97	85	32

Note. NA: Not applicable.

Materials

Flavour stimuli. As a result of the pilot study, kale and carrot capsules were used instead of juice to minimize the effects of the maternal response to bitter-tasting food on fetal movement as well as facilitate participation, particularly for participants who might be bitter-taste sensitive. Following the pilot study, approximately 400 mg of organic kale or carrot capsule including only kale or carrot powder were used throughout the longitudinal study.

The pilot study proved that pregnant mothers would not take capsules without knowing their contents and the opportunity to check their safety. Hence, details of the contents of the capsules were provided to mothers during informed consent, making it impossible to blindly administer the capsules.

Maternal dietary intake measures. Using an adapted questionnaire on the frequency of vegetable consumption in terms of bitter (i.e., kale, lettuce, leeks, Brassica vegetables) and non-bitter (i.e., carrot, potatoes, peas, beans, sweetcorn) vegetables was recorded at 32, 36 weeks and at the postnatal data collection time. Understanding the naturalistic consumption of kale and carrot and those with similar flavour profiles was important because it would have been unethical to ask mothers not to consume bitter and non-bitter-tasting foods during the study period. During consent and prior to each procedure, mothers were informed that the questionnaire was for research purposes and not a diagnostic screen for eating behaviours or eating disorders.

The Sheffield Pregnancy Food Frequency Questionnaire (Mouratidou et al., 2006) was used as a reference from which eight questions were adapted. The left column in Table 2.4 describes the eight questions that mothers answered whereas the right column describes the reference questions (Mouratidou et al., 2006).

Table 2.4. Adapted vegetable food frequency questionnaire

Questions adapted for this study	The Sheffield Pregnancy FFQ (Mouratidou et al., 2006)
How many times a week nowadays do you eat / drink…?	How many times a week nowadays do you eat / drink…?
Q1. carrots (raw, cooked or boiled)	Q26. carrots, tinned carrots
Q2. carrot juice/smoothie	Q30. fruit juice

Q3. kale (raw, cooked or boiled)	Q23. cabbage, Brussel sprouts, kale and other green leafy vegetables
Q4. kale juice/smoothie	Q30. fruit juice
Q5. chips, roast potatoes, boiled, mashed, jacket potatoes, sweet potatoes?	Q20. mushy peas, fish and chips
	Q15. boiled, mashed, jacked potatoes
	Q14. roast potatoes (cooked in fat)
Q6. peas, beans, sweetcorn	Q20. mushy peas, fish and chips
	Q22. peas, sweetcorn, broad beans
Q7. brassica vegetables	Q23. cabbage, Brussel sprouts, kale and other green leafy vegetables
Q8. other green vegetables (lettuce, leeks etc.)	Q24. other green vegetables (cauliflower, runner beans, leeks)
	Q27. salad (lettuce, tomato, cucumber etc.)

Note. Q: Question. FFQ: Food frequency questionnaire.

Following the Sheffield FFQ, 5-point Likert scale (1 = Rarely or never, 2 = Once a fortnight, 3 = 1-3 times, 4 = 4-7 times, 5 = More than once a day) was used to score the items. Higher scores indicated a higher frequency of vegetable consumption. Cronbach's alpha indicated a high level of reliability in prenatal and postnatal datasets (Cronbach's α =.80, .87, respectively). The ratings were summed and resulted in six scores as follows:

Carrot consumption score: Q1+Q2 (minimum: 1, maximum: 10)
Kale consumption score: Q3+Q4 (minimum: 1, maximum: 10)
Non-bitter vegetable consumption score: Q5+Q6 (minimum: 1, maximum: 10)
Bitter vegetable consumption score: Q7+Q8 (minimum: 1, maximum: 10)
Overall non-bitter consumption score: Q1+Q2+Q5+Q6 (minimum: 1, maximum: 20)
Overall bitter consumption score: Q3+Q4+Q7+Q8 (minimum: 1, maximum: 20)

Maternal Psychological Measures. Given that maternal stress, anxiety, and depression affect fetal and infant behaviour (Austin et al., 2005; Federenko & Wadhwa, 2004; Reissland et al., 2018; Wu et al., 2022), mothers were assessed prior to each scan and at postnatal data collection point using widely used and well-validated self-report measures to control for the potential effects of maternal mental health on fetal and infant behaviours. All women were asked to complete the Hospital Anxiety and Depression Scale (HADS, Zigmond & Snait, 1983) to describe their feelings over the past week. The HADS has two subscales, HADS-A (Anxiety / 7 questions) and HADS-D (Depression/ 7 questions), and a total score range of 0 (minimum) to 21 (maximum). Anxiety statements are such "*I feel tense or wound up*" or "*I get sudden feelings of panic*". Depression statements are such "*I still enjoy the things I used to*" or "*I can enjoy a good book or radio or TV program*". Mothers also completed the Perceived Stress Scale (PSS, Cohen et al., 1983) to evaluate stress levels during the month prior to each ultrasound scan. The PSS has 10 items, and the answers are based on 5-point Likert (ranging from 0 = 'never' to 4 = 'very often' stressed). The PSS questions are such "*How often have you been upset because of something happened unexpectedly*?" or "*How often have you felt that difficulties were piling up so high that you could not overcome them*?". During consent and prior to each procedure, mothers were informed that these questionnaires were for research purposes and not a diagnostic screening of mental health (see appendix 5 and 6 for the HADS and PSS questionnaires, respectively).

Fetal measurements before and during each 4D scan. To ensure that fetuses were healthy and within the expected developmental range, fetal measurements were collected before and during the ultrasound scanning. Prior to recruitment, using the National Health Services (NHS) Personal Child Health Record ('the little red book'), it was confirmed that no abnormalities were detected at the 20-week scan and all mothers had normal volumes of amniotic fluid. Research on investigating fetal movements has usually assessed head-

circumference at 20 weeks and fetus sex to control for any potential effects on the movements (e.g., Reissland et al., 2020a, 2021). Therefore, these measurements were recorded and incorporated as covariates in the statistical analysis where appropriate. At the beginning of each scan, sonographers checked heartbeat, femur length and head circumference to confirm that the fetuses were within the expected developmental range. All fetuses involved in the project were confirmed to be healthy and in the expected developmental range at both 32 and 36 weeks. Gestational age at each scan was recorded to ensure that there was no significant difference between the groups.

Table 2.5. Fetus information

	Kale Flavour Group	Carrot Flavour Group	Control Group
	n = 34	*n* = 35	*n* = 30
Fetal sex	Female = 17 Male = 17	Female = 17 Male = 18	Female = 15 Male = 15
Fetal head circumference at 20 weeks (mean)	168.07 (1.424)	164.35 (1.023)	171.23 (1.493)
Fetal exact age (mean) at 32 weeks 36 weeks	32.09 (.070) 35.97 (.089)	32.12 (.096) 36.04 (.098)	32.09 (.100) 35.83 (.095)

Note: Values in parentheses are standard errors.

Checklist. From 36 weeks, mothers were asked to consume at least four daily kale or carrot capsules per week for three consecutive weeks (min = 12, max = 21 capsules) based on their group allocation and to record the date and time they consume the capsule on a standard checklist which was provided by the researcher (see appendix 7 for the checklist). The mothers emailed the checklist to the researcher every week. The total number of capsules

consumed in the last month of pregnancy was calculated to be used in the statistical analysis of the postnatal study as a covariate to control for the potential effects of the amount of flavour exposure on infant movements.

Demographic information and birth outcomes. Ethnicity, maternal level of education, maternal age, and maternal pre-pregnancy BMI was recorded. In terms of birth outcomes, gestational age at birth, infant sex, birthweight, Apgar scores and delivery type were also recorded from the "little red book". The information on the feeding method was collected to determine whether the new-born was breastfed and/or bottle-fed until the postnatal odour test was performed. The variables that were significantly associated with fetal or infant behaviour were included as covariates in the statistical analysis where appropriate.

Table 2.6. Demographics and birth outcomes

Demographics in the prenatal dataset			
	Kale	Carrot	Control
Sample size	*n* = 34	*n* = 35	*n* = 30
Maternal age	29.88 (.774)	31.26 (.792)	28.4 (1.033)
BMI	25.86 (.571)	26.15 (.604)	25.55 (.307)
Level of Education	GCSE* (2) College / A level (13) Degree (14) Postgraduate (5)	College / A level (22) Degree (11) Postgraduate (2)	GCSE (3) College / A level (10) Degree (10) Postgraduate (7)
Birth outcomes in the prenatal dataset			
Birth weight (grams)	3346.26 (40.296)	3255.42 (61.727)	3428.12 (99.775)
Gestational age at birth (weeks)	39.3 (.170)	39.39 (.185)	39.83 (.182)

Demographics in the postnatal dataset

	Kale	Carrot
Sample size	*n* = 14	*n* = 18
Maternal age	30.5 (1.287)	32.33 (.946)
BMI	25.75 (.865)	25.99 (.976)
Level of education	GCSE*:1 College / A-level: 3 Degree: 7 Postgraduate degree: 3	GCSE: 0 College / A-level: 8 Degree: 9 Postgraduate degree: 1
Infant sex	Female:5 Male: 9	Female:11 Male: 7

Birth outcomes in the postnatal dataset

Birth weight (grams)	3246.86 (61.27)	3406.22 (111.29)
Gestational age at birth (weeks)	39.14 (.398)	39.37 (.346)
Delivery type	Vaginal birth: 12 Caesarean section: 2	Vaginal birth: 13 Caesarean section: 5
Feeding type	Breastfeeding: 5 Bottle-feeding: 5 Both type of feeding: 4	Breastfeeding: 9 Bottle-feeding: 8 Both type of feeding: 1
Infant age at test (min:2 weeks max:4 weeks)	3.05 (.230)	3.07 (.203)

*General Certificate of Secondary Education.

Odour stimuli. At postnatal testing, kale, carrot, and control odour were presented to the neonates to measure their odour-elicited reactions after repeated exposure in the last three weeks of pregnancy. Kale and carrot powder inside of the vegetable capsules was used to prepare the kale and the carrot odour for neonatal testing to have an equivalent stimulus in

both the prenatal and postnatal stages. To prepare the odour stimuli, first, a cotton swab was moistened with water. Then the moistened cotton swab was dipped into kale or carrot powder. A cotton swab moistened with water was used as a control (neutral) stimulus (see appendix 8 for the information leaflet to prepare the kale and carrot odour).

Procedure

Participants were involved in a number of stages including a single dose flavour exposure at 32 and 36 weeks; repeated flavour exposure from 36 weeks until birth; and neonatal assessment in the first month after birth.

1. ***Single dose flavour exposure at 32 and 36 weeks.*** Fetuses were exposed to one of the following, at 32- and 36-weeks' gestation (same flavour each time).
    - KALE group (a single dose of a kale powder capsule, n = 34)
    - CARROT group (a single dose of a carrot powder capsule, n = 35)
    - CONTROL group (from archived data, no flavour exposure before or during the scan, n = 30)

Based on the results of pilot scans, mothers (n = 99) in the main study ingested the vegetable capsules with a mouthful of water around 20 minutes before 4D scanning (M = 23.56 min, SE = .59). Water, at room temperature, was slowly consumed to limit any thermal effects on fetal movement. The volume of ingested liquids was similar for each participant. After swallowing the capsule, the mothers filled out questionnaires on vegetable consumption, anxiety, and perceived stress. Following the completion of the waiting time, mothers underwent 4D ultrasound scan. The exact time from the consumption of the capsules to the start of the 4D ultrasound scan was recorded by the researcher.

The scans took place at private ultrasound clinics in Chester-Le-Street, Darlington, or Middlesbrough, depending on the participant's preference. All scans were collected at approximately the same time of the day (3 p.m.). During the scans, mothers lay on their back

or side in a darkened room, as in their 12 and 20-week scans, and they were able to see their unborn baby on the screen. The scan room was dimly lighted and quiet.

Three qualified and experienced sonographers conducted the ultrasound scans. All sonographers were blinded to the project hypotheses and the condition of the participant. Ultrasound scans were recorded (at 24 fps) for offline analysis with a GE Voluson E8/E10 Expert Ultrasound System with a RAB6-RS transducer (General Electric, Boston, MA). Before beginning the ultrasound, sonographers took measures of the fetuses' heart rates, femur lengths, and head circumferences to make sure they were healthy and within the normal developmental range. Following this, the fetal face and upper torso were visualized by 4D colour full frontal or facial profile ultrasound recordings. If the fetus is facing away from the probe (or when the mother was uncomfortable in a position), the sonographer paused the 4D imaging until a clear view could be established. Conventional 2-dimensional (2D) images were also collected to use as a reference for fetal movements captured in 4D scans. In this thesis, 2D and 4D ultrasound images were recorded side-by-side for all scans. An example of using 2D as a reference to describe mouth movements is shown in Fig. 2.4.

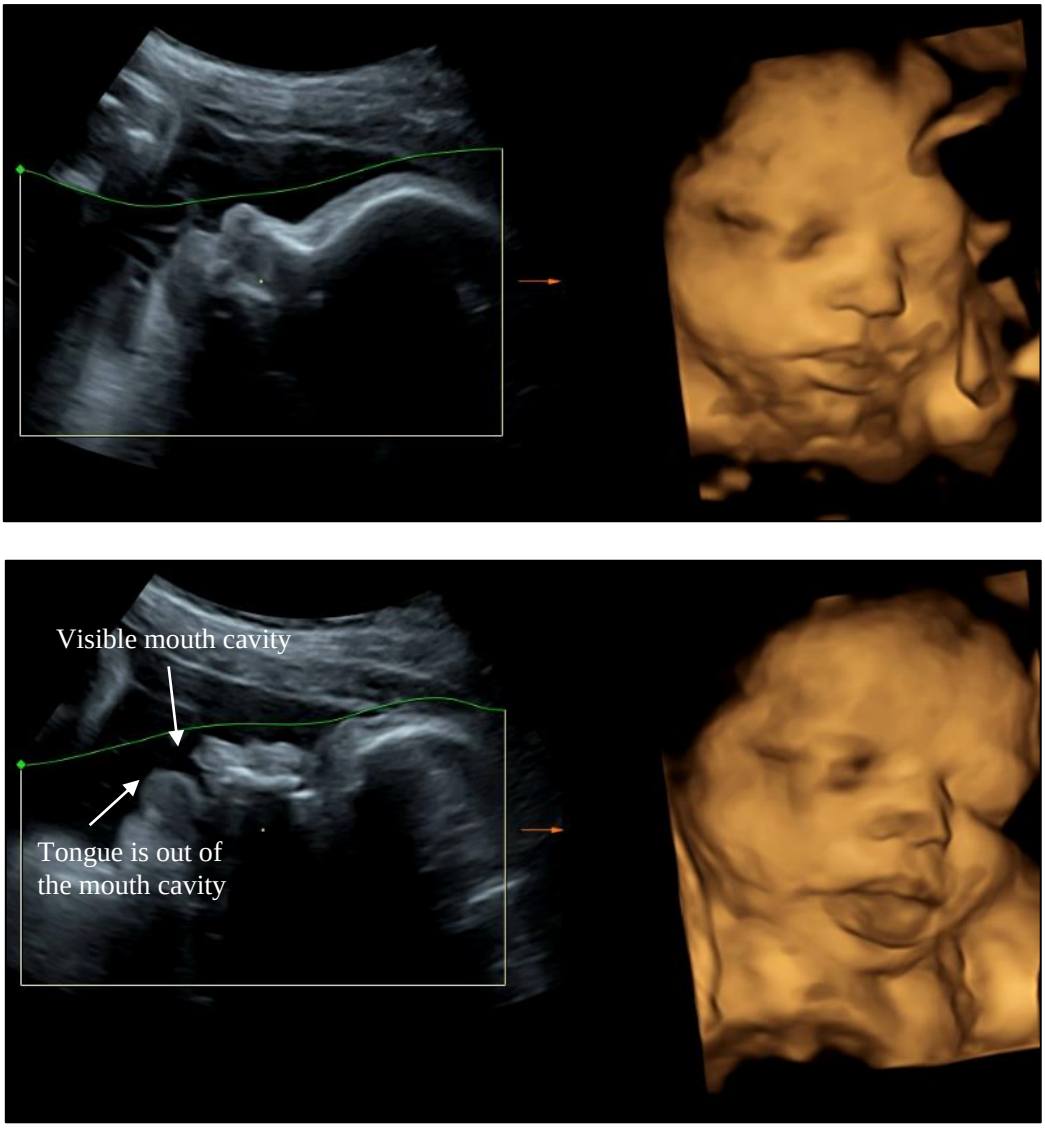


Fig. 2.4. Example of recording 2D and 4D side-by-side.

Note. The top panel demonstrates a neutral face. The bottom panel demonstrates the usage of 2D as a reference when the same fetus displayed tongue show (FM19) and lips parting (FM25).

2. ***Repeated flavour exposure from 36 weeks until birth.*** In the vegetable exposure groups (kale: n = 14, carrot: n = 18), mothers consumed one capsule (of the flavour group to which they were originally allocated) at least four days/week for three consecutive weeks, with daily documentation of capsule intake during this period via the checklist provided by the researcher.

3. ***Neonatal assessment within the first postnatal month.*** After the birth of the baby, a neonatal assessment in the participant's home settings was conducted, recording neonatal facial responses to kale, carrot, and control odours within the first month (*M* = 3.06 weeks, *SE* = 0.15, min = 2 weeks, max = 4 weeks; kale: *n* = 14, carrot: *n* = 18). Mothers were instructed to feed their babies around 30 mins before the session. Breastfeeding mothers were told to avoid consuming foods containing carrot or kale before feeding. None of the neonates had been introduced to solid food before the assessment and all sessions were completed in the afternoon around 3 p.m. Mothers answered questionnaires on birth outcomes, vegetable consumption and mental health while the researcher was preparing the odour stimuli. Once the three stimuli were prepared, the neonate was placed in a baby bouncer facing towards the camera. The assessment in this stage followed a similar procedure to the first test conducted in a study to test neonatal hedonic reactions to anise odour which was experienced prenatally (Schaal et al., 2000). Two sets of kale, carrot and control odour stimuli were presented in a random order and neonatal facial reactions to each stimulus were recorded for offline coding (see Table 2.7 for an example of stimuli presentation). The total duration of the experiment was around eight mins with a total of two minutes of odour presentation and a total of six mins of intervals.

Table 2.7. Example of odour presentation

Baseline	20 seconds
The first set of odour presentations	
Kale 1	20 seconds
Interval	60 seconds
Carrot 1	20 seconds

Interval	60 seconds
Control 1	20 seconds
Interval	60 seconds
The second set of odour presentations	
Kale 2	20 seconds
Interval	60 seconds
Carrot 2	20 seconds
Interval	60 seconds
Control 3	20 seconds
Interval (post stimuli)[a]	10 seconds
End of the test	

[a] Neonatal facial responses were recorded for 10 more seconds after the last stimuli presentation.

Due to Covid restrictions, some of the postnatal sessions (*n* =10) had to be switched to online platforms such as Zoom or Microsoft Teams without compromising the quality of the data. In this case, the parents conducted the session in their home environment, with full guidance and support from the researcher before and during the session. Around two days before the session, participants received a package including instructions on how to conduct the assessment. The package included an instructional video and two information sheets (see appendix 8 for the information sheets). The instructional video was prepared to show how to conduct the experiment. One of the information sheets featured instructions on how to prepare the materials, the test environment, and the infant for the session, as well as what to do before and during the experiment. The second one

contained details about the two sets of odour presentations, including the stimulus sequence, duration, and interval periods.

Both in-person and online sessions followed the same experimental procedure, and the method of testing did not statistically affect the study outcomes. On the day of the experiment, the researcher first had a short chat with the parents to answer any questions. Once the baby and parents were ready, the assessment was conducted under the guidance of the researcher, following the stimuli order. All sessions were recorded using the online platform for offline behavioural coding. Most of the videos obtained using online platforms were of high quality (see Fig. 2.5). There were only two low-quality sessions, which were excluded from the analysis.

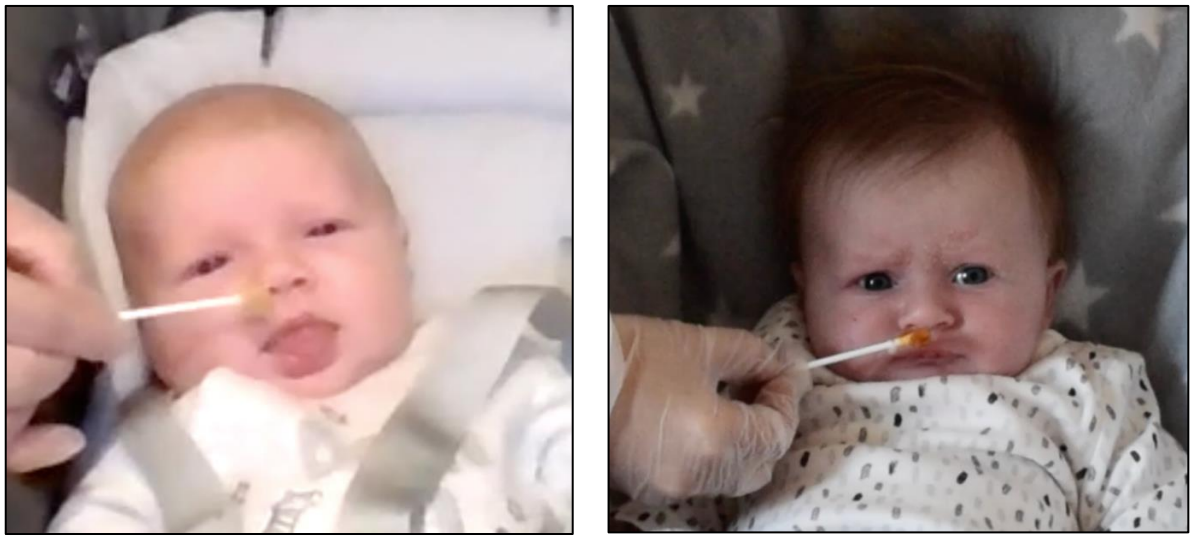


Fig. 2.5. Example comparing video quality between online platform records (left) and camera records (right).

Method of coding fetal and neonatal facial movements

Following FACS, coding was performed by systematically assessing various components of the fetal and neonatal face based on the muscular activity using the final coding scheme including the 17 independent movements.

In this thesis, these 17 independent FMs were assigned to either cry-face or laughter-face gestalt following the Fetal Observable Movement System and the Baby Facial Action Coding System. Five movements (FM1, 4, 10, 16, 20) were coded for the cry-face gestalt and two movements (FM12, 19) were coded for the laughter-face gestalt. Six FMs (FM6, 9, 11, 25, 26, 27) were coded for both gestalts as these can be seen in both. Four facial movements (FM2, 18, 24, 28) were assigned to a particular gestalt neither in FOMS nor BABYFACS, and therefore they were exploratory acknowledging that they can occur in both facial gestalts. When an FM (e.g., lips parting-FM25) was assigned to both gestalts, the definition of facial gestalt depends on reactivity in the other part of the face. For example, when lips parting occurs with upper-lip raiser (FM10), the interpretation of this facial gestalt was cry-face gestalt because upper-lip raiser is classified as cry-face. On the other hand, if a lips parting (FM25) occurs together with the lip corner puller (FM12), the final face was interpreted as laughter-face gestalt because the lip-corner puller is classified as the laughter-face. Table 2.8 demonstrates the classification of 17 discrete FMs.

Table 2.8. Classification of facial movements (FMs)

FMs and underlying muscles	Laughter-face gestalt	Cry-face gestalt
FM1: inner-brow raiser		√
FM2: outer-brow raiser[a]	√	√
FM4: brow lowerer		√

FM6: cheek raiser	√	√
FM9: nose wrinkle	√	√
FM10: upper-lip raiser		√
FM11: nasolabial furrow	√	√
FM12: lip-corner puller	√	
FM16: lower-lip depressor		√
FM18: lip pucker[a]	√	√
FM19: tongue show	√	
FM20: lip stretch		√
FM24: lip presser[a]	√	√
FM25: lips parting	√	√
FM26: jaw drop	√	√
FM27: mouth stretch	√	√
FM28: lip suck[a]	√	√

[a]Exploratory movements that can be seen in any of the gestalts.

A facial gestalt can involve two, three, four or more movements which can occur simultaneously or within one second of each other (Reissland et al., 2011; 2013). The sum of the movements that contribute to the gestalt shows how complex the gestalt is. For example, a gestalt with five individual movements is considered more complex than a gestalt with only two movements. Research show that the complexity of facial gestalt increases significantly between 24- and 36-weeks gestational age, suggesting that older fetuses can generate more complex gestalt than younger fetuses (Reissland et al., 2011; 2013). In this thesis, the

complexity of cry-face and laughter-face gestalts was examined between 32- and 36-weeks' gestation.

Beyza Ustun, who is the main coder, coded all the ultrasound scans as well as the neonatal assessment videos. Fetal and neonatal facial movements were coded frame-by-frame using the offline video recordings in the Observer® 15XT by Noldus (The Netherlands). Frame-by-frame coding was a substantial time commitment. It took approximately 10 hours to complete the coding of one 20–minute ultrasound scan. In terms of the coding duration in the postnatal period, the researcher coded for the duration of odour stimuli presentation (20 sec) and of post-stimuli presentation (10 sec). The total duration of coding for each participant was three minutes [(30 sec) X 2 sets) X 3 stimulus] and it took around 1.5 hours to code for one postnatal video.

During the 4D ultrasound scan, the fetal face was not always visible enough to code due to fetal positioning (e.g., arms in front of the face) or image quality. Therefore, stop and start codes were used to identify the sections of the scans where the fetal face was of good enough quality to be coded (see Fig. 2.6).

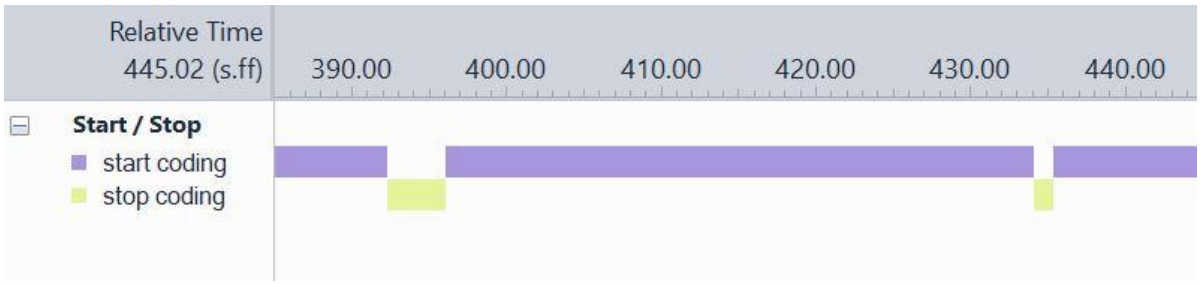
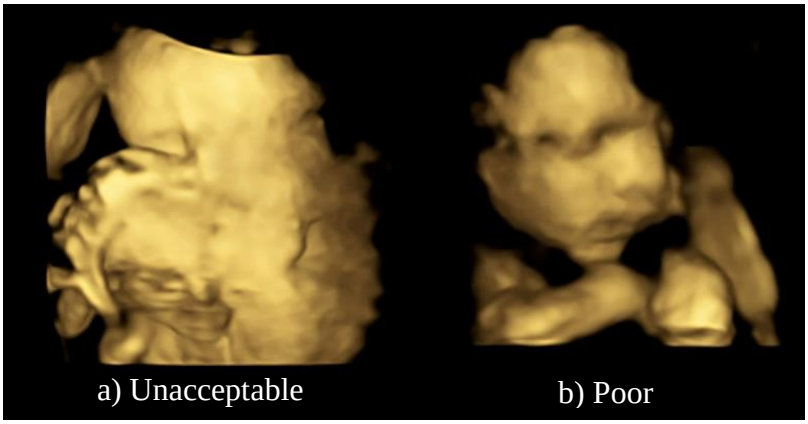


Fig. 2.6. Visual graph of start and stop codes of the ultrasound scans in the Observer.

Regarding the image quality, when the fetal face was not visible or of poor quality (see Fig.2.7, images a-b), the section was marked with a 'stop' code whereas if the face was visible with a clear view (see Fig.2.7, images c-e), the section was marked with 'start' code.



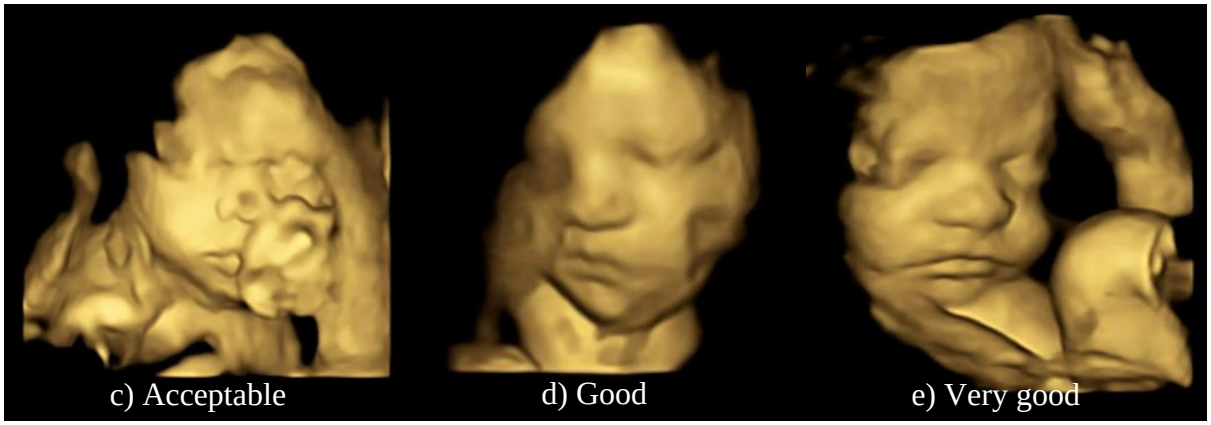


Fig. 2.7. Examples of fetal image quality.

Prior to the onset of the coding, each fetal scan and neonatal video was observed at full speed from beginning to end to establish familiarity with the neutral face and judge the presence of 17 facial movements. The steps to code a fetal face are as follows:

1. ‘Start-stop’ codes were marked to identify the visible sections of the ultrasound scans where the fetal face was of good enough quality to be coded.
2. The codable length was determined based on the ‘start-stop’ codes. The sum of the duration of ‘start’ codes was referred as the codable length.
3. Discrete facial movements were coded frame-by-frame beginning with the lower face and moving to upper face throughout the total codable footage of the video.

4. The relative frequency of discrete facial movements per minute was computed because the length of the codable scans varied across the dataset.
5. The number of discrete facial movements appearing simultaneously or within one second of one another was combined to identify facial gestalts (cry-face or laughter-face).
6. The relative frequency of facial gestalt per minute was calculated.
7. Gestalts were classified by the number of simultaneous FMs they contained - single, double, triple, quadruple or higher - to assess their complexity.

The equation for calculating the relative frequency of a movement or a gestalt per minute is as follows:

$$Relative\ frequency\ of\ facial\ movement = \left(\frac{Total\ number\ of\ facial\ movements\ or\ gestalts}{Codable\ scan\ length}\right) * 60$$

The steps to code a neonatal face were the same as the fetal stage apart from 'stop-start' codes. In the neonatal stage, the total codable length was predetermined as three minutes. Although the duration of odour presentation was the same for all postnatal videos, the relative frequency of individual movements and gestalts per minute was calculated to be able to compare fetal and neonatal responses equivalently.

Examples of facial movements on fetal and infant face. The images below illustrate fetal facial movements and gestalts coded in the prenatal stage. For comparison, the images on the left panel represent the baseline and the images on the right panel represent the movements produced by the same fetus. All images were obtained from the data of this thesis.

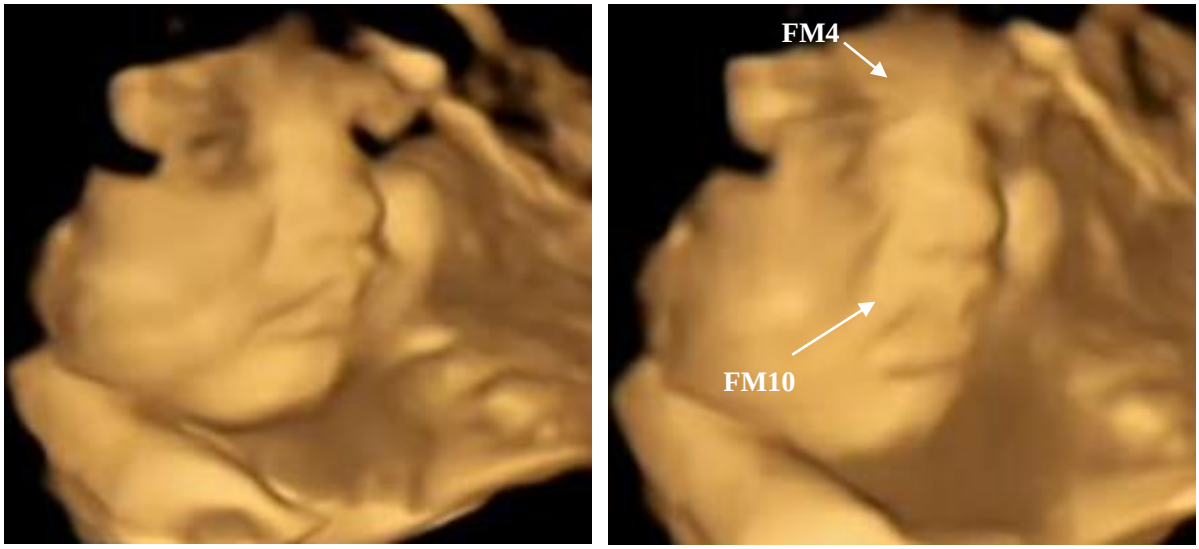


Fig. 2.8. A cry-face gestalt (fetal) involving brow lowerer (FM4) and upper-lip raiser (FM10).

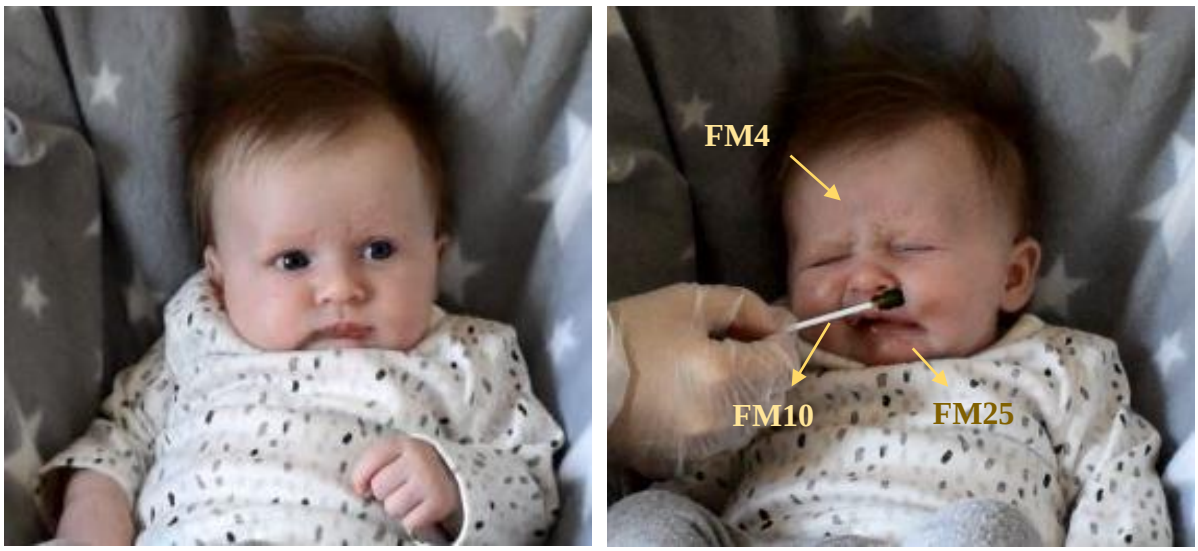


Fig. 2.9. A cry-face gestalt (neonatal) involving brow lowerer (FM4) and upper-lip raiser (FM10), and lips parting (FM25).

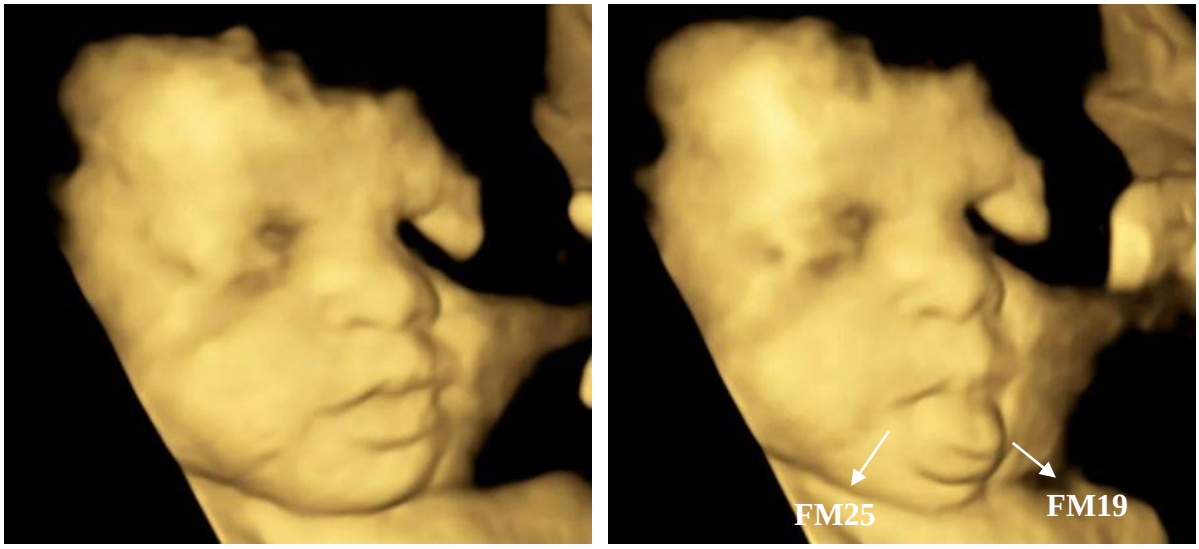


Fig. 2.10. A laughter-face gestalt (fetal) involving tongue show (FM19) and lips parting (FM25).

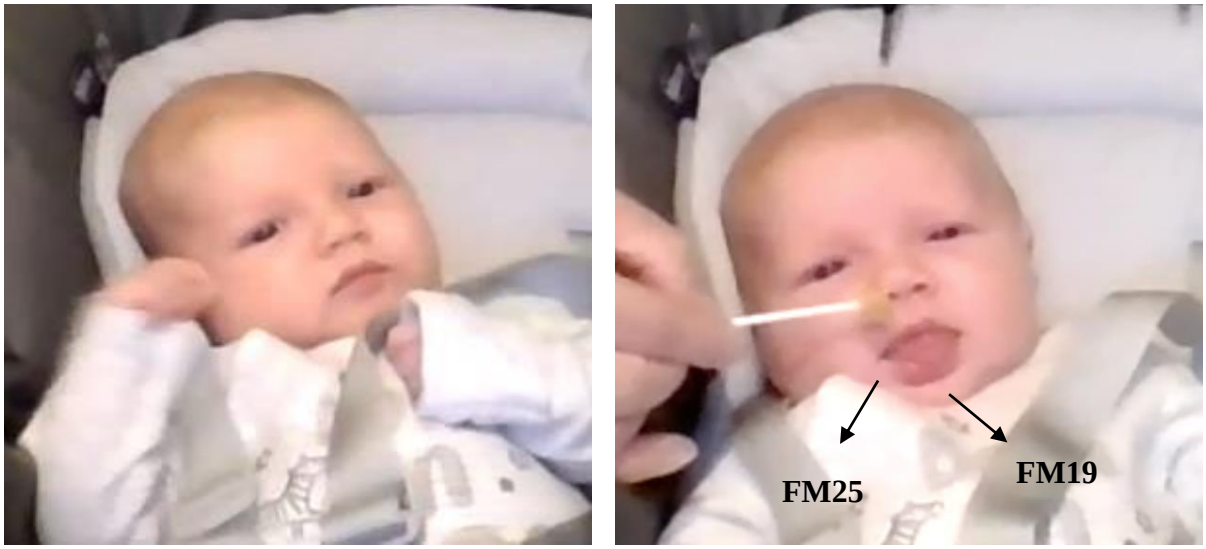


Fig. 2.11. A laughter-face gestalt (neonatal) involving tongue show (FM19) and lips parting (FM25).

Reliability of coding. Intercoder reliability was achieved at the prenatal stage by two independent FOMS-qualified coders on 20% of the total number of scans with an excellent overall agreement (Cohen's kappa 0.90, min: 0.81 - max: 0.98). A certified FACS coder (NR)

and two FOMS-qualified coders performed intercoder reliability on 15% of the total number of fetal / infant assessments at the postnatal stage, and an excellent overall agreement was achieved (Cohen's kappa 0.95, min:0.93 – max:0.96). Intra-reliability was applied to 10% of the prenatal and postnatal datasets to ensure consistency of codes across time, and reliability revealed a Cohen's kappa mean of 0.97 and 0.98, respectively.

Outcome measures

Prenatal stage. Relative frequency of individual facial movements and facial gestalts per minute of a codable scan (Between-group comparison). Developmental complexity in facial gestalt by calculating the number of discrete movements contributing to the gestalt from 32 to 36 weeks (Within-group comparison).

Postnatal stage. Relative frequency of individual facial movements and facial gestalts per minute of a codable postnatal video (Between-group comparison). The developmental trend of the relative frequency of individual facial movements and facial gestalts per minute of codable scan or neonatal video between 32 weeks, 36 weeks, and postnatal measurement (Within-group comparison).

Chapter 3

Flavour Sensing in Utero and Emerging Discriminative Behaviours in the Human Fetus[3]

Abstract

The diet of pregnant women exposes fetuses to a variety of flavours consisting of compound sensations involving smell, taste and chemesthesis. The effects of such prenatal flavour exposure on chemosensory development have so far been only measured postnatally in human infants. This article reports the first direct evidence of human fetal responsiveness to flavours transferred via maternal consumption of a single-dose-capsule by measuring frame-by-frame fetal facial movements. Pregnant women and their fetuses based in northeast England were involved in this study from 32 to 36 weeks' gestation. Fetuses exposed to carrot flavour (n = 35) showed "lip-corner puller" and "laughter-face gestalt" more frequently, whereas fetuses exposed to kale flavour (n = 34) showed more "upper-lip raiser", "lower-lip depressor", "lip stretch", "lip presser", and "cry-face gestalt" in comparison with the carrot group and a control group not exposed to any flavours (n = 30). The complexity of facial gestalts increased from 32 to 36 weeks in the kale condition, but not in the carrot condition. Findings of this study have important implications for understanding the earliest evidence for fetal abilities to sense and discriminate different flavours.

[3] This research is published in Psychological Science.

Statement of Relevance

Through their developing sensory abilities, fetuses are alert to aspects of their environment in the womb. For example, by swallowing and inhaling the amniotic fluid, a fetus can sense the flavours of food eaten by its mother. However, the current literature on human studies has exclusively focused on postnatal outcomes of prenatal flavour exposure. Instead, by analysing their facial reactions, this article presents direct, novel evidence that fetuses can discriminate different flavours in amniotic fluid. It was found that when fetuses were exposed to carrot flavour, they were more likely to show “laughter-face” reactions, and when they were exposed to kale flavour, they were more likely to show “cry-face” reactions. Also, facial responses to flavours became more complex as fetuses matured. This study sheds new light not only on fetal sensory abilities but also on the specificity of facial responses to different flavours relating to their discriminative abilities.

Introduction

Amniotic fluid is the first place where fetuses start to sense their environment, specifically their chemical environment (Brumley & Robinson, 2010). This experience provides continuous sensory information, such as taste and smell, from fetal to neonatal life (Mellor, 2019; Schaal, 2005). The continuity, based on early familiarization, allows new-borns to adapt to the postnatal environment (Mellor, 2019).

Among a huge variety of other compounds linked with maternal-fetal genotype and metabolism as well as the maternal environment, the prenatal environment is permeated with aroma compounds conveyed through the mother's diet (Schaal, 2016). This is the case for both human (Hauser et al., 1985; Mennella et al., 1995; 2001; Schaal et al., 2000) and non-human (Figueroa et al., 2013; Hepper & Wells, 2006; Lévy et al., 2020; Smotherman & Robinson, 1987) fetuses who encode and memorise incoming chemical stimuli. In human fetuses, taste buds develop anatomically at 8 weeks' gestation and can detect tastants from 14 weeks' gestation (Witt & Reutter, 1998). Additionally, fetal nasal orifices are open to allow amniotic fluid to access olfactory sensory neurons, which can sense odour-active molecules from 24 weeks' gestation (Witt, 2020). Hence, although they continue to develop anatomically and functionally after birth, fetal chemosensors are sufficiently mature to detect flavours including tastants and odorants in the amniotic fluid in the last trimester of pregnancy (Forestell & Mennella, 2015; Schaal, 2016). Throughout this article, flavour exposure or experience was referred to, acknowledging that taste, olfaction and trigeminal chemesthesis cannot be dissociated in the womb and that their effects on the fetus may involve one or several of these chemosensory inputs.

To date, the impact of fetal flavour exposure has been investigated using several indirect strategies. First, before ultrasound visualization was possible, fetal chemosensation and swallowing activity of amniotic fluid were inferred from changes in the pregnant

mother's waist size, especially in cases of polyhydramnios, amniotic fluid being in excess because of low fetal swallowing (de Snoo, 1937). Second, preterm infants were studied based on the assumption that their chemosensory reactivity could be extrapolated to same-age fetuses for both olfaction (Schaal et al., 2004) and taste (Maone et al., 1990; Tatzer et al., 1985).

A third strategy consisted of testing term-born neonates in the hours or days following birth with chemostimuli that they were assumed to be exposed to prenatally. For example, Hepper (1995) found that neonates 15 to 28 hours old did not show aversive reaction to garlic odour (relative to a control odour) if their mothers had eaten at least four meals containing garlic per week during the last month of pregnancy. Supporting this finding, Schaal et al. (2000) showed that neonates up to 4 days of age, whose mothers consumed anise-flavoured sweets in the last 2 weeks of pregnancy, compared to a nonexposed group, preferred the anise odorant. Additionally, Mennella et al. (2001) tested the effects of maternal carrot juice consumption on 5- to 6-month-old infants' response to carrot flavour and found that those exposed to carrot flavour during the last trimester of pregnancy displayed fewer negative facial expressions towards carrot flavoured cereal than plain cereal. These human findings, added to those of studies on fetal rats, lambs, piglets, and dogs (Figueroa et al., 2013; Hepper & Wells, 2006; Lévy et al., 2020; Smotherman & Robinson, 1987), support the claim that there is evidence of stable, long-term retention of fetal experiences of flavour that can affect food acceptance postnatally.

A fourth strategy consisted of visualizing the spontaneous, immediate responses of surgically prepared fetuses to flavours in utero. This approach has been used in various nonhuman models (Schaal et al., 1991; Smotherman et al., 1991). However, human fetal studies in this field have yet to be undertaken. The aim of the present study was to fill this

gap in testing human fetal reactions via ultrasound scanning immediately after exposing them to target flavours ingested by the pregnant mother.

A human fetus can display facial movements (FMs) ascertained by the mobilization of individual facial muscles (Reissland et al., 2016). These FMs can be classified with an objective coding scheme, in which each FM refers to a specific collection of muscular movements. For example, FM11 refers to the appearance of a nasolabial furrow that is formed by the action of the zygomaticus minor muscle, and the lower lip being pulled down towards the chin by the depressor labii inferioris muscle generates FM16 (Ekman & Friesen, 1978).

Multiple muscular actions induce complex facial expressions, called “gestalts”. The gestalts are the combination of FMs occurring simultaneously or within 1 s of one another, which may consist of double, triple, quadruple, or more fetal FMs (Reissland et al., 2011, 2013). The more FMs contribute to the gestalt, the more complex it is. With fetal age, the complexity of the gestalt increases as evidenced by the increased number of co-occurring movements (Reissland et al., 2011, 2013). These gestalts resemble facial expressions associated postnatally with positive or negative hedonic responses, visible from the second trimester of pregnancy and increasing with gestation (Reissland et al., 2011, 2013). However, it is not yet possible to ascertain hedonic co-occurrence in fetuses. For instance, the "cry-face gestalt" can be described as a series of coordinated FMs encompassing lower-lip depressor (FM16) and nasolabial furrow (FM11), which would be perceived as a cry face by an adult observer but might not be associated with a “sad” hedonic response (for examples of different facial gestalts, see Figs.3.1 and 3.2).

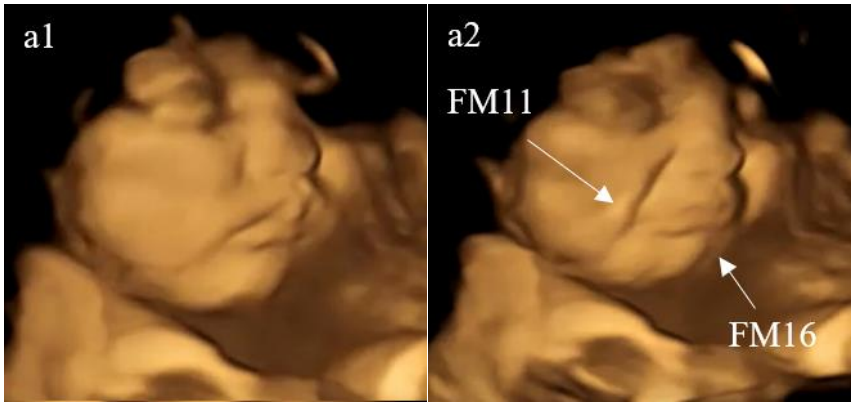
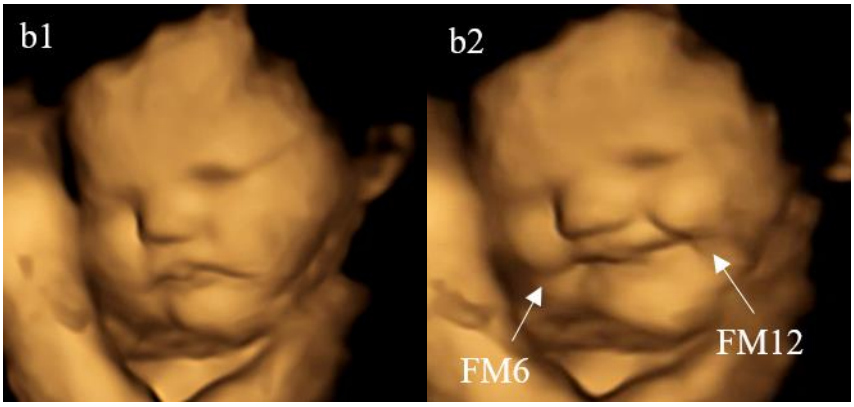


Fig. 3.1. Example of cry-face gestalt of a kale-exposed fetus. (a1) baseline, (a2) cry-face gestalt. FM11: nasolabial furrow; FM16: lower-lip depressor.

Fig. 3.2. Example of laughter-face gestalt of a carrot-exposed fetus. (b1) baseline, (b2) laughter-face gestalt. FM6: cheek raiser; FM12: lip-corner puller



The present study coded 4D ultrasound scans with the validated Fetal Observable Movement System (Reissland et al., 2016) to examine how fetuses from 32 to 36 weeks of gestational age react to different flavours transferred to the fetal environment by maternal ingestion. FMs obtained from scans from three groups of fetuses exposed to either carrot flavour, kale flavour, or no flavour were compared. Although there is no chemical evidence on the stability of compounds of kale or carrot until they reach the amniotic fluid, multiple data exist on the mother-to-fetus transfer of vegetable aromas (for a review, see Spahn et al., 2019). For example, carrot flavour transfer is inferred by a change in infant behaviour measured a posteriori (Mennella et al., 2001). Similarly, neonates whose mothers consumed more green vegetables in pregnancy showed higher liking scores for target odours (Wagner et al., 2019). Regarding the taste of vegetables, the complex carrot flavour can be described by

adult judges as “sweet” because of its sugar content but also sometimes as having “fruity”, “woody”, or even “petrol” flavours, likely a result of terpenoids present in carrot (e.g., β-carotene; Alasavar et al., 2001). Kale was chosen because it conveys more bitterness to infants than other green vegetables such as spinach, broccoli, or asparagus (Johnson et al., 2021).

Because these two flavours do not share the same flavour profile, different FM profiles were expected to be observed in kale-exposed fetuses and carrot-exposed fetuses. New-borns exposed to carrot juice prenatally had less nose wrinkling, brow lowering, upper lip raising, gaping, and head turning than nonexposed new-borns (Mennella et al., 2001). In contrast, new-borns drop the corners of their mouths and raise their upper lips when exposed to a bitter solution (Steiner, 1979).

On the basis of this current evidence, it was hypothesised first that there will be a significant difference in facial reactions as expressed in facial muscle movements and gestalts between the three groups of fetuses. It was predicted that higher rate of “laughter-face” reactions (fetal movements and gestalts) in the carrot group and higher rate of “cry-face” reactions (fetal movements and gestalts) in the kale group in comparison with each other and the control group. Second, because of the developmental progression in chemosensory perception and maturation of facial neuromuscular structures, irrespective of what flavour they were exposed to, it was expected to observe developing complexity in these facial gestalts from 32 to 36 weeks.

Methods

Ethics

This study was conducted in accordance with the Declaration of Helsinki, and ethical permission for the research reported in this article was granted by Durham University (PSYCH-2019-03-12T15_59_32-wvgf27). All mothers gave written informed consent.

Participants

One-hundred mothers between the ages of 18 and 40 years (M = 29.92 years, SD = 5.03) with healthy, singleton fetuses were enrolled in this study. The target sample size of 100 was chosen before recruitment to obtain 85% power (f = .25) for the main comparison, that is, fetal FMs depending on the flavour exposure. Given that a recent study (Reissland et al., 2020a) reported a large and significant effect size in fetal movements at 32 weeks, medium and even large effect sizes are not unusual in this type of research. Data collection was opted to stop in the study when a predetermined number of participants was reached. Although 100 women were recruited, all scans could not be included in the study for a variety of reasons. Some women were unable to attend their 36-weeks scans because of COVID-19 pandemic restrictions (n = 11) or because they had given birth before their 36-weeks scans were to take place (n = 3). In addition to the factors mentioned above, not all scans were of good enough quality to be coded and analysed (see Table 3.1).

Table 3.1. Distribution of mothers over flavour stimuli and number of scans coded

Time point	Kale flavour	Carrot flavour	Control	Total
Recruited	35	35	30	100
At 32 weeks	34	33	30	97
At 36 weeks	26	27	30	83
At 32 and 36 weeks	26	25	30	81

Note: [34+33+30(32-weeks)] + [26+27+30(36-weeks)] = 180 scans from 99 fetuses (49 female, 50 male).

All participants were White British, living in the northeast of England. Experimental group participants (carrot: n = 35, kale: n = 34) were recruited in a private ultrasound clinic in Newcastle, UK. For the control group (n = 30), 4D ultrasound scans of healthy fetuses were

obtained from anonymised archived data in the Fetal and Neonatal Research Lab at Durham University. All mothers had a healthy 20-week anomaly scan including the evaluation of a normal amount of amniotic fluid. They had no known allergies or mental health and/or medical conditions, had a pre-pregnancy body mass index (BMI) of 18.5 to 30 (M = 25.87, SE = .30), and were non-smoking. All control group mothers fulfilled the same inclusion criteria as the experimental groups. Simple randomisation (via online tool at http://www.randomizer.org) was used to assign each participant to one of the experimental groups and to select control group participants from 75 eligible archived data.

Flavour stimuli

Participants in carrot and kale groups received one organic carrot or kale capsule, respectively. The reason for using a capsule was to limit as much as possible the degradation of the flavours before the absorptive digestive process, because the capsules are formulated to reach the small intestine by resisting the gastric passage (Marzorati et al., 2015). In addition, maternal likes or dislikes could be controlled by giving capsules with almost no smell and taste compared with the direct consumption of carrot and kale. One capsule contained approximately 400 mg of carrot/kale powder. This amount corresponds to approximately 50 g of raw vegetables (e.g., one medium carrot or 100 g of chopped kale). The shell of the capsules consists of hydroxypropyl methylcellulose, which is suitable for vegans and vegetarians (Marzorati et al., 2015).

Procedure

For this study, at 32- and 36-weeks' gestation, all women underwent nonmedical 4D ultrasound scans in accordance with the British Medical Ultrasound Society guidelines (Society of Radiographers & British Medical Ultrasound Society, 2021). All participants were asked not to ingest anything in the hour prior to their appointment to optimise the effect of the flavour stimuli.

In the experimental groups, participants had abstained from consuming any food and/or drink involving carrot and kale on the day of the scan. Experimental group mothers swallowed one vegetable capsule (carrot/kale) with a mouthful of water approximately 20 min (M = 23.56, SE = .59) before each scan. The waiting time was based on the time needed for capsule shell to dissolve in the small intestine (Marzorati et al., 2015). Control group mothers were not exposed to any flavourant before and/or throughout the scans. Mothers could not be blinded to the condition because of the colour of the flavour stimuli. Because maternal mental health variables were identified as significant covariates of fetal movement (Federenko & Wadhwa, 2004), mothers were asked to complete the Perceived Stress Scale (Cohen et al., 1983) and Hospital Anxiety and Depression Scale (Zigmond & Snaith, 1983) before each scan. These measurements were used in the statistical analyses where appropriate.

To control for the effects of maternal vegetable consumption on fetal exposure to the stimulus, the frequency of their effective intake of non-bitter (carrot and other non-bitter vegetables including potatoes, peas, beans, and sweet corns) and bitter (kale and other bitter vegetables including lettuce, leeks, and Brassica vegetables such as broccoli and cauliflower) vegetables in the week prior to each scan were assessed (see Table 3.2; for the questionnaire, see Table 2.4 in the general methodology chapter).

Table 3.2. Mean frequencies of vegetable consumption in the week prior to scans

	Kale flavour group (n = 34)		Carrot flavour group (n = 35)	
	32 weeks	36 weeks	32 weeks	36 weeks
Kale (minimum = 0, maximum = 10)	2.38 (0.119)	2.85 (0.214)	2.31 (0.090)	2.33 (0.109)
Carrot (minimum = 0, maximum = 10)	3.65 (0.126)	3.89 (0.186)	3.66 (0.136)	4.04 (0.139)

Overall (bitter vegetables) (minimum = 0, maximum = 20)	7.94 (0.457)	8.31 (0.377)	7.65 (0.343)	8.04 (0.254)
Overall (non-bitter vegetables) (minimum = 0, maximum = 20)	9.85 (0.274)	10.08 (0.295)	9.84 (0.331)	10.39 (0.302)

Note: Values in parentheses are standard errors.

Following the completion of waiting time, mothers underwent 4D ultrasound scans for around 25 min. To minimize possible effects attributable to different levels of fetal movements, each scan started at approximately the same time of day (~3 p.m.). Throughout the scans, by three expert sonographers who were blinded to the study objectives and the group allocation, all mothers laid on their back or on their side in a darkened, quiet room, as they did in their 12- and 20-week scans. They were able to see their unborn child on a screen. At the start of each scan, the sonographer measured femur length, head circumference, and heartbeat to ensure that fetuses were healthy and in the expected range of development. Real-time 4D ultrasound recordings and traditional 2D images were visualised side by side to observe the face and upper torso of the fetus. The scans were recorded (at 24 fps) for off-line analysis using GE Voluson E8/E10 Expert Ultrasound System with a RAB6-RS transducer (General Electric, Boston, MA). All mothers received a copy of their scans.

Behavioural coding scheme and method of coding

By following procedures validated in previous research, 17 independent FMs were selected to analyse fetal FMs in response to different flavours (see Table 3.3). Fetuses in the last trimester of pregnancy are able to display these muscle movements (Reissland et al., 2016). The movements, apart from FM11, FM16 and FM24, were selected on the basis of previous research reporting postnatal flavour-elicited reactions (e.g., Mennella et al., 2001; Rosenstein & Oster, 1988; Schaal et al., 2000). Preliminary analysis of the current sample before deciding on the final coding scheme revealed that fetuses were able to express

nasolabial furrow (FM11), lower-lip depressor (FM16) and lip presser (FM24), along with other movements defined in the literature, when exposed to flavours.

In terms of classification of movements, eleven movements were attributed to the gestalts following the Fetal Observable Movement System coding scheme (Reissland et al., 2016). Two movements (FM20 and FM26) were linked to the gestalts following the Baby Facial Action Coding System (Oster, 2006). The rest of the FMs were not specified in the literature; thus, they were exploratory. In the case of the FMs that were assigned to both gestalts, such as cheek raiser (FM6), the final interpretation in corresponding gestalts of these actions depends on reactivity in the other part of the face.

Table 3.3. Fetal facial movements (FMs) coded and the muscle configurations of facial gestalts

Fetal FM and underlying muscles	Laughter-face gestalt	Cry-face gestalt
FM1: inner-brow raiser		✓
FM2: outer-brow raiser[a]	✓	✓
FM4: brow lowerer		✓
FM6: cheek raiser	✓	✓
FM9: nose wrinkle	✓	✓
FM10: upper-lip raiser		✓
FM11: nasolabial furrow	✓	✓
FM12: lip-corner puller	✓	
FM16: lower-lip depressor		✓
FM18: lip pucker[a]	✓	✓
FM19: tongue show	✓	
FM20: lip stretch		✓
FM24: lip presser[a]	✓	✓
FM25: lips parting	✓	✓

FM26: jaw drop	✓	✓
FM27: mouth stretch	✓	✓
FM28: lip suck[a]	✓	✓

[a]These FMs were not assigned to any gestalt in the Fetal Observable Movement System or the Baby Facial Action Coding System. Thus, these can be seen in either facial gestalt.

All coding was completed by trained, blinded coders (one main Facial Action Coding System certified coder – Beyza Ustun, two reliability coders) using the Observer (15XT; Noldus, the Netherlands). A lab assistant, who was not involved in coding and analysing, fully-anonymised the data so that the coders were blinded to both experimental and control groups. The coders first observed the scan and marked with "start-stop" codes those sections of the scans where the fetal face was visible enough to code. Although the codable scan duration varied between fetuses because of their position in the womb, the mean of codable length in each group was similar (see Table 3.4). Therefore, the relative frequency of fetal FMs per minute was calculated by dividing the number of the given FMs by the codable time and multiplying the result by 60. Second, the total codable footage of each scan was coded frame by frame to identify discrete FMs displayed by the fetus. Third, facial gestalts were identified by summing the number of movements in the gestalt, and the relative frequency of gestalts per minute was measured. Fourth, to measure the complexity of gestalts, gestalts were classified by the number of concurrent FMs they comprised - single, double, triple, and quadruple or higher. Interobserver reliability analyses were applied on 20% of the data set, and the overall agreement was 0.90, with a range of 0.81 to 0.98 using Cohen's kappa.

Table 3.4. The mean of codable scan length (in seconds)[4]

Gestational age	Kale Flavour Group Mean (SE)	Carrot Flavour Group Mean (SE)	Control Group Mean (SE)
At 32 weeks	487.56 (49.786)	425.49 (60.501)	458.25 (49.159)
At 36 weeks	499.85 (60.694)	467.03 (60.255)	508.21 (46.774)

Statistical analyses

This study involved longitudinal sampling and mixed design on three different groups (between-subjects design) - two experimental (carrot exposure, kale exposure) and one control (no flavour exposure). All dependent variables – namely, the frequency of discrete fetal FMs, facial gestalts, and the level of complexity - were log-transformed to normalise the data set. The log-transformed scores were transformed back for data presentation.

First, a one-way analysis of variance (ANOVA) was computed to assess whether maternal (age and BMI) and fetal (head circumference at 20 weeks, gestational age at birth, and birth weight) variables differed between groups. Second, Pearson chi-square test was used to test whether there were any significant differences between the groups in terms of fetal sex. Third, Pearson correlations were used to test whether dependent variables were correlated with maternal mental health variables (stress, anxiety, and depression) and level of education. For missing data in the maternal and fetal independent variables, series' mean estimations were used. Additionally, an independent-samples *t* test was conducted to assess whether there were differences between the experimental groups in terms of the frequency of maternal vegetable consumption as assessed by questionnaire and waiting time after mother's flavour ingestion. Covariates that significantly correlated with the dependent variables were included in further analyses. A multivariate analysis of covariance (MANCOVA) and a series

[4] This table was provided as supplementary material in the published article.

of post-hoc analyses, using the Scheffé criterion for significance, tested the impact of the type of flavour stimuli (between groups) on the frequency of discrete FMs and on facial gestalts to interpret fetal reactions to flavours. When the data were available at both time-points, a general linear mixed model for repeated measures (3 groups x 2 gestational age) was used to assess fetal neuro-motor-maturation from 32 to 36 weeks in terms of the complexity of FM profiles and the effect of flavour-based reactivity. Series' mean estimations were used for missing data ($n = 6$) of the maternal demographics. An alpha level of .05 were used for all statistical analyses, which were carried out using IBM SPSS.

Results

In this repeated-measures study, fetal 4D ultrasounds at 32 weeks ($n = 97$) and 36 weeks ($n = 81$) of gestational age were coded for fetal FMs and facial gestalts in three different groups (kale, carrot, control).

Covariates

Predictors of fetal movements, including fetal sex, maternal age, maternal pre-pregnancy BMI, gestational age at birth, and birth weight were initially examined but removed from further analyses because these measures did not significantly differ between groups ($p > .05$). However, head circumference at 20 weeks' gestation was significantly different between groups ($p = .002$) and was therefore controlled in further analyses. An independent-samples *t* test confirmed that there were no significant differences between experimental groups in both 32 and 36 weeks, in the mothers' waiting time for the scan to start after they had consumed the vegetable capsule, and the frequency of vegetable consumption (kale, carrot, overall bitter, overall non-bitter; see appendix 9). None of the FMs were significantly associated with maternal mental health variables at 32 weeks. However, maternal stress scores at 36 weeks' gestation had a significant relationship with fetal lower-

lip depressor (FM16); therefore, it was included in subsequent analysis (p = .045; see appendix 10).

Average time elapsed from ingestion to appearance of the first fetal FMs

In the carrot group, the mean of first FM was observed after 29.20 min (*SE* = 1.652) at 32 weeks and after 28.29 min (*SE* = 1.917) at 36 weeks. Similarly, fetuses reacted to kale flavour stimuli after 30.16 min (*SE* = 1.67) at 32 weeks and after 28.83 min (*SE* = 1.118) minutes at 36 weeks.

Analyses of discrete fetal FMs

32 weeks' gestation. On the basis of 97 ultrasound scans, the analyses of discrete fetal FMs showed that the relative frequency of upper-lip raiser (FM10), $F(2, 94) = 16.12$, $p < .001$, $\eta_p^2 = .26$; lower-lip depressor (FM16), $F(2, 94) = 16.08$, $p < .001$, $\eta_p^2 = .26$; lip stretch (FM20), $F(2, 94) = 39.45$, $p < .001$, $\eta_p^2 = .46$; and lip presser (FM24), $F(2, 94) = 27.28$, $p < .001$, $\eta_p^2 = .37$, was significantly greater in the kale group than in the carrot and control groups. Moreover, the relative frequency of lip-corner puller (FM12) was found to be significantly higher in the carrot group (*M* = .35, *SE* = .037) than in the control (*M* = .15, *SE* = .021) and kale group (*M* = .03, *SE* = .008) at 32 weeks, $F(2, 94) = 43.52$, $p < .001$, $\eta_p^2 = .48$ (see Fig. 3.3).

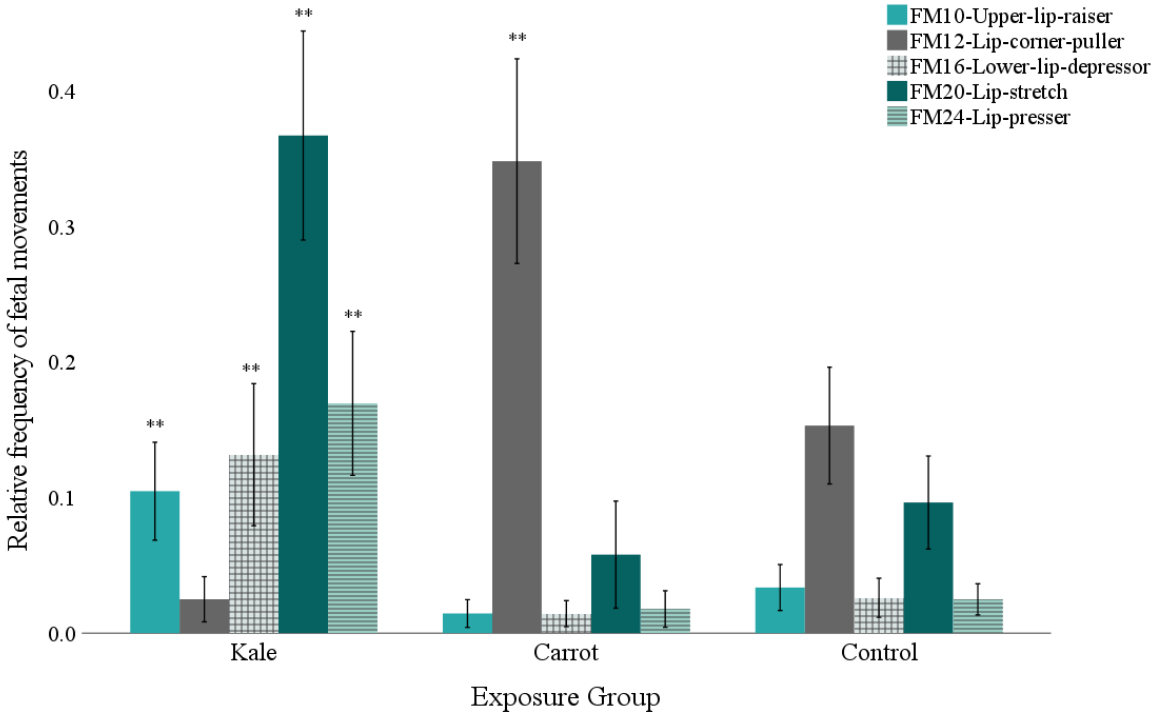


Fig. 3.3. Relative frequency of fetal facial movements at 32 weeks split by exposure group. Note: Error bars represent 95% confidence intervals. Asterisks indicate significant differences (**$p < .001$) in between-groups analyses.

36 weeks' gestation. On the basis of 83 ultrasound scans, the relative frequency of upper-lip raiser (FM10), $F(2, 80) = 4.83$, $p = .010$, $\eta_p^2 = .11$; lower-lip depressor (FM16), $F(2, 80) = 10.80$, $p < .001$, $\eta_p^2 = .21$; lip stretch (FM20), $F(2, 80) = 25.07$, $p < .001$, $\eta_p^2 = .39$; and lip presser (FM24), $F(2, 80) = 20.81$, $p < .001$, $\eta_p^2 = .32$ was significantly greater in the kale group than in the control and carrot groups. Similar to the results at 32 weeks, the relative frequency of lip-corner puller (FM12) was greater in the carrot group ($M = .39$, $SE = .034$) than in the control group ($M = .16$, $SE = .032$) and kale group ($M = .02$, $SE = .034$), $F(2, 80) = 30.54$, $p < .001$, $\eta_p^2 = .43$. Carrot-group fetuses ($M = .09$, $SE = .014$) displayed higher frequency of lip pucker (FM18) in comparison to kale-group fetuses ($M = .01$, $SE = .015$), $F(2, 80) = 7.03$, $p = .002$, $\eta_p^2 = .15$. There was no significant difference between the three

groups in any other discrete FMs (FM1, 2, 4, 6, 9, 11, 19, 25, 26, 27, and 28) or the total number of FMs at 32 and 36 weeks between the three groups (see Fig. 3.4).

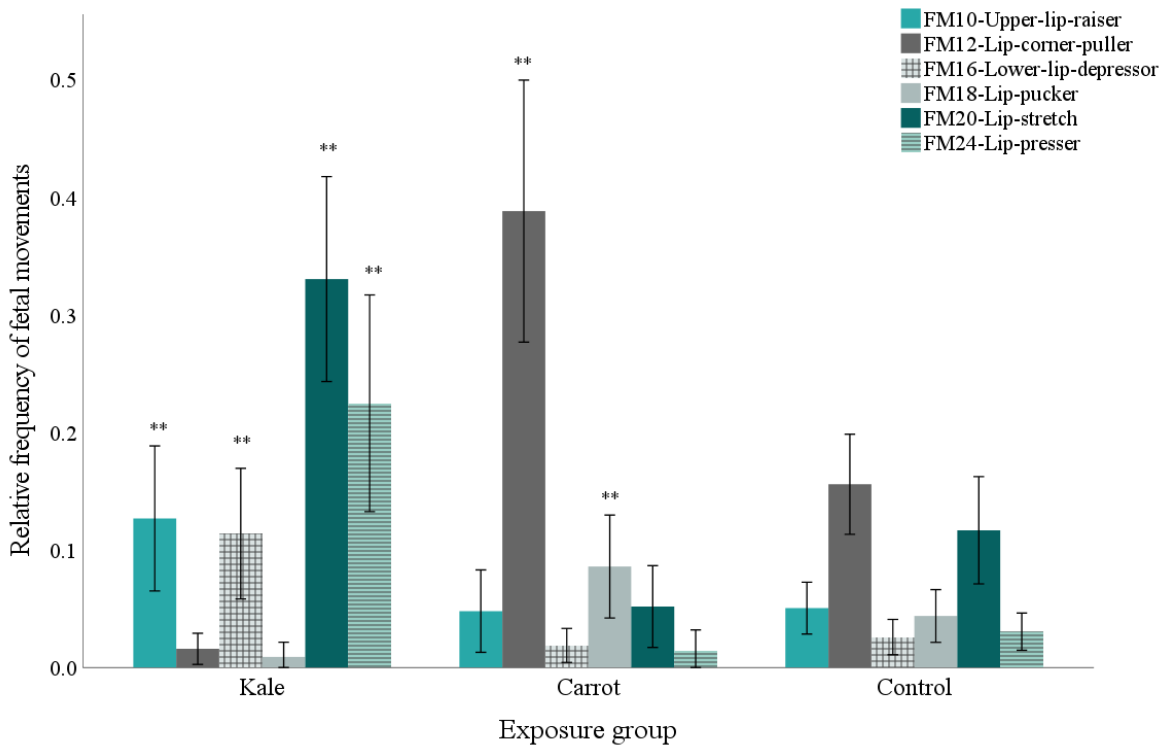


Fig. 3.4. Relative frequency of fetal facial movements at 36 weeks split by exposure group. Note: Error bars represent 95% confidence intervals. Asterisks indicate significate differences (**$p < .001$) in between-groups analyses.

Laughter-face gestalt versus cry-face gestalt

Using Roy's largest root in MANCOVA results, there was a significant effect of flavour exposure on the facial gestalts at 32 weeks, $F(2, 94) = 93.82$, $p < .001$, $\eta_p^2 = .67$. Separate univariate ANOVAs on the outcome variables revealed a significant effect of flavour stimuli on the relative frequency of cry-face gestalt, $F(2, 94) = 38.74$, $p < .001$, $\eta_p^2 = .45$, with a greater frequency in the kale group ($M = .71$, $SE = .046$) than in the control group ($M = .24$, $SE = .049$) and carrot group ($M = .18$, $SE = .047$). In addition, there was a

significant effect of flavour stimuli on the relative frequency of laughter-face gestalt at 32 weeks, $F(2, 94) = 28.82$, $p < .001$, $\eta_p^2 = .38$, with the carrot group showing a greater frequency ($M = .48$, $SE = .04$) than the control group ($M = .19$, $SE = .042$) and the kale group ($M = .05$, $SE = .04$).

At 36 weeks, Roy's largest root revealed a significant difference on the facial gestalts depending on flavour exposure, $F(2, 80) = 40.41$, $p < .001$, $\eta_p^2 = .50$. The relative frequency of cry-face gestalt was higher in the kale group ($M = .70$, $SE = .065$), $F(2, 80) = 14.97$, $p < .001$, $\eta_p^2 = .27$, whereas the relative frequency of laughter-face gestalt was higher in the carrot group ($M = .49$, $SE = .06$), $F(2, 80) = 9.8$, $p < .001$, $\eta_p^2 = .20$ (see Fig. 3.5).

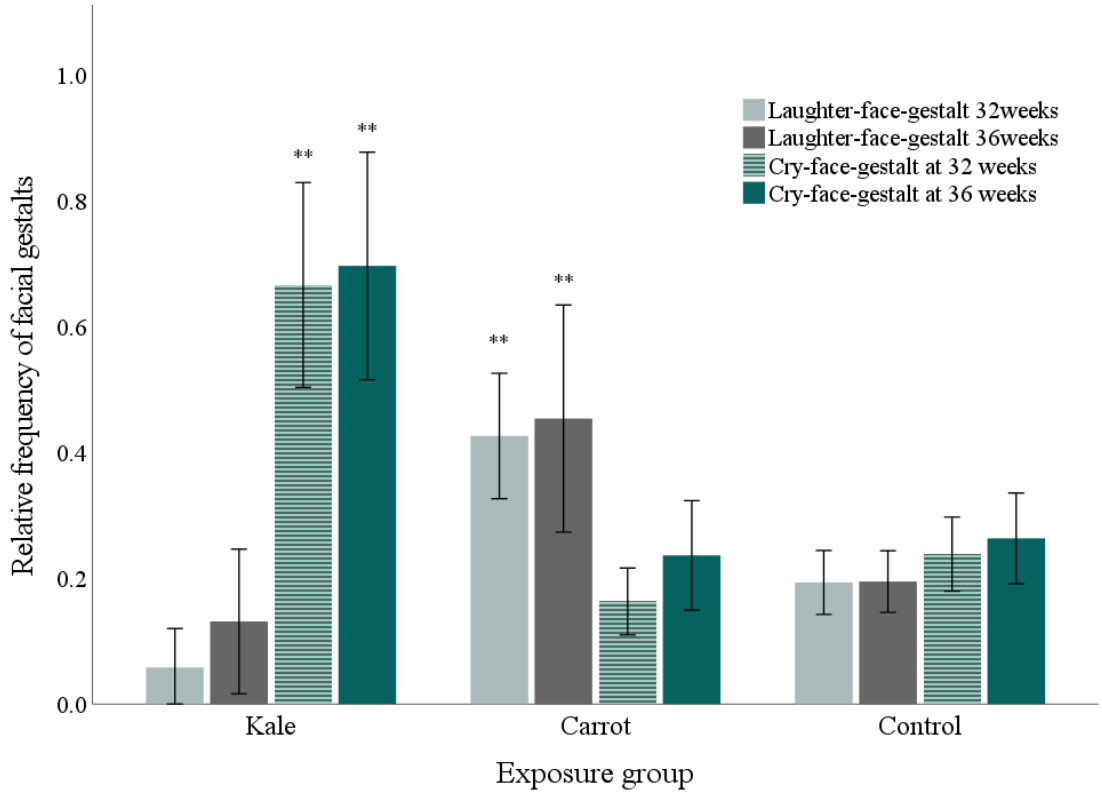


Fig. 3.5. Relative frequency of facial gestalts at 32 and 36 weeks split by exposure group. Note: Error bars represent 95% confidence intervals. Asterisks indicate significant differences (**$p < .001$) in between-groups analyses.

Combinations of fetal FMs into complex face gestalts

Whereas facial gestalts consisting of double and triple movements did not show a significant increase in frequency, the overall facial gestalt made of quadruple or more movements increased from 32 to 36 weeks, $F(1, 78) = 3.88$, $p = .052$, $\eta_p^2 = .05$. The highest increase rate of these facial gestalts, comprising four or more movements, from 32 weeks ($M = .04$, $SE = .009$) to 36 weeks ($M = .08$, $SE = .016$), was found in the kale group (see Fig. 3.6), whereas no significance changes were observed in the carrot and control groups.

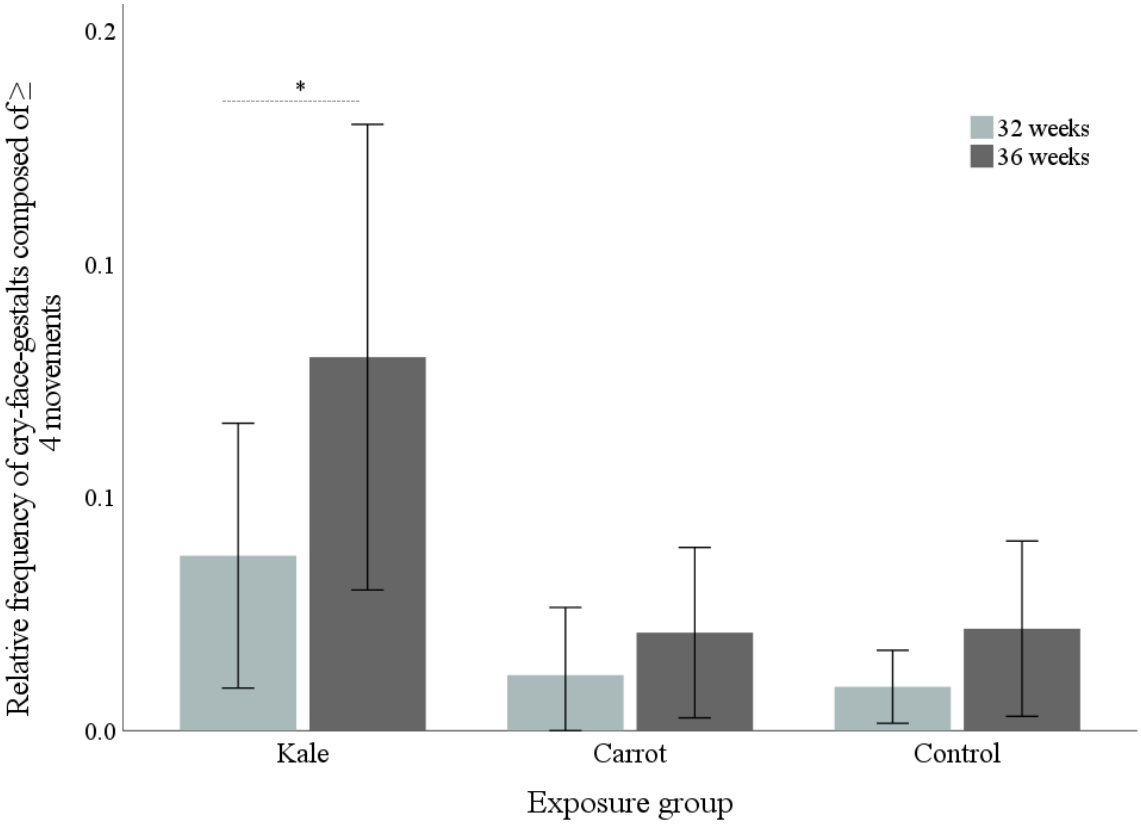


Fig. 3.6. Relative frequency of cry-face gestalts composed of four or more facial movements at 32 and 36 weeks split by exposure group.

Note: Error bars represent 95% confidence intervals. The asterisk indicates a significant difference (*$p < .05$) in between-group analyses.

Discussion

This study assessed whether human fetuses aged 32 to 36 weeks react to flavours taken in by their mother. The main results show that the intake of a single dose of flavourant by a pregnant woman activates fetal FMs as well as a combination of movements that together form a laughter-face or a cry-face gestalt. Moreover, fetuses express different frequencies of facial gestalts in relation to the type of flavour they are exposed to, namely, more laughter-face gestalts when exposed to a carrot flavour and more cry-face gestalts when experiencing a kale flavour. Finally, as fetuses develop from 32 to 36 weeks, they react to kale flavour, but not to carrot flavour, with an increasing number of different FMs coalescing in more complex facial gestalts. These findings are discussed in more detail below.

The fetus reacts to dietary flavours ingested by the mother

Previous studies have sensorily monitored changes in flavour quality and intensity of the amniotic fluid after the ingestion of odorants or tastants by mammalian females (Hauser et al., 1985; Mennella et al., 1995). The transplacental transfer of flavour molecules has rarely been assessed in tracing them using instrumental chemistry (Schaal, 2005), but numerous physiological and pharmacokinetic studies of drugs have shown that most molecules can cross the placenta, some by passive diffusion and some by facilitated diffusion (Syme et al., 2004). Human studies of fetal chemoreception so far have not included instrumental chemical verification of effective transplacental flavourant transfer into the amniotic fluid but rely only on differential postnatal behavioural outcomes to the flavourants ingested by pregnant women. The present study examined fetal chemosensory reactivity and was able to demonstrate that pregnant women's flavour intake affects the fetal reactions *in utero* directly and quasi-immediately.

Fetal reactions observed here in both flavour groups compared with the control group provide evidence that the ingestion of only 400 mg of powdered carrot or kale in a capsule

was sufficient to reach fetal chemoreceptors. This effect occurs within a relatively short time: Around 30 min after maternal ingestion of the flavour capsules, observable facial reactions were detected in the fetuses. Thus, in this short time, the flavour content of the capsules undergoes digestion, absorption into mothers' bloodstream, metabolization and circulation through the placenta and fetus, collection in the amniotic fluid, and fetal chemoreceptors. So far, little is known about the biotransformation and transplacental kinetics of flavourants ingested by mothers, but this relatively short transfer time is compatible with the pharmacokinetic data on drugs in the human materno-fetal system (Brown et al., 1990).

Fetal behaviour in utero is specific to flavour input

The morphological specificity of fetal reactions observed in the present study aligns with expectations based on neonatal studies on flavour (Maone et al., 1990; Rosenstein & Oster, 1988; Steiner, 1979; Tatzer et al., 1985).

In the kale-exposed group, at 32 and 36 weeks, the relative frequency of upper-lip raiser (FM10), lower-lip depressor (FM16), lip stretch (FM20), and lip presser (FM24), as well as their combinations leading to the cry-face gestalt, was higher in comparison with carrot-exposed and control fetuses. In the case of exposure to carrot flavour, at 32 and 36 weeks, lip-corner puller (FM12) alone and facial configurations involving movements making up the laughter-face gestalt were relatively more frequent in the carrot-exposed group compared with both the control and kale-exposed groups. However, lip pucker (FM18) appeared at 36 weeks but not at 32 weeks. It is known that fetuses can pucker their lips around 32 to 34 weeks (Piontelli, 2015). Individual differences in lip pucker (FM18) performance may explain the nonappearance of this movement at 32 weeks.

Although lip presser (FM24) and lip pucker (FM18) are not assigned to any of the gestalts in the literature, the findings suggest that FM24 is a reaction to kale flavour and FM18 to carrot flavour. The evidence that fetuses react with differential FMs depending on

the flavour of maternal intake indicates that they discriminate these two flavours by at least 32 weeks' gestation.

Development of flavour-elicited facial expressivity

The present study demonstrates that it is possible to visualize the short-term development of facial expressive reactivity during late pregnancy. Specifically, the combination of four or more FMs contributing to the cry-face gestalt in the kale-group fetuses increases from 32 to 36 weeks. This finding supports the concept that fetuses exhibit complex motor behaviours associated with cry-face and such behaviours increase with gestational age (Reissland et al., 2011). In contrast, in the current sample, there was no evidence that the complexity of the gestalt expression related to the perception of carrot flavour increases with fetal maturation. This contrasting result can be explained by the anatomical substrate engaged in generating laughter-face and cry-face gestalts. The laughter-face gestalt, which occurs in the carrot flavour group, is anatomically more straightforward to generate because the activation of a sole FM12 suffices for its appearance. It thus develops earlier (Kurjak et al., 2003) compared with the complex cry-face gestalt, which occurs more in the kale flavour group. The cry-face gestalt needs the activation of multiple FMs to end with a readable gestalt. Therefore, a developmental trend might not be observed that late in gestation.

Limitations

For this well-powered study, adding a control group from archived data, because of limited resources, may constitute a limitation of the research. Although the archived data include the same stress, anxiety, and depression measures as the original study, information in the control group on the frequency of vegetable consumption could not be collected before ultrasound scans. Given that infants' chemosensory responses may be influenced by habitual vegetable consumption by pregnant mothers (Wagner et al., 2019), this might also affect fetal reactivity. However, it was assumed that the data not collected are unlikely to affect the

results because the frequency of vegetable consumption was not associated with the FMs of fetuses in both experimental groups.

Another potential confounding factor in the study is the impact of genetic variations in bitter taste perception due to the expression of the TAS2R38 gene (Feeney, 2011). Given that genetically mediated sensitivity to bitter tastants has been associated with food acceptance in infants (Cont et al., 2019), it was acknowledged that fetuses in this study were likely to comprise a mixture of super-tasters, medium tasters and nontasters based on single-nucleotide polymorphisms of the TAS2R38 gene. Although capsules were used to bypass mothers' taste sensitivity, variance in the fetal gene encoding TAS2R38, which dictate individual gustatory differences, may have an impact on fetal FM profile. Additionally, mothers in the experimental groups might have seen the shades of colour of the powder inside the translucent capsule, but they could neither taste nor smell the content. Nonetheless, further work is needed to determine whether taster status moderates fetal FM profiles in response to flavour experiences in utero.

Conclusion

The present article is the first longitudinal study to indicate that fetuses are capable of *prenatally* detecting chemosensory information conveyed by flavour compounds originating in maternal diet. This was shown by fetal facial reactions after either carrot or kale flavour exposure. Fetuses from 32 to 36 weeks react to kale flavour with a cry-face gestalt increasing in complexity as they mature. Because the laughter-face gestalt is less complex than the cry-face gestalt, an increase in complexity was not observed in this case.

Results of this study have important implications for our understanding of the development of human oral and nasal chemoreception, including the nature and timing of behavioural reactions to prenatal flavour exposure, fetal engagement of memory for flavours,

and the potential role played by prenatal to postnatal continuity in perception and reactivity to the chemical environment.

Given that experimental evidence indicates that prenatal flavour experience is embedded and is accessed postnatally in humans (Mellor, 2019; Schaal, 2005), it could be argued that repeated prenatal flavour exposures may lead to preferences for certain flavour profiles that are consistent with flavour experienced postnatally in very different contexts. Future studies need to follow up with postnatal behavioural analyses to assess how prenatal flavour exposure can exert influences on postnatal food preferences in the short and long term.

Chapter 4

Chemosensory continuity from prenatal to postnatal life in humans: A systematic review and meta-analysis[5]

Abstract

Throughout pregnancy, fetuses are exposed to a range of chemosensory inputs influencing their postnatal behaviours. Such prenatal exposure provides the fetus with continuous sensory information to adapt to the environment they face once born. This study aimed to assess the chemosensory continuity through a systematic review and meta-analysis of existing evidence on chemosensory continuity from prenatal to first postnatal year. Web of Science Core Collections, MEDLINE, PsycINFO, EBSCOhost eBook collection was searched from 1900 to 2021. Studies identified from the search were grouped according to type of stimuli the fetuses were exposed to prenatally that the neonatal infants' responses to were being evaluated, namely flavours transferred from the maternal diet, and the odour of their own amniotic fluid. Of the 12 studies that met the eligibility criteria for inclusion (k = 6, k = 6, respectively in the first and the second group of studies), and eight studies (k = 4, k = 4, respectively) provided sufficient data suitable for meta-analysis. Infants, during their first year of life, oriented their heads for significantly longer durations in the direction of the prenatally experienced stimuli with large pooled effect sizes (flavour stimuli, d = 1.24, 95% CI [0.56, 1.91]; amniotic fluid odour, d = 0.853; 95% CI [.632, 1.073]). The pooled effect size for the duration of mouthing behaviour was significant in response to prenatal flavour exposure through maternal diet (d = 0.72; 95% CI [0.306, 1.136]), but not for the frequency

[5] This research is under peer-review for publication in PLOSONE.

of negative facial expressions (*d* = -0.87, 95% CI [-2.39, 0.66]). Postnatal evidence suggests that there is a chemosensory continuity from fetal to the first year of postnatal life.

Introduction

Fetuses are reactive to their environment in the womb based on their developing sensory abilities (André et al., 2018; Bossy, 1980), which allow postnatal detection of, for example, the odour of amniotic fluid, and flavour cues from the diet of the pregnant mother (Schaal, 2016). Research suggests that fetal experiences have an impact on the behaviour of infants (Gonzalez-Gonzalez et al., 2006; Lecanuet et al., 1995; Smotherman & Robinson, 1988). Therefore, it is argued that postnatal reactions are influenced after birth not only by perceptual capacities, but also by prenatal sensory inputs and the ability to embed, and access this learning when faced with stimuli familiarized in the womb (André et al., 2018; Mellor, 2019).

Transnatal chemosensory transmission, also known as transnatal chemosensory continuity, refers to the transition from intra-uterine to extra-uterine life (André et al., 2018; Mellor, 2019). This continuity, because of early familiarization, facilitates new-born infants' ability to adapt to their postnatal environment (Nicklaus & Schwartz, 2019; Schaal, 2016; Schaal et al., 2004). Taste, olfaction, and trigeminal chemesthesis cannot be dissociated in the intra-uterine environment, and thus this article refers to flavour exposure acknowledging that their effects on the fetus may involve one or several of these chemosensory inputs (Rozin, 1982). Postpartum reactions, that provide indirect evidence of chemosensory transmission, to such prenatal flavour exposure, may be observed for weeks, months, and potentially years after birth.

It is essential to understand how the evidence supporting transnatal continuity is to be assessed and managed in practice. Such a process might facilitate programming healthy behaviours since sensory abilities are already functional at the fetal stage (André et al., 2018; Browne, 2008). Different postnatal behaviours, food acceptance or preference for familiar

flavour, can be attributed to prenatal perception of the formation of discriminative abilities (Forestell & Mennella, 2015).

Existing relevant systematic reviews (Nehring et al., 2015; Spahn et al., 2019) have been conducted assessing the effects of pre and/or postnatal flavour exposure on postnatal behavioural outcomes until the age of 2 years old (Spahn et al., 2019) and the age of 9 years old (Nehring et al., 2015). Their results showed that infants can recall the volatiles transmitted to amniotic fluid from the maternal diet. In this systematic review, in addition to the infant reactions to the flavours transmitted via maternal diet, infant responses to the odour of their own amniotic fluid were also investigated, which would provide further evidence of chemosensory continuity from prenatal to postnatal life. The current review is the first undertaking a meta-analysis to determine pooled summary effect sizes of the chemosensory transmission from fetal life to the first postnatal year of life, in human participants. Unlike previous reviews, the current study focused on the first postnatal year since experiences during this critical stage of development can have long-term consequences, especially in terms of food-related behaviours (Savage et al., 2021; Schwartz et al., 2011b; Stettler, 2007).

The current systematic review and meta-analysis was therefore designed to synthesize the findings from two related bodies of research: 1) studies that have investigated whether infants (Participant) have different behavioural profiles (Outcome) when they are exposed to flavour stimuli (Exposure) via maternal diet during pregnancy, and 2) studies that have investigated whether infants (Participants) have different behavioural profiles (Outcome) to the odour of their own amniotic fluid that they have been exposed to through gestation (Exposure). In the first group of studies, the behavioural responses of infants whose mothers ingested the target flavour to those who did not experience the same flavour were compared. In the second group of studies, infant reactions to the odour of familiar amniotic fluid odour (collected from their own mother) were compared either to their reactions to the odour of

unfamiliar amniotic fluid (collected from a different mother) odour or to a control odour such as distilled water.

Methods

The methods used for this systematic review and meta-analysis followed the PRISMA guidelines (Moher et al., 2009).

Search methods for identification of the studies

A literature search was conducted using four electronic databases (Web of Science Core Collections, MEDLINE, Psych INFO and EBSCOhost eBook collection) for studies conducted in the date range 1900 and December 2021. The search terms are presented in Table 4.1. The reference list of all papers identified from the keyword search, were manually screened to identify any further studies of interest to ensure literature saturation.

Table 4.1. Search terms

Prenatal terms	AND	**Chemical sense and food terms**	AND	**Exposure or continuity terms**	AND	**Subject terms**	AND	**Behaviour terms**	NOT	**Animal terms**
Prenatal		Taste*		Exposure		F$etus*		Preference*		Animal*
OR		OR		OR		OR		OR		OR
Early		flavo$r*		Experience		Baby*		Acceptance		Cat*
OR		OR		OR		OR		OR		OR
Pregnancy		odo$r		Learning		Infant*		behavi$r*		Dog*
OR		OR		OR		OR		OR		OR
Intrauterine		Chemical sense*		Perception		Newborn*		facial expression*		Bird*
OR		OR		OR		OR		OR		OR
in the womb		Smell		Ingestion		Postnatal		Response*		Mice*
OR		OR		OR		OR		OR		OR
Perinatal		Gustatory		Transnatal continuity		Neonate*		facial movement*		Rat*
OR		OR		OR				OR		OR
Maternal		Olfactory		Transnatal transmission				Liking		Piglet*

OR	OR	OR	OR
Pregnant woman*	Amniotic fluid	Disliking	Mammal*
OR	OR	OR	OR
Mother*	Food	Intake	Monkey*
	OR	OR	OR
	beverage*	Refusal	Lamb*
	OR	OR	OR
	dietary supplement*	Appetite	Rabbit*
		OR	
		Attraction	

Note. "AND" showing that studies required having one term from each column, "OR" showing that any of those terms is adequate for eligibility, "NOT" showing that any of those terms is adequate for ineligibility.

Selection of studies

The database searches were imported into EndNote X20. Duplicates were automatically removed while importing. Before the screening, one author (Beyza Ustun) checked and removed any remaining duplicates manually. To ensure that there was no double counting, the studies with the same cohort of participants published in multiple publications were coded as a single study. The primary author (Beyza Ustun) examined the titles and abstracts of studies to evaluate their fit to the inclusion criteria. Full texts of relevant studies were screened for further analysis of inclusion criteria, with a second reviewer (JC or NR), consulted to resolve any discrepancies.

Inclusion and exclusion criteria

The target population was healthy (as indicated in the studies) mother-infant dyads from prenatal life to the first postnatal year.

In the first group of studies that reviewed the effects of specific flavour exposure studies were included if they reported a measure of a specific prenatal flavour exposure transferred via maternal diet and postnatal behavioural responses (i.e., orofacial reactions and

head orientation) to that flavour. Infant head orientation to a stimulus experienced and rehearsed in the prenatal environment indicates a preference for the stimulus (Soussignan et al., 1997, 1999; Steiner et al., 2001). Furthermore, orofacial responses have been found to be a robust indication of hedonic discrimination of infants up to one-year-old (Soussignan et al., 1997, 1999; Steiner et al., 2001). Studies were included if they reported a comparison between groups of infants ≤ one-year if age with and without prenatal exposure to the specific flavour exposure. Inclusion criteria for studies were not restricted to randomized designs. No restrictions on inclusion were applied for duration or type of flavour exposure during pregnancy, fetal age at time of exposure, type of postnatal stimuli (taste, odour, or both), or type of maternal intake route (e.g., intake via food, beverages, or dietary supplement). Since breastmilk contains maternal dietary aromas (Hausner et al., 2010), the results from the breastfeeding period were excluded if a study evaluated the effects of a particular flavour exposure during both pregnancy and breastfeeding.

In the second group of studies on the infants' responses to the odour of their own amniotic fluid, studies were included if they reported a measure of infant behaviours (i.e., orofacial responses and head orientation) at ≤ one-year after birth in response to the odour of their own amniotic fluid compared to their response to a control condition such as distilled water or amniotic fluid from another mother. Inclusion criteria for studies were not restricted to randomized designs. If different comparators were used in a study and one of the comparators involved pairing with distilled water, this control odour was chosen in the analysis because this type of water is purified and devoid of contaminants.

Both groups of studies in the systematic review excluded: animal studies where animals were a total or a part of the sample of a study, studies that were not published in the English language, unpublished studies, reviews, meta-analyses, letters, opinions, conference or poster abstracts, studies focusing on medical/ health or birth outcomes, studies reporting

unhealthy samples with diagnosed disease or condition. Studies reporting mothers with gestational diabetes, mothers with allergies, obesity, or hyperemesis were excluded as were studies reporting fetal anomalies at 12 or 20 weeks, malnourished fetuses, preterm delivery (< 37 weeks), or low birth weight (≤ 2500 g). Studies involving pregnant mothers who knowingly smoked or used Nicotine Replacement Therapy were also excluded. In longitudinal studies where infant behaviours were assessed at multiple points, the earliest time point was selected to capture the earliest infant reactions after birth. No restrictions were applied regarding maternal age, race, ethnicity, socioeconomic status, parity, or study sample size.

Data extraction and management

All data were extracted in a pre-defined form by the primary author (Beyza Ustun) and were confirmed by the other authors (JC and NR). The data extraction form summarized study and participant characteristics (sample size, infant age at testing, when applicable gestational age during exposure), infant behavioural measurement, outcomes, study effect sizes and controlled confounders. The form also extracted information about the flavour type, testing stimulus, and type of control condition, if relevant.

Assessment of risk of bias (RoB) in included studies

The risk of bias of each included study was assessed using either ROBINS-I or ROB 2 tools (Sterne et al., 2016, 2019) which are recommended by the Cochrane Collaboration. All studies were checked for overall risk-of-bias judgement with a range of components (see Table 4.2). Each component was rated as low risk, some concern, and high risk. Any disagreements were discussed in consultation with the other authors (JC or NR) until all disagreements were resolved.

Table 4.2. Risk of bias for individual studies

Using the ROBINS-I tool

Study	Outcome assessed for risk of bias	Bias due to confounding	Bias in selection of participants into the study	Bias in classification of interventions	Bias due to deviations from intended interventions	Bias due to missing data	Bias in measurement of outcome	Bias in selection of the reported result	Overall bias
Marlier 1998a	Head orientation (duration)	Low	Low	Low	Low	Low	Low	Low	Low
Marlier 1998b	Head orientation (duration)	Low	Low	Low	Low	Low	Low	Low	Low
Hepper 1995	Head orientation (duration)	Some concern	Low	Low	Low	Low	Low	Low	Some concern
Schaal 1995a	Head orientation (duration)	Low	Low	Low	Low	Low	Low	Low	Low
Schaal 1998	Head orientation (duration)	Low	Low	Low	Low	Low	Low	Low	Low
Schaal 2000	Negative facial expressions (frequency)	Low	Low	Low	Low	Low	Low	Low	Low
Schaal 2000	Head orientation (duration)	Low	Low	Low	Low	Low	Low	Low	Low
Schaal 2000	Mouthing behaviour (duration)	Low	Low	Low	Low	Low	Low	Low	Low

Wagner 2019	Mouthing behaviour (duration)	Low	Low	Low	Low	Low	Low	Low	Low

Using the ROB 2 tool							
Study	**Outcome assessed for risk of bias**	**Bias arising from the randomization process**	**Bias due to deviations from intended interventions**	**Bias due to missing data**	**Bias in measurement of outcome**	**Bias in selection of the reported result**	**Overall bias**
Mennella 2001	Negative facial expressions (frequency)	Low	Low	Low	Low	Low	Low

Data synthesis and analysis

First, a narrative descriptive summary of the findings from the studies included in this systematic review was provided. This narrative overview summarizes the main findings across studies based on postnatal behavioural outcomes of infants when re-exposed to prenatal stimuli.

Studies were categorized with respect to infant responses to prenatal chemosensory stimuli. If there was a minimum of two studies (Valentine et al., 2010) using the same infant assessment method and the studies provided sufficient data to calculate effect sizes, a meta-analysis was carried out using Comprehensive Meta-Analysis (Borenstein et al., 2013). Effect sizes (Cohen's d values) were calculated for each study and Cohen's convention was used to assess effect sizes, with 0.2 indicating a small effect, 0.5 a moderate effect, and 0.8 indicating a large effect (Cohen, 1992). Heterogeneity was assessed using Cochran’s Q and I2. It was concluded that there was evidence for heterogeneity when the p-value for Cochran’s Q was significant ($p < .05$) and if the I2 was greater than 50% (Higgins & Thompson, 2002). A fixed effect or random effects model was reported depending on whether the effect sizes were homogeneous. The number of missing studies that would need to be retrieved for the effect size to be non-significant was estimated using Rosenthal's Fail-safe N (Rosenthal, 1978).

Results

Study selection

The literature search yielded 1,927 potentially relevant articles. After removing duplicates (automatically and manually) and ineligible records depending on language and publication type 1,673 studies remained. Having examined the title and abstract of those studies, 57 full text articles were evaluated in detail. Of those, 45 were excluded, leaving a total of six studies (Faas et al., 2000, 2015; Hepper, 1995; Mennella et al., 2001; Schaal et al., 2000; Wagner et al., 2019) meeting the eligibility criteria for the first group of studies and six

studies (Marlier et al., 1998a, 1998b; Schaal et al., 1995a, 1998; Varendi et al., 1996, 1998) meeting the inclusion criteria for the second group of studies. The most common reason for exclusion from the systematic review was using postnatal exposure to flavour as an independent variable (k = 18). Because of sufficient results reported and having more than one study to conduct a meta-analysis, four studies (Hepper, 1995; Mennella et al., 2001; Schaal et al., 2000; Wagner et al., 2019) were included in the first group (flavour stimuli from maternal diet) and four studies (Marlier et al., 1998a, 1998b; Schaal et al., 1995a, 1998) were included in the second group (amniotic fluid odour). Where possible, the authors of the studies reporting insufficient data were contacted to obtain further information. Fig. 4.1. shows the PRISMA flowchart of the search strategy and study selection. The overall risk of bias in included studies was mostly judged to be low, only one study (Hepper, 1995) was judged to have some concerns due to not reporting confounding variables.

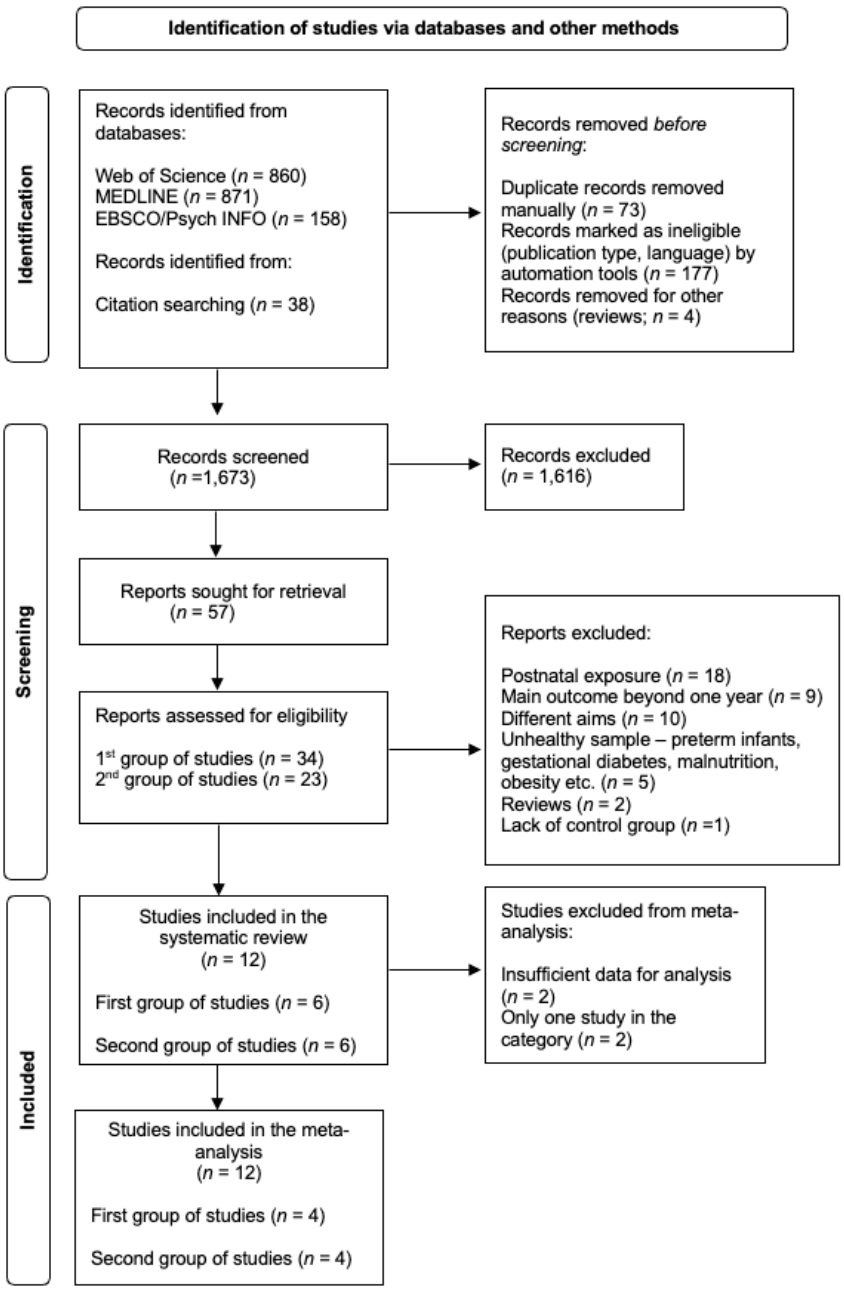


Fig. 4.1. Flow diagram of studies

Study characteristics

Studies included in the review came from different countries: France (*k* = 8), Argentina (*k* = 2), USA (*k* = 1), and Northern Ireland (*k* = 1). There were two randomized controlled studies (Mennella et al., 2001; Varendi et al., 1998) and one non-RCT (within-between subject) (Schaal et al., 2000), the remainder were longitudinal cohort studies (Faas et

al., 2000, 2015; Hepper, 1995; Marlier et al., 1998a, 1998b; Schaal et al., 1995a,1998; Varendi et al., 1996; Wagner et al., 2019). Characteristics of these studies are described in Table 4.3 and Table 4.4.

Table 4.3. Studies that compared the behavioural responses of infants whose mothers did or did not ingest the target flavour

Reference (First author, year, country)	Sample size	Gestational age during exposure	Flavour ingested by mothers	Stimuli used at infant testing	Infant age at testing	Outcome	Infant behavioural measurement	Effect size Cohen's d (SEd)	Covariance and confounders controlled
Faas 2000 (Argentina)	50 mother-infant dyads (17 exposed, 33 non-exposed)	During pregnancy	Alcohol flavour	Ethanol odour	24-48 h	When the primary stimulus was ethanol, new-borns whose mothers were frequent drinkers had significantly higher head and facial movements in response to ethanol (p < .05).	Head orientation and facial responses Not included in the meta-analysis (insufficient data to compute an effect size) A set of 11 odour stimuli were given, primarily ethanol (Ethanol-Lemon-Ethanol) or primarily lemon (Lemon-Ethanol-Lemon). The first and last odours were provided five times in a sequence, but the middle (dishabituation) odour was given only once.		
Faas 2015 (Argentina)	43 mother-infant dyads (16 exposed, 33 non-exposed)	During pregnancy	Alcohol flavour	Ethanol odour	7-14 d	When the primary stimulus was ethanol, new-borns of frequent drinkers showed significantly higher frequencies of appetitive reactions to the ethanol odour in comparison to new-borns of infrequent drinkers (p < .03). Duration of appetitive responses, frequency of aversive responses towards ethanol sequence were not significantly affected by maternal alcohol intake.	Appetitive responses (frequency) Not included in the meta-analysis, insufficient number of studies in the category. These measurements were not included in the meta-analysis (insufficient data to compute an effect size).	1.368 (0.476)	Gestational age at birth, infant sex, birthweight, birth height, delivery type, head circumference, Apgar scores, maternal age, parity, maternal age, infant age at assessment.
Hepper 1995 (Northern Ireland)	20 mother-infant dyads (10 exposed, 10 non-exposed)	In the last month of pregnancy	Garlic flavour	Garlic odour	15-28 h	New-born exposed to garlic flavour oriented their head towards the garlic odour for longer (p = .016).	Head orientation (duration)	1.189 (0.485)	Infant age at testing.

Mennella 2001 (USA)*	29 mother-infant dyads (15 exposed, 14 non-exposed)	Three consecutive weeks during last trimester of pregnancy	Carrot juice	Carrot flavour	5.7 mo	Infants exposed to carrot juice had fewer negative facial expressions to carrot-flavoured cereal ($p < .05$).	Negative facial responses (frequency)	-0.152 (0.392)	Race, singletons vs twins, breastfeeding, maternal age, infant age at testing, infant BMI, infant sex, mothers' eating habits.
Schaal 2000 (France)	23 mother-infant dyads (Day1:11 exposed, 12 non-exposed; Day4: 10 exposed, 10 non-exposed)	In the last two gestational weeks	Anise flavour	Anise odour	8 h–4 d	New-born (day1) exposed to anise flavour had fewer negative facial responses in response to anise odour ($p < .001$).	Negative facial responses (frequency/ day1)	-1.717 (0.368)	Gestational age at birth, delivery type, parity, maternal age, Apgar scores, infant sex, birthweight.
						New-born (day4) exposed to anise in utero oriented their head towards the anise odour for longer ($p < .001$).	Head orientation (duration/ day4)	1.287 (0.491)	
						New-born (day1) exposed to anise flavour had longer mouthing responses in response to anise odour ($p = .02$).	Mouthing (duration / day1)	1.084 (0.458)	
Wagner 2019 (France)	79 mothers-infant dyads	In the last two months	Green vegetables	2-isobutyl-3-methoxypyrazine**	8-12 mo	At 8 months, neonates whose mothers consumed more green vegetables in pregnancy showed higher liking scores for the corresponding odour** ($p < .001$).	Mouthing (duration)	0.629 (0.240)	Maternal age, infant age, oronasal affections, infant gender, feeding style, breastfeeding

Note. h = hours, mo = months, d = days. * The analysis on the lactation period were not included in the review due to review aims. ** 2-isobutyl-3-methoxypyrazine, trimethylamine odorant corresponds to green vegetable food category.

Table 4.4. Studies that investigated whether infants have different behavioural profiles to the odour of their own amniotic fluid (AF) compared to unfamiliar AF or control

Reference (First, author, year, country)	Sample size	Attraction response to	Infant age at testing	Compared with (Control condition)	Outcomes	Infant behavioural measurement	Effect size Cohen's d (SEd)	Covariance and confounders controlled
Marlier 1998a (France)	38 mother-infant dyads	The odour of own AF	2-4 d	Distilled water	Infants head orientation was significantly longer duration towards own AF than distilled water ($p <$ .001).	Head orientation (duration)	1.067 (0.203)	Gestational age at birth, infant sex, ethnicity, Apgar scores, birthweight, delivery type, maternal age, maternal socioeconomic level, feeding type, parity, arousal level of infants at testing.
Marlier 1998b (France)	22 mother-infant dyads	The odour of own AF	15-57 h	Distilled water	Infants head orientation was significantly longer towards own AF than distilled water ($p <$ .01).	Head orientation (duration)	0.902 (0.253)	Gestational age at birth, infant sex, delivery type, Apgar scores, birthweight, maternal age, parity, socioeconomic level, breastfeeding, testing time, infants' hunger/satiety, and arousal level.
Schaal 1995a (France)	37 mother-infant dyads	The odour of own AF	13-47 h	Distilled water	Infants head orientation was significantly longer towards own AF than distilled water ($p <$ .0007)	Head orientation (duration)	0.649 (0.181)	Gestational age at birth, infant sex, delivery type, Apgar scores, birthweight, maternal age, parity, ethnicity, smoking status, socioeconomic level, breastfeeding, feeding type, age at testing.

Schaal 1998 (France)	12 mother-infant dyads	The odour of own AF	48-96 h	Non-familiar AF	New-born turned their nose significantly longer to their own AF than non-familiar AF (p = .01).	Head orientation (duration)	0.882 (0.34)	Gestational age at birth, infant sex, delivery type, Apgar scores, birthweight, maternal age, parity, infant age at test, breastfeeding, time on testing day, hunger/satiety, and arousal level.
Varendi 1996 (France)	30 mother-infant dyads	The odour of breast moistened with their own AF	6-23 min	Breast not moistened with own AF	A significant majority of new-born chose to feed on the breast moistened with their own AF (p <.001).	Head orientation (duration)	Not included in the meta-analysis (insufficient data to compute an effect size)	
Varendi 1998 (France)	32 mother-infant dyads	The odour of own AF	31-90 min	No odour	The crying times were significantly shorter in AF odour group than control group (p =.02).	Crying (duration)	-0.891 (0.371)	Maternal smoking status, delivery type, infant axillary temperature, parity, maternal age, infant sex, birthweight, infant age at test.

Note. min = minutes, h = hours, d = days, AF = Amniotic fluid.

Association between prenatal flavour exposure through maternal diet and infant behaviours

Table 4.3 shows the six studies (Faas et al., 2000, 2015; Hepper, 1995; Mennella et al., 2001; Schaal et al., 2000; Wagner et al., 2019) that had investigated whether infants show different behavioural profiles when they are exposed to flavour stimuli via maternal diet during pregnancy. The most used measures of infants' responses to prenatally exposed flavours were orofacial responses (k = 5) using action units based on muscular activation (Oster, 2006). Infant behavioural responses in the studies reviewed were mainly recorded offline and later analysed by trained coders who were blind to the hypotheses, condition, and type of stimulus. Only one study (Hepper, 1995) did not provide information about whether the behaviours were coded offline.

Flavours of alcohol (k = 2), anise (k = 1), carrot (k = 1), garlic (k = 1), green vegetables (k = 1) through maternal ingestion of foods or beverages during pregnancy were analysed. The two studies on alcohol flavour reported that all infants were healthy during the pre and postnatal periods. Despite showing evidence of the transfer of alcohol flavour from mother to fetus during pregnancy, two studies (Faas et al., 2000, 2015) were excluded from the meta-analysis due to insufficient data (Faas et al., 2000) to compute an effect size and for being the only study that measured appetitive responses (Faas et al., 2015). These two studies presented a series of 11 odour stimuli to test the odour-elicited reactions. The study (Faas et al., 2015) reported that babies born to frequent drinkers and stimulated with ethanol on 10 of 11 trials (Ethanol–Lemon–Ethanol sequence) exhibited significantly higher frequencies of appetitive responses relative to babies born to infrequent drinkers (p < .025), and the analysis produced the following individual effect size, d = 1.368 (0.476). Regarding the *timing* of prenatal flavour exposure, fetuses were exposed to specific target flavours mostly in the last two months of pregnancy. Only two studies (Faas et al., 2000, 2015) measured the weekly

flavour exposure throughout pregnancy. In terms of the *amount*, two studies (Mennella et al., 2001; Schaal et al., 2000) determined a minimum amount of flavour exposure four days a week. The *duration* of the flavour exposure in the studies was between one week (Hepper, 1995) and one month (Mennella et al., 2001). Examining studies, in terms of the *measurement of flavour consumption*, four observational studies measure the consumption of target flavour in the mothers' diet (Faas et al., 2000, 2015; Hepper, 1995; Wagner et al., 2019). One study (Mennella et al., 2001) gave the target flavour directly to mothers to eat. One study (Schaal et al., 2000) grouped the mothers based on their habitual intake of target flavour. In this study, the mothers were assigned to the experimental group if their diet involves the consumption of the target flavour. Experimental group mothers consumed the target flavour experimentally during the study. Total intake was calculated based on this experimental consumption as well as self-reported food consumption including target flavour.

In the meta-analysis (k = 4), there were 151 infants aged from 8 hours to 8 months showing reactions to specific flavours in three subcategories: reactions via the frequency of negative facial expressions, via the duration of head orientation and via the duration of mouthing behaviour towards the odour exposed during pregnancy.

The effects of prenatal flavour exposure on frequency of infant negative facial expressions. Facial configurations of nose wrinkling, brow lowering, upper lip raising, lip corner-depressing, lip stretching, gaping and head-turning away from a stimulus are defined as negative facial responses in new-borns, and are considered to express aversion towards a stimulus (Oster & Ekman, 1978; Rosenstein & Oster, 1988). It was hypothesized that infants who had been exposed to specific flavour in utero would postnatally show fewer negative facial responses to the matching flavour, compared to infants whose mothers did not ingest the flavour during pregnancy.

Two studies (Mennella et al., 2001; Schaal et al., 2000) were included in the meta-analysis measuring the frequency of negative facial expressions to the flavours that they were exposed to in the last trimester of pregnancy. Across these two studies, 52 infants (26 exposed, 26 not exposed) between 0.5 hours and 5.7 months old were assessed on the frequency of negative facial responses towards the flavour and odour stimuli that they experienced prenatally. Individual study effect sizes were -0.15 (Mennella et al., 2001; CI= -0.921 to 0.616, p = .70) and -1.72 (Schaal et al., 2000; CI= -2.906 to -0.527, $p \leq .005$). Because of the heterogeneity in the effect sizes between the studies (Q = 4.69, p = .030, I^2 = 78.68%), the random effects model is reported. The combined effect size for negative facial responses is not significant (d = -0.87, 95% CI = −2.39 to 0.66; Z = -1.11, p = .266). This result shows that the frequency of negative facial responses towards the target flavour was not significantly different between infants prenatally exposed to those flavours and infants who were not exposed (see Fig. 4.2).

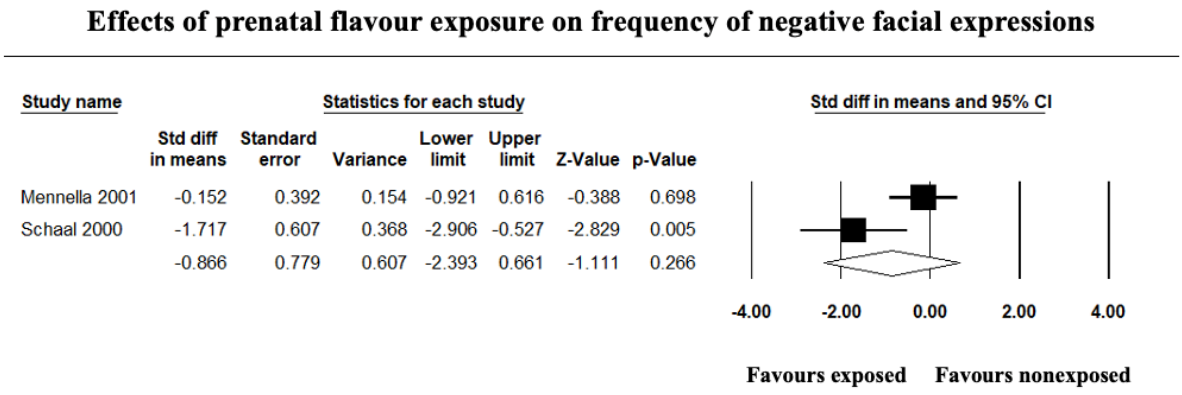


Fig. 4.2. Forest plot of meta-analysis of the effects of prenatal flavour exposure on negative facial expressions.

Note: Diamonds represent the overall effect sizes, with squares representing individual studies. The size of the squares represents the weights assigned to each study.

The effects of prenatal flavour exposure on duration of infant head orientation. Two studies (Hepper, 1995; Schaal et al., 2000) were included in the meta-analysis of the duration of head orientation towards the odour that was exposed during pregnancy. Across the two studies, 40 infants (20 exposed, 20 not exposed) between 15 and 110 hours old were assessed, by using a two-odour choice test, on the duration of head orientation towards different odours. Individual study effect sizes were 1.19 (Hepper, 1995; CI= 0.238 to 2.14, p = 0.014) and 1.29 (Schaal et al., 2000; CI= 0.324 to 2.25, p = 0.009). There is no evidence of heterogeneity (Q = 0.02, p = .887, I^2 < 0.01%), and therefore, the fixed effect model is reported. The combined effect size for head orientation is significant (d = 1.24; 95% CI = 0.56 to 1.91; Z = 3.58, p < .001). This demonstrates that infants prenatally exposed to odours through maternal diet had significantly longer duration of head orientation towards the same olfactory stimulus (see Fig. 4.3).

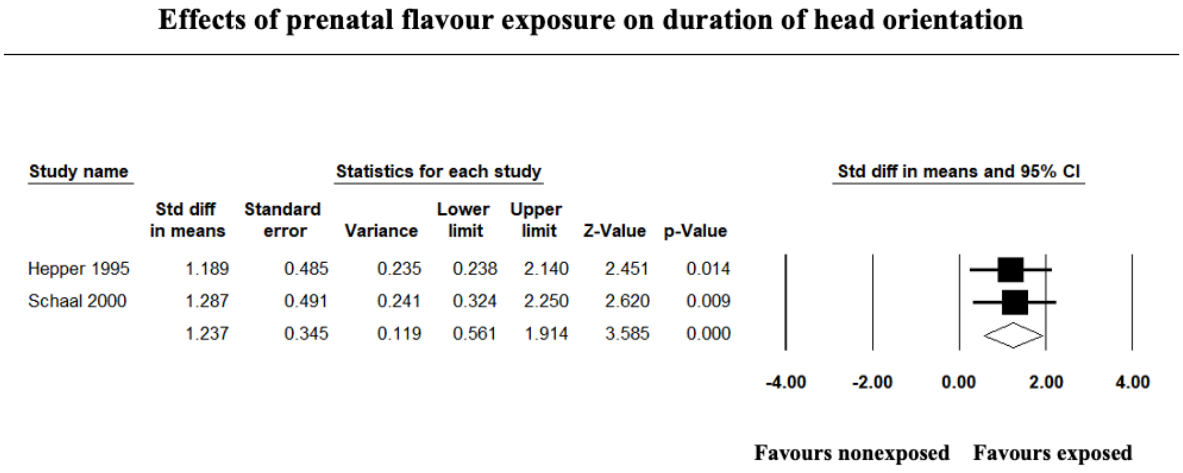


Fig. 4.3. Forest plot of meta-analysis of the effects of prenatal flavour exposure on duration of head orientation.

Note: Diamonds represent the overall effect sizes, with squares representing individual studies. The size of the squares represents the weights assigned to each study.

The effects of prenatal flavour exposure on duration of infant mouthing behaviour. Two studies (Schaal et al., 2000; Wagner et al., 2019) were included in the meta-analysis of the duration of mouthing behaviour to the prenatally exposed flavours. A total of 102 infants between 8 hours and 8 months old were assessed on the effects of anise or green vegetable exposure. Individual study effect sizes were 0.629 (Wagner et al., 2019; CI= 0.158 to 1.1, p=.009) and 1.035 (Schaal et al., 2000; CI=0.164 to 1.906, p=.02). There is no evidence of heterogeneity (Q = 0.646, p = .421, I^2 < 0.01%), and therefore, the fixed effect model is reported. The pooled effect size for mouthing behaviour is significant (d = 0.72; 95% CI = 0.306 to 1.136; Z = 3.409, $p \leq .001$) demonstrating longer duration of mouthing behaviours to prenatally exposed flavours (see Fig. 4.4).

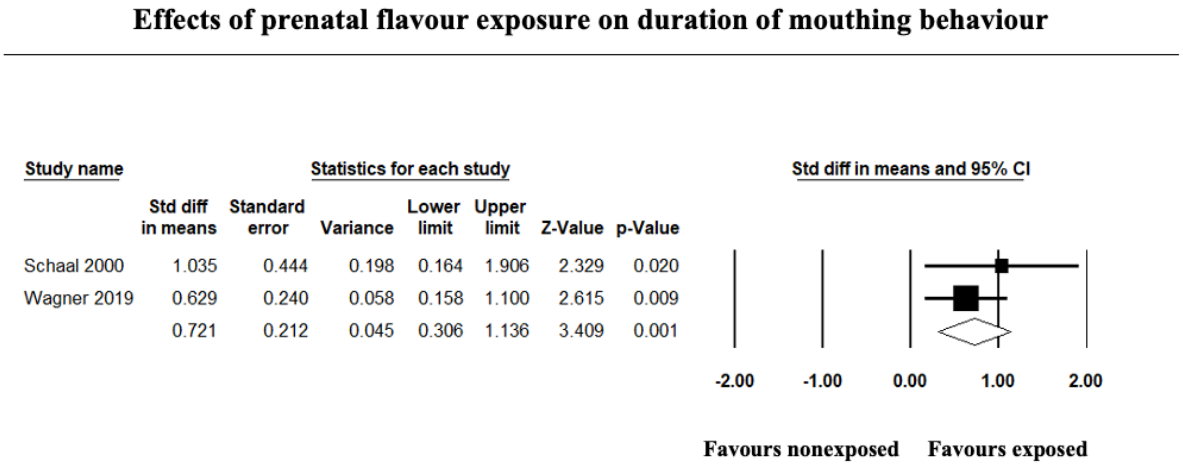


Fig. 4.4. Forest plot of meta-analysis of the effects of prenatal flavour exposure on duration of mouthing behaviour.

Note: Diamonds represent the overall effect sizes, with squares representing individual studies. The size of the squares represents the weights assigned to each study.

Association between the odour of amniotic fluid and infant behavioural profile

There are five studies that have investigated whether infants have different behavioural profiles to the odour of their own amniotic fluid that they have been exposed to throughout gestation (see Table 4.4). Five observational studies (Marlier et al., 1998a, 1998b; Schaal et al., 1995a, 1998; Varendi et al., 1996) assessed infant reactions to the familiar amniotic fluid measuring the duration of the head orientation towards the familiar stimulus, and one randomized control study (Varendi et al., 1998) measured the duration of crying. To measure head orientation towards a familiar amniotic fluid odour, all studies used a two-choice test involving the presentation of two stimuli placed symmetrically on either side of the infant's head. As a control stimulus, studies used distilled water (k = 3), non-familiar amniotic fluid (k = 1), the natural odour of the mother's breast (k = 1), and no odour stimulus (k = 1).

All studies except one (Varendi et al., 1996) measured only nasal chemoreception. In that study, infants were able to lick (oral response) and/or smell (nasal response) the mother's breast which was moistened by their own amniotic fluid. However, this study was not included due to insufficient data to compute the effect size, although they reported that a majority of new-born chose to feed on the breast moistened with their own amniotic fluid. Also, the study that measured the duration of crying (Varendi et al., 1998) was not included due to being the only study with this behavioural outcome. This study did however find a significant effect - crying times were significantly shorter in the amniotic fluid odour group than in the control group (d = - 0.891, p = .02).

In the meta-analysis, a total of 109 infants between five and 96 hours old were assessed by a two-choice odour test, on the duration of head orientation towards their own amniotic fluid odour paired with a control condition (e.g., distilled water, unfamiliar amniotic fluid or natural mother's breast odour). Individual study effect sizes ranged between 0.649

(Schaal et al., 1995a) and 1.067 (Marlier et al., 1998a). The effect sizes in these studies are homogeneous (Q = 2.417, p = .49, I^2 < 0.01 %), and therefore, the fixed effect model is reported. The combined effect size for the duration of head orientation is significant (d = 0.853; 95% CI = .632 to 1.073; Z = 7.58, p < .001, fail-safe N = 55). Visual inspection of the funnel plot (see Fig. 4.5) suggested a relatively symmetric distribution of study findings.

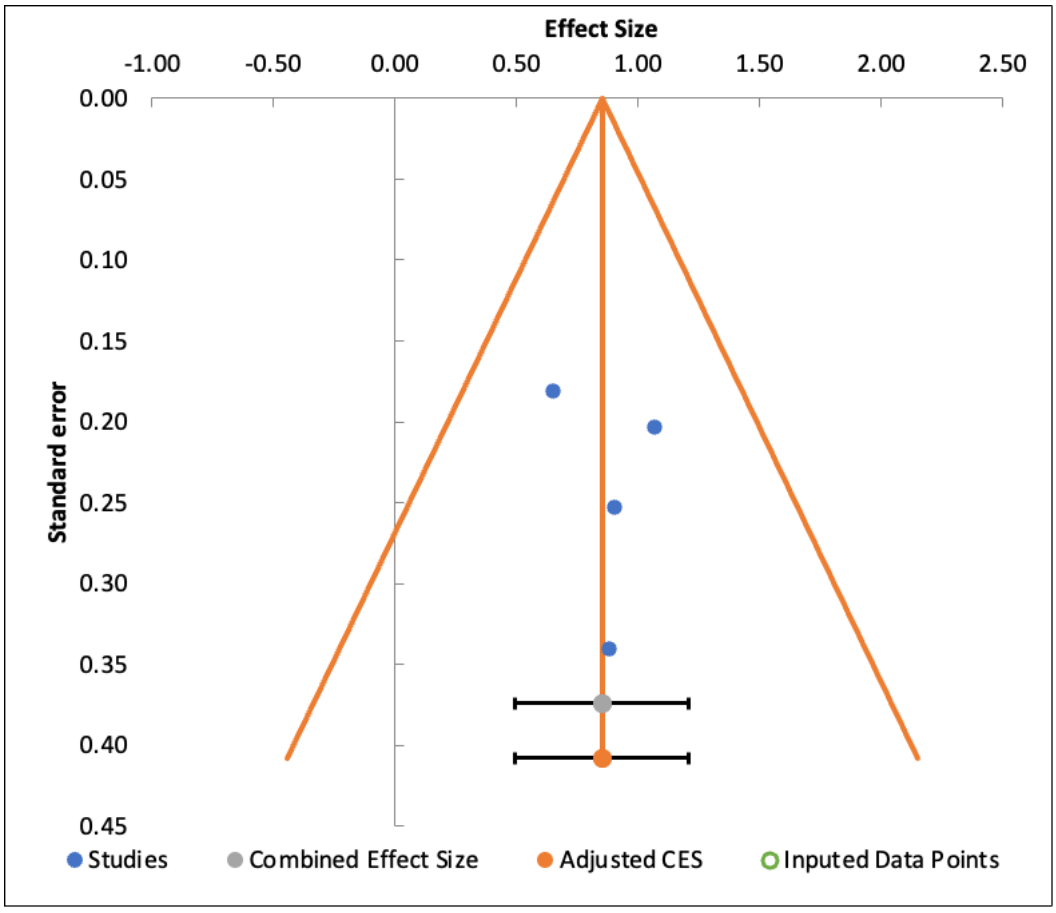


Fig. 4.5. Funnel plot for the association between the odour of amniotic fluid and the duration of head orientation.

Results showed that infants oriented their heads for significantly longer towards their own amniotic fluid compared to the control condition during the first two postnatal days (see Fig. 4.6).

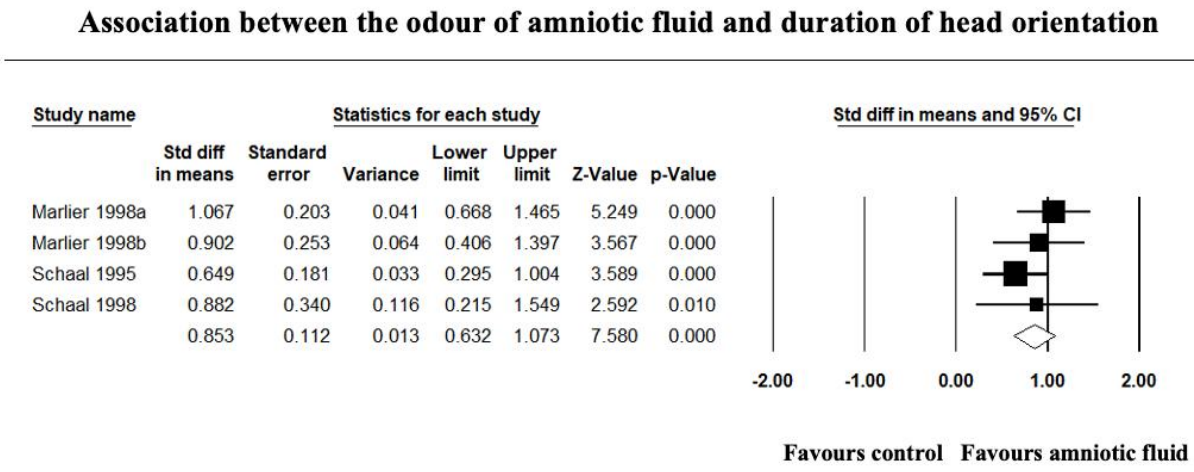


Fig. 4.6. Forest plot of meta-analysis of the association between the odour of amniotic fluid and duration of head orientation.

Note: Diamonds represent the overall effect sizes, with squares representing individual studies. The size of the squares represents the weights assigned to each study.

Discussion

The aim of this systematic review and meta-analysis was to examine whether there is a transnatal chemosensory continuum from fetal to the first year after birth. To answer this question, infant responses to flavours transferred via maternal diet prenatally and infant responses to their own amniotic fluid odour were analysed separately to distinguish between different (but related) media of transmission. Overall, the results presented here indicate that there is a chemosensory continuity from prenatal to the first year of postnatal life, although the effect of flavour exposure through maternal diet was not consistent across all types of postnatal reactions.

Studies included in the first group highlight that the effects of maternal consumption of specific flavours, including alcohol, carrot, anise, garlic and green vegetables influence infant responses to these flavours hours, days, and months after birth. Although, the findings

cannot be extrapolated to all flavours that the mother consumes during pregnancy, it supports the current evidence (Nicklaus & Schwartz, 2019; Schaal, 2006) that maternal dietary aromas are transferred to the fetal environment and that these flavour cues are sensed by fetuses and later accepted by infants. Thus, manipulating maternal diet can shape postnatal food preferences (Hetherington et al., 2015; Scaglioni et al., 2018).

In the meta-analysis of postnatal reactions to prenatally introduced flavours via maternal consumption of flavours, there were three subcategories: frequency of negative facial expressions, duration of head orientation, and duration of mouthing behaviour and. The studies compared infants who were exposed to a specific flavour at any point during pregnancy to infants whose mothers did not consume that flavour. Most studies did not use randomized designs. Prenatal flavour exposure through maternal diet resulted in a large significant effect in longer duration of head orientation (Hepper, 1995; Schaal et al., 2000) and mouthing behaviour (Schaal et al., 2000; Wagner et al., 2019) to the familiar flavour, which can be considered to represent preference, attraction, or acceptance (Hausner et al., 2010; Soussignan et al., 1997, 1999; Steiner et al., 2001). Although the pooled effect size was significant, the results in head orientation should be interpreted with caution because Hepper (1995) resulted in some concern in risk of bias analysis.

In contrast, the combined effect size of the other two studies that looked at negative facial expressions was non-significant. One of the two studies (Schaal et al., 2000) in that category found a significant individual effect size indicating that those infants born to mothers who ingested anise flavour in the last two weeks of pregnancy had fewer negative facial expressions after birth to anise odour. Although infants whose mothers consumed carrot juice showed fewer negative facial expressions towards carrot cereal over to plain cereal at approximately 6 months (Mennella et al., 2001), the effect size of the comparison between exposed and unexposed infants, the comparators included in the meta-analysis, when

fed with carrot-flavoured cereal (Mennella et al., 2001) was not significant. This result might be explained with the difference between the type of stimuli used at infant testing. Schaal et al. (2000) measured infant olfactory responses whereas Mennella et al. (2001) assessed gustatory responses. Despite the nonsignificant results between groups, the individual effect size could not be dismissed in one of the studies (Schaal et al., 2000) as well as the significant effect reported that infants exposed to carrot in the amniotic fluid preferred carrot-flavoured cereal over plain cereal (Mennella et al., 2001). Previous research indicated that negative facial configurations are more discriminating than positive ones, particularly when evaluating neonatal odour-elicited behaviours (Rosenstein & Oster, 1988; Soussignan et al., 1997, 1999; Steiner, 1979). Hence, it can still be argued that negative facial expressions are an effective method for measuring new-borns' facial reactions to stimuli.

The second group of studies increased our further understanding of the chemosensory continuum from prenatal to postnatal life by analysing infant reactions to the odour of their own amniotic fluid, this has not been examined in previous systematic reviews. This analysis showed that when infants are introduced postnatally to their own amniotic fluid odour, they displayed longer head orientation and shorter crying periods from birth to four postnatal days. Corroborating previous research findings (André et al., 2018; Liley, 1986; Mellor, 2019), these results showed that fetal chemosensory abilities are functional to detect olfactory molecules in their own amniotic fluid (André et al., 2018; Browne, 2008; Soussignan et al., 1997) and that these prenatal olfactory molecules were recalled after birth, resulting in soothing effect and preference for familiar chemosensory inputs. Furthermore, fetal nasal perception was sufficient to mediate the neonatal selective response because all studies included in the meta-analysis measured responses to olfactory stimuli.

The results indicate that human fetuses can detect flavour signals from their mothers' diets as well as the odour of their own amniotic fluid and embed information during

pregnancy for postnatal use. Repeated exposure to specific flavour through maternal consumption during the last two months of pregnancy is the optimal period to have the effects on postnatal behaviours. The effects of prenatal chemosensory inputs on postnatal outcomes were observed mostly in the first couple of weeks after birth but there were also effects after 6 and 8 months of life.

Strengths and limitations

This review advances current knowledge of prenatal and postnatal chemosensory continuity. The current systematic review is the first study which uses meta-analysis to examine whether there is transnatal chemosensory continuity from fetal to neonatal life by focusing on two related fields of research. A six-component search strategy was applied spanning four databases, as well as manual searches, to identify all relevant academic articles on this topic. The review does, however, have limitations, including the quantity of studies and confounding factors. Firstly, only four different studies were included in each group of meta-analysis because of a lack of studies in the subcategory or insufficient data reported. Secondly, individual variables such as fetal sex, birth outcomes, genetic determinants of chemosensory perception, maternal eating habits, socio-economic status, and ethnicity may affect the individual response to prenatal chemosensory inputs (Ashman et al., 2016; Beauchamp & Moran, 1982; Emmet et al., 2015; Mennella, 2005; Nisbett & Gurwitz, 1970; Okubo et al., 2014; Rauber et al., 2013). Additionally, because all studies investigating infant reactions up to one year old were included, the probability that flavour exposure during breastfeeding and/or complementary feeding can influence infant responses needs to be considered (Delaunay-El Allam et al., 2010; Hausner et al., 2010; Mennella et al., 2017; Schwartz et al., 2011a, 2011b). However, in all included studies, infant testing was completed when they were under the age of six months, which minimizes the effects of complementary

feeding considering that most infants have probably not yet been introduced to solid foods at this age (Delaunay-El Allam et al., 2010; Schwartz et al., 2011a).

Research recommendations

Measuring postnatal behavioural reactions to prenatally exposed flavours, odours, or tastes may provide indirect evidence of flavour transfer from maternal diet to fetal environment, fetal chemosensory abilities, fetal memory for flavours and thus chemosensory continuity from prenatal to postnatal life. Research on the fetal behaviour can provide us a direct measurement to understand how a fetus perceives and responds to the prenatal flavour environment. Longitudinal prospective studies starting from fetal stage until infancy, childhood and adulthood are required to explore chemosensory continuity over the life span. In conclusion, the results of this systematic review and meta-analysis support the hypothesis that there is transnatal continuity between prenatal and postnatal life.

Chapter 5

Facial reactions of human neonates to odours experienced in utero: A comparison to fetal reactions

Abstract

Fetuses are exposed to a variety of flavours, including olfactory and taste molecules, which cross the placenta from the mother's diet, and such prenatal experience extends postnatally. Experimental research to date has only investigated the effects of prenatal flavour exposure on postnatal outcomes. This study reports the first longitudinal study of pre-to-post repeated exposure by comparing fetal and infant facial responses to the odour they experienced repeatedly in the womb. Two groups of mother-infant dyads (carrot: n = 18, kale: n = 14) were followed from 32 weeks' gestation until their first postnatal month. Both groups of infants showed a greater frequency of laughter-face reactions and fewer frequency of cry-face reactions to the odours they had experienced prenatally, in comparison to the non-exposed and control odours. The longitudinal findings showed that the frequency of laughter face was increased whereas cry-face reactions were decreased from the fetal to neonatal period by repeated exposure in the last trimester. These findings suggest that prenatal exposure to a certain vegetable flavour heightens the preference for matching odour in the first postnatal month.

Introduction

Fetal chemo-perception, through their developing gustatory and olfactory abilities, is functional in the last trimester of pregnancy, enabling fetuses to sense olfactory molecules that pass from the mother's diet into the amniotic fluid while the nasal cavity is filled with it (Schaal et al., 1995a; Schaal, 2016; Ustun et al., 2022; Witt & Reutter, 1998; Witt, 2000).

Research have shown that these olfactory compounds, found in the womb, influence neonatal orofacial reactions to matched odour outside the womb (Hepper 1995; Schaal et al., 2000; Wagner et al., 2019). For example, new-borns displayed selective reactions to the odour of their own amniotic fluid compared to the odour of the unfamiliar amniotic fluid or control stimuli evidencing that the new-borns recognise odour cues that were encountered in the environment of the aquatic amniotic fluid (Contreras et al., 2013; Marlier et al., 1998a, b; Schaal et al., 1995a ,b, 1998; Soussignan et al., 1997; Varendi et al., 1996). Furthermore, human studies measuring facial responses to odorants exposed in amniotic fluid have reported a lower frequency of negative facial responses to anise odour in the group of new-borns whose mothers had consumed anise flavourings in the past two weeks before the labour compared to the group of new-borns whose mothers did not ingest the anise flavour (Schaal et al., 2000). Similarly, prenatal exposure to odour compounds such as vanilla, alcohol, anise, onion, garlic, apple, and cineole, has also been shown to induce a postnatal preference for these stimuli in rats, piglets, and dogs (Eade et al., 2010; Figueroa et al., 2013; Gaztañaga et al., 2017; Hepper & Wells, 2006).

Such prenatal exposures also influence the subsequent odour-liking responses in the first year of life (Wagner et al., 2019). Repeated flavour exposure in the womb provides fetuses with time to learn and assimilate the chemosensory information to access familiar flavour cues postnatally (Forestell & Mennella, 2015; Mellor, 2019; Schaal 2005). For instance, Wagner et al. (2019) found that infant reactions to the odour of often disliked foods,

such as green vegetables, were modified into liking responses via repeated exposure to target odour compounds during pregnancy. Together, these studies, based on postnatal investigations, suggest that there is a perinatal continuity not only in sensing stimuli but also in olfactory / gustatory memory. However, to date, there is no longitudinal investigation of the effects of prenatal flavour exposure from fetal to neonatal life.

The aim of this research was to address this gap by comparing responses from fetal face (pre-repeated-exposure) and neonatal face (post-repeated-exposure) to investigate the effects of repeated exposure. Based on previous research suggesting that prenatal flavour exposure positively influences preference for postnatal odour-elicited responses, it was hypothesised that regardless of the type of vegetable flavour exposed in the womb, new-borns will show more laughter-face responses to the target odour they repeatedly experienced prenatally compared to nontarget or control odours, whereas they will show more cry-face reactions to the nontarget odour they were not repeatedly exposed to during pregnancy compared to exposed or control odours. Second, it was hypothesised that the frequency of laughter-face reactions to the exposed flavour will be higher in the neonatal testing than it will be in the fetal testing whereas the frequency of cry-face reactions to the exposed odour will be lower in the neonatal testing than it will be in fetal testing.

Methods

Ethics

This study was conducted in accordance with the Declaration of Helsinki, and ethical permission was granted by Durham University (PSYCH-2019-03-12T15_59_32-wvgf27). Informed written consent was obtained from all mothers included in the study.

Participants

Thirty-five participants who were followed longitudinally from 32 weeks until the first postnatal month were recruited in this study. Although all participants in the prenatal

cohort (n = 99) consented to participate, 64 of them could not take part in the postnatal cohort due to Covid-19 pandemic restrictions. One infant was excluded because the mother did not consume the minimum amount of flavour after 36 weeks' gestation. Additionally, the data of two participants were removed due to technical problems during the video recording. Therefore, thirty-two healthy infants (16 female, 16 male) were included in the current study 14 of whom were exposed to kale flavour and 18 to carrot flavour in the last trimester of pregnancy. Sensitivity analysis indicated that 32 participants would be powered to detect (at 85%) effect sizes of Cohen's f = .32 to compare neonatal FMs depending on the flavour exposure.

All mother-infant dyads were White British, based in the northeast of England. Healthy mothers aged between 18-40 years old, with no known allergies, had a pre-pregnancy body mass index (BMI) of 18.5 to 30, no prescription drug or recreational drug use, and no smoking (including e-cigarettes and Nicotine Replacement Therapy) or alcohol consumption during pregnancy. All infants were born healthy (Apgar scores at 1 min ≥ 8 and at 5 min ≥ 9), with any known allergies, with a birth weight of ≥ 2500g and no NICU admission (see Table 5.1).

Table 5.1. Maternal-infant descriptive information

	Kale Flavour Group (n = 14)	Carrot Flavour Group (n = 18)
Infant sex	Female:5 Male: 9	Female:11 Male: 7
Head-circumference (HC) at 20-weeks in cm	M = 173.74 (2.025)	M = 164.19 (1.626)
Gestational age at birth	M = 39.14 (.398)	M = 39.37 (.346)
Birth weight in grams	M = 3246.86 (61.27)	M = 3406.22 (111.29)
Infant age at test	M = 3.05 (.232)	M = 3.07 (.203)

Maternal age in years	*M* = 30.50 (1.287)	*M* = 32.33 (.946)
Maternal pre-pregnancy BMI	*M* = 25.75 (.864)	*M* = 25.99 (.657)
Delivery type	Vaginal birth: 12 Caesarean section: 2	Vaginal birth: 13 Caesarean section: 5
Feeding type	Breastfeeding: 5 Bottle-feeding: 5 Both type of feeding: 4	Breastfeeding: 9 Bottle-feeding: 8 Both type of feeding: 1

Note: Values in parentheses are standard errors.

Fetal testing procedure and prenatal flavour exposure

Mothers were randomly assigned to the kale or carrot groups. Depending on their group, mothers consumed one capsule containing around 400 mg of either kale or carrot powder around 20 mins before undergoing a 4D ultrasound scan lasting around 25 minutes at 32- and 36-weeks' gestation (more detailed information is provided in Chapter 3). After the 36 weeks ultrasound scan, mothers were asked to consume the same flavour capsule daily at least for four days per week for three consecutive weeks. One infant's data was excluded because the mother only consumed three capsules after 36 weeks' gestation. The day, time, and amount of the capsules consumed were recorded using a weekly checklist to confirm that the number of capsules consumed did not significantly differ between the groups. The minimum number and frequency of capsule consumption were chosen based on previous studies that reported a significant effect of repeated prenatal flavour exposure on infant preference for a particular flavour (Mennella et al., 2001; Schaal et al. 2000). The average number of flavour capsules consumed was 14 (*SE* = .424, min:12, max:21) in the three weeks before birth (see Table 5.2). All mothers were instructed to consume the capsules between 10 a.m. and 3 p.m. each day to minimise any potential effects attributable to different levels of neonatal responses.

Table 5.2. Average amount of flavour exposure after 36 weeks' gestation

	Kale group (n = 14)	Carrot group (n = 18)
Flavour exposure in number of capsules	12.79 (.447) Min: 12, Max: 18	14.94 (.586) Min: 12, Max: 21
Flavour exposure in grams	5.11 (.179) Min:4.80, max:7.20	5.98 (.234) Min: 4.80, Max: 8.40

Note: Values in parentheses are standard errors. Min = minimum statistic, Max = maximum statistic.

Maternal vegetable consumption and mental health during the study

To control for any potentially confounding effects of maternal habitual intake of vegetables, the frequency of non-bitter (carrot and other non-bitter vegetables including potatoes, peas, beans, and sweet corn) and bitter vegetables (kale and other bitter vegetables including lettuce, leeks, and Brassica vegetables such as broccoli and cauliflower) were measured at three-time points, in the week prior to fetal scanning at 32 and 36 weeks and prior to infant assessment (see Table 5.3).

Exposure to maternal mental health problems during pregnancy and in the first postnatal year affects fetal and infant behaviour (Austin et al., 2005; Federenko & Wadhwa, 2004; Hanington et al., 2010; Perren et al., 2005). Therefore, maternal mental health including anxiety, depression, and perceived stress was recorded in the week prior to fetal scanning at 32 and 36 weeks and prior to the neonatal assessment which took place between two and four postnatal weeks (Cohen et al., 1983; Zigmond & Snaith, 1983).

Table 5.3. Mean frequencies of maternal vegetable consumption at three time-points

Maternal vegetable consumption	Kale group mothers (n = 14)	Carrot group mothers (n = 18)
Kale at 32 weeks (min=0, max=10)	3.36 (.599)	2.39 (.164)
Kale at 36 weeks	3.29 (.485)	2.31 (.198)

(min=0, max=10)		
Kale at postnatal stage (min=0, max=10)	3.43 (.499)	2.28 (.158)
Carrot at 32 weeks (min=0, max=10)	4.07 (.399)	3.89 (.212)
Carrot at 36 weeks (min=0, max=10)	3.93 (.385)	3.94 (.232)
Carrot at postnatal stage (min=0, max=10)	4.07 (.221)	3.83 (.202)
Other bitter at 32 weeks (min=0, max=10)	5.79 (.408)	5.78 (.348)
Other bitter at 36 weeks (min=0, max=10)	5.64 (.509)	5.69 (.326)
Other bitter at postnatal stage (min=0, max=10)	5.57 (.359)	5.72 (.266)
Other non-bitter at 32 weeks (min=0, max=10)	6.00 (.257)	6.44 (.305)
Other non-bitter at 36 weeks (min=0, max=10)	6.21 (.318)	6.31 (.313)
Other non-bitter vegetables at postnatal stage (min=0, max=10)	6.07 (.286)	6.17 (.232)
Overall bitter at 32 weeks (min=0, max=20)	9.14 (.895)	8.17 (.398)
Overall bitter at 36 weeks (min=0, max=20)	8.93 (.822)	8.00 (.365)
Overall bitter at postnatal (min=0, max=20)	9.00 (.770)	8.00 (.323)
Overall non-bitter at 32 weeks (min=0, max=20)	10.07 (.486)	10.33 (.464)
Overall non-bitter at 36 weeks (min=0, max=20)	10.14 (.592)	10.25 (.496)
Overall non-bitter at postnatal (min=0, max=20)	10.14 (.417)	10.00 (.379)

Note: Values in parentheses are standard errors.

Postnatal stimuli

During olfactory testing, three stimuli (carrot odour, kale odour, and control odours) were presented to the neonates in random order. Cotton swabs were odorised by using the kale and carrot powder, which was inside the flavour capsules, that mothers consumed before the ultrasound scans and during the last trimester of pregnancy. Hence, fetuses and neonates were exposed to relatively equivalent stimuli at testing. Postnatal odour stimuli were prepared by soaking the cotton swabs into the water and then adding either kale or carrot powder. A cotton swab soaked only in water was used as the control stimulus.

Infant testing procedure

The procedure was partially based on a previous experimental study that investigated infant reactions to the anise odour, they were exposed to in the last two weeks of pregnancy, at birth and on the fourth day after birth (Schaal et al., 2000). While separate contributions of odour, taste and trigeminal chemesthesis cannot be distinguished in the amniotic fluid environment, it is feasible experimentally to postnatally assess odour-elicited and/or taste-elicited reactions. Most research, including the current one, which has measured infant odour-elicited reactions over a short time after birth, (within the first two weeks) showed that infant reactions map onto ingestive behaviour (e.g., Marlier et al., 1997; Schaal et al., 2000). It is important to measure infant reactions as soon as possible post birth given that the effects of prenatal exposure might be obscured by their postnatal experience. However, it was not possible to visit mothers and their neonates at their home right after birth, therefore in our research infant reactions were assessed in a home setting at a one-time point in the first postnatal month (M = 3.06, SE = 0.15, min: 2 weeks max: 4 weeks).

The assessment was mostly (n = 22; kale:11, carrot:11) carried out by a researcher. However, due to the restrictions during the outbreak of the COVID-19 pandemic, some infants had to be tested by their own parents (n=10; kale:5, carrot:5) using online platforms

such as Zoom or Microsoft Teams. In this case, full guidance, and support on how to conduct the assessment were provided to parents prior to and during the session (see appendix 8).

All sessions were recorded, using a camera or online platform recording, for offline behavioural coding. To optimise the effect of the odour stimuli presented to the neonates, mothers were asked to feed their babies (breastfeeding or bottle-feeding) around 30 min before the session. Breastfeeding mothers were asked to avoid consuming foods containing carrot or kale before feeding. None of the neonates had been introduced to solid food by the time of their assessment and all sessions were completed in the afternoon around 3 p.m.

The experiment was conducted in a silent room. The TV was turned off, and there were no other distractions in the room (e.g., other people, animals, toys). The room temperature was normal (e.g., 25 °C). Only the experimenter and the parents were in the testing environment.

Before starting the experiment, *first,* the camera was arranged to be positioned against a plain background to ensure that the focus was on the infant's face and upper body during the experiment. *Second*, the carrot, kale and control odours were prepared while wearing different odourless, disposable gloves for each odour preparation. *Third,* the infant was secured upright in a bouncer, facing the camera. The process was the same when the parents conducted the session.

Three odours (Kale, Carrot and Water) were presented in two sets to the neonate during the experiment (e.g., Kale-Carrot-Water in the first round, Carrot-Water-Kale in the second round). The order of odour stimuli was randomised between the sets and between participants. Neonates were in a calm, active, and awake state before and during the odour presentation (as defined by Prechtl, 1974). Odours were presented under the infant's noses (midline), using ~ 20 cm long cotton swabs. Each odour was presented for 20 seconds with a 60-second interval between stimuli presentations. If neonates cried during the assessment, the

experiment was stopped until they were calm and soothed. None of the infants had any respiratory problems before or during the experiment.

Behavioural coding to analyse fetal and infant responses

The behavioural coding scheme and method of coding, including the details of ultrasound scan coding, have been described in full in Chapter 3 where fetal data are reported (Ustun et al., 2022) as well as in Chapter 2, and therefore only a brief description is provided here.

The coding process was the same in both the prenatal and postnatal stages. In both prenatal (32 and 36 weeks) and postnatal stages, 17 individual FMs and two facial gestalts (cry-face and laughter-face) were coded using the Observer®(15XT). Five FMs, including inner-brow raiser (FM1), brow lowerer (FM4), upper-lip raiser (FM10), lower-lip depressor (FM16), lip stretch (FM20), were coded as contributing to cry-face gestalt. Two FMs, including lip-corner puller (FM12) and tongue show (FM19), were coded as contributing to laughter-face gestalt. The remaining 10 FMs, including outer-brow raiser (FM2), cheek raiser (FM6), nose wrinkle (FM9), nasolabial furrow (FM11), lip pucker (FM18), lip presser (FM24), lips parting (FM25), jaw drop (FM26), mouth stretch (FM27), lip suck (FM28), can be observed in both gestalts.

In terms of the coding duration in the postnatal period, the duration of odour stimulation (20 sec) and post-stimuli (10 sec) were coded. The total duration of coding for each odour presentation was 60 seconds [(20 sec +10 sec) x 2 sets]. The relative frequency of FMs and facial gestalts per minute was calculated in both prenatal and postnatal stages to have a consistency between the three-time points. Inter-coder reliability analysis was applied to 15% of the dataset by three independent coders (Cohen's kappa 0.95, min:0.93 – max:0.96).

Fig. 5.1. Example of infant 'cry-face' responses to unfamiliar odours.

Note. Top panel: age at test 4 weeks; cry-face reactions to kale odour displayed by an infant prenatally exposed to carrot: left image illustrates a cry-face involving inner-brow raiser (FM1) + brow lowerer (FM4), right image illustrates a cry-face involving brow lowerer (FM4) + nose wrinkle (FM9) + lips parting (FM25). Bottom panel: age at test 3.6 weeks; cry-face reactions to carrot odour displayed by an infant prenatally exposed to kale: left image illustrates a cry-face involving nasolabial furrow (FM11) + lip stretch (FM20), right image illustrates a cry-face involving brow lowerer (FM4), nose wrinkle (FM9) + upper-lip raiser (FM10) + lips parting (FM25) + jaw drop (FM26).

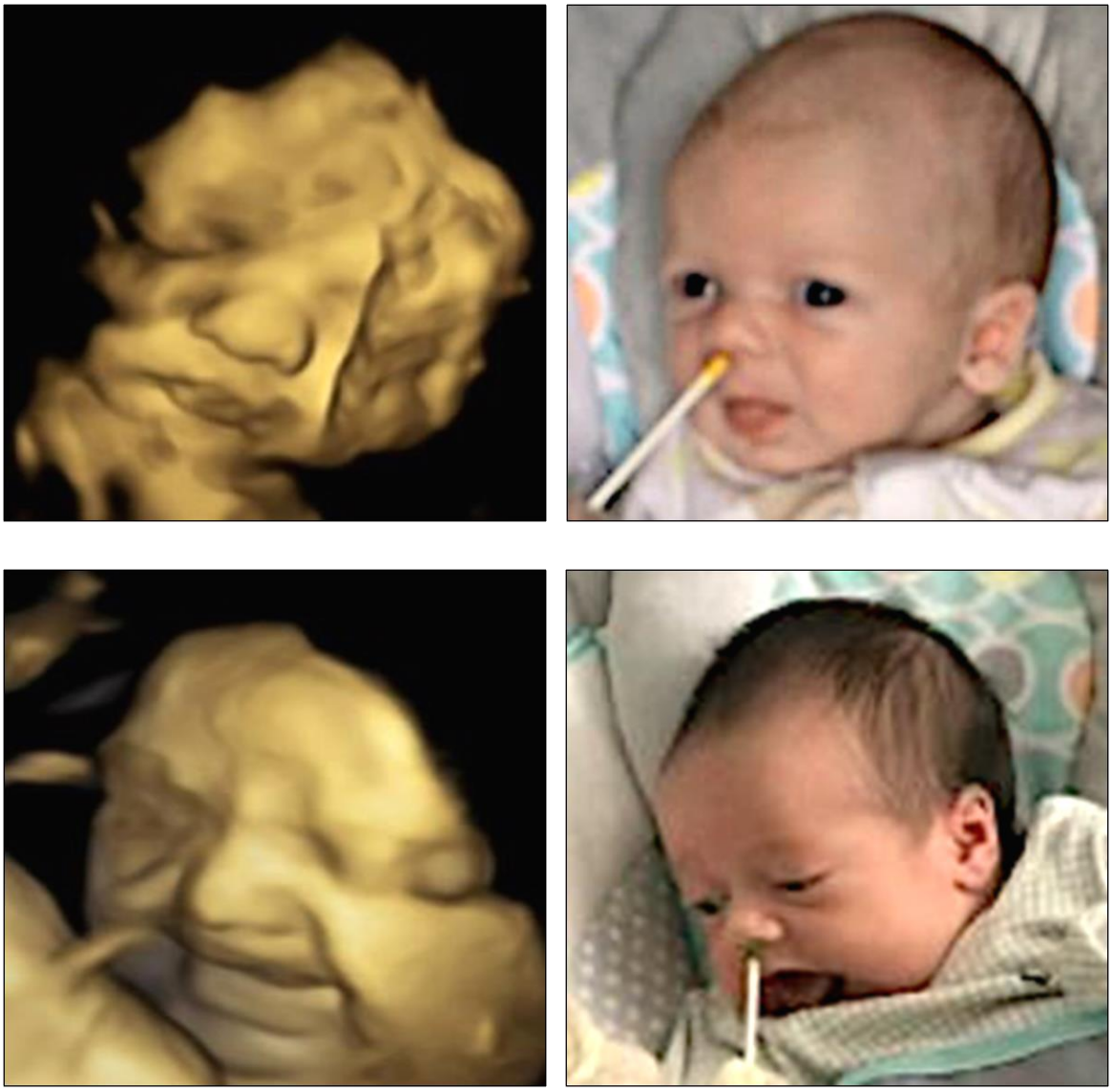


Fig. 5.2. Examples of the trajectory of reactions to familiar odour.

Note: Top panel: left and right images illustrate laughter-face reactions to carrot odour involving tongue show (FM19) + lips parting (FM25) at 32.5 gestational weeks (before repeated exposure) and involving tongue show (FM19) + lips parting (FM25) at 4 postnatal weeks (after repeated exposure), respectively. Bottom panel: left image illustrates a cry-face reaction to kale odour involving brow lowerer (FM4) + lip stretch (FM20) + lips parting (FM25) at 32.3 gestational weeks (before repeated exposure) and right image illustrates a laughter-face reaction involving tongue show (FM19) + lips parting (FM25) to kale odour at 3.6 postnatal weeks (after repeated exposure).

Statistical analyses

The study involved longitudinal sampling and a mixed design of two different flavour groups (kale and carrot) at three-time points (32-weeks, 36-weeks, first month after birth). All dependent data, the frequency of discrete facial movements and facial gestalts per minute, were log-transformed to normalise the dataset. For graphical data presentation, non-normalised scores were used.

The correlations of maternal (age, pre-pregnancy BMI and mental health), fetal (head circumference at 20 weeks, gestational age at birth, and birth weight) and infant (age at testing) variables with dependent variables (facial movements and gestalts) were tested using Pearson correlations. Additionally, maternal vegetable consumption and the number of kale and carrot exposures with capsules were tested using Pearson correlations to test any effects on the dependent variables. Feeding type (breastfeeding, bottle-feeding, both) delivery type (vaginal, caesarean) and maternal level of education was tested using one-way ANOVA to detect any significant covariances on the dependent variables. Independent *t* tests were used to test the effects of infant sex and postnatal testing method (in-person vs online) on dependent variables. The covariates were included in further analyses if they were significantly related to any of the fetal and/or infant reactions.

A multivariate analysis of covariance (MANCOVA) and a series of post hoc analyses were conducted to compare the mean frequency per minute of infant reactions to kale, carrot, and control odours between the kale and carrot exposure groups. To assess the developmental trend of the frequency of facial gestalts and discrete facial movements to the flavour they were exposed prenatally, a General Linear Model (GLM) for repeated measures including 3 different time points (32-weeks' gestation, 36-weeks' gestation, first postnatal month) was applied in both the kale and carrot exposure groups (within-group comparisons). An alpha

level of .05 was considered for all statistical analyses, which were conducted using the Statistical Package for Social Sciences (SPSS 27.0).

Results

Covariates

Covariates of dependent variables, including infant sex, maternal age, pre-pregnancy BMI, gestational age at birth, birth weight, delivery type, feeding type, maternal level of education, postnatal testing methods (in person vs online), the number of capsules consumed after 36 weeks' gestation were initially examined but removed from further analyses because these measures did not significantly affect fetal and infant facial movements. In terms of maternal vegetable consumption, kale, carrot and other bitter vegetable consumption at three-time points, other non-bitter vegetable consumption at 36 weeks was not included in the further analysis because they did not significantly relate to the dependent variables. In terms of maternal mental health, depression scores at 32 weeks and stress scores at 36 weeks were also removed from further analysis due to non-significant results. However, fetal head-circumference at 20 weeks' gestation, infant age at testing, maternal other non-bitter vegetable consumption at 32 weeks and postnatal stage, maternal anxiety scores at three time-points, maternal depression scores at 36 week and postnatal stage, maternal stress scores at 32 and postnatal stage were significantly related to one or more of the dependent variables and included in further analysis to control for significant covariances (see appendix 11).

Facial responses to different odours

Mixed MANCOVAs were performed with each odour (kale, carrot, and control odour) as the within-subject variable and the prenatal flavour exposure condition (kale flavour or carrot flavour) as the between-subject variable.

Analyses of discrete FMs. When the carrot odour was presented, the relative frequency of lip-corner puller (FM12), $t(28) = -3.614$, $p = .001$, $d = 1.911$; lip pucker (FM18), $t(29) = -$

2.268, p = .031, d = 2.25, was significantly greater in the group of neonates exposed to the carrot than the group of neonates who exposed to kale in utero. However, the relative frequency of upper-lip raiser (FM10), $t(16)$ = 2.679, p = .017, d = 1.451; lower-lip depressor (FM16), $t(16)$ = 2.404, p = .029, d = .4713; lip stretch (FM20), $t(16)$ = 3.162, p = .006, d = 1.319, lip presser (FM24), $t(19)$ = 2.098, p = .049, d = .7616, was significantly greater in the group of neonates who exposed to kale than the group of neonates who exposed to carrot in utero (see Fig.5.3).

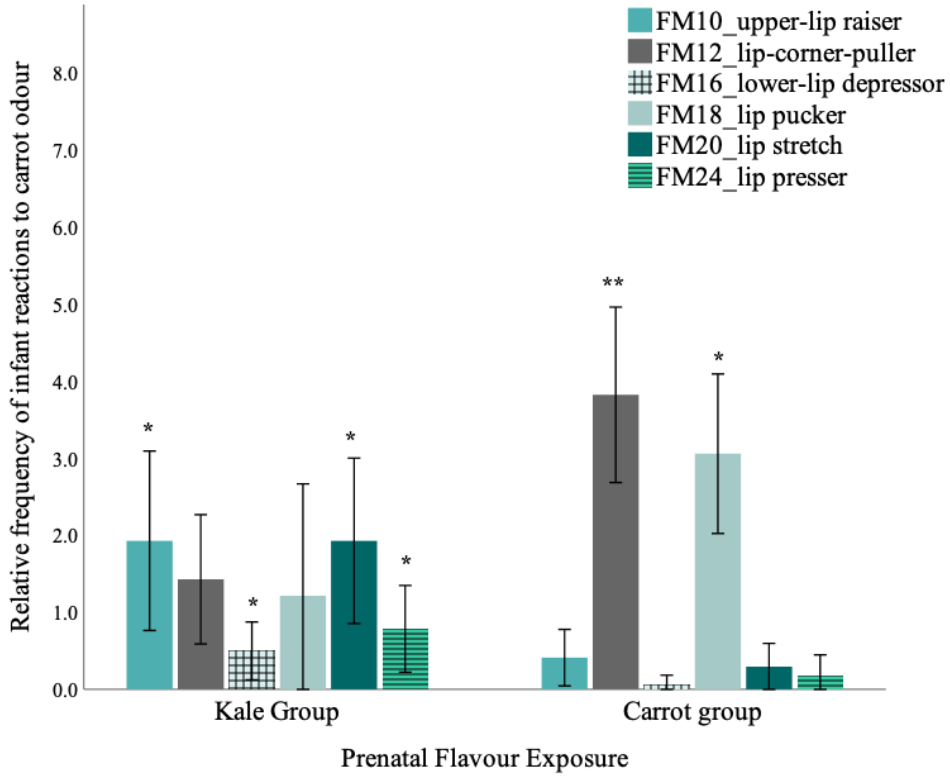


Fig. 5.3. Relative mean frequency of infant discrete facial reactions to carrot odour split by prenatal exposure group.

Note: Error bars represent 95% CI, ** p < .001 in between-group analyses.

When the kale odour was presented, the relative frequency of lip-corner puller (FM12), $t(14) = 5.687$, $p < .001$, $d = 1.192$; lip pucker (FM18), $t(17) = 2.481$, $p = .024$, $d = 1.520$ was significantly greater in the group of neonates who exposed to kale than the group of neonates who exposed to carrot in utero. However, the relative frequency of lower-lip depressor (FM16), $t(17) = -2.695$, $p = .015$, $d = 2.975$; lip stretch (FM20), $t(18) = -5.439$, $p < .001$, $d = 2.586$; lip presser (FM24), $t(16) = -2.757$, $p = .014$, $d = 4.061$, was significantly greater in the group of neonates who exposed to the carrot than the group of neonates who exposed to kale in utero (see Fig.5.4).

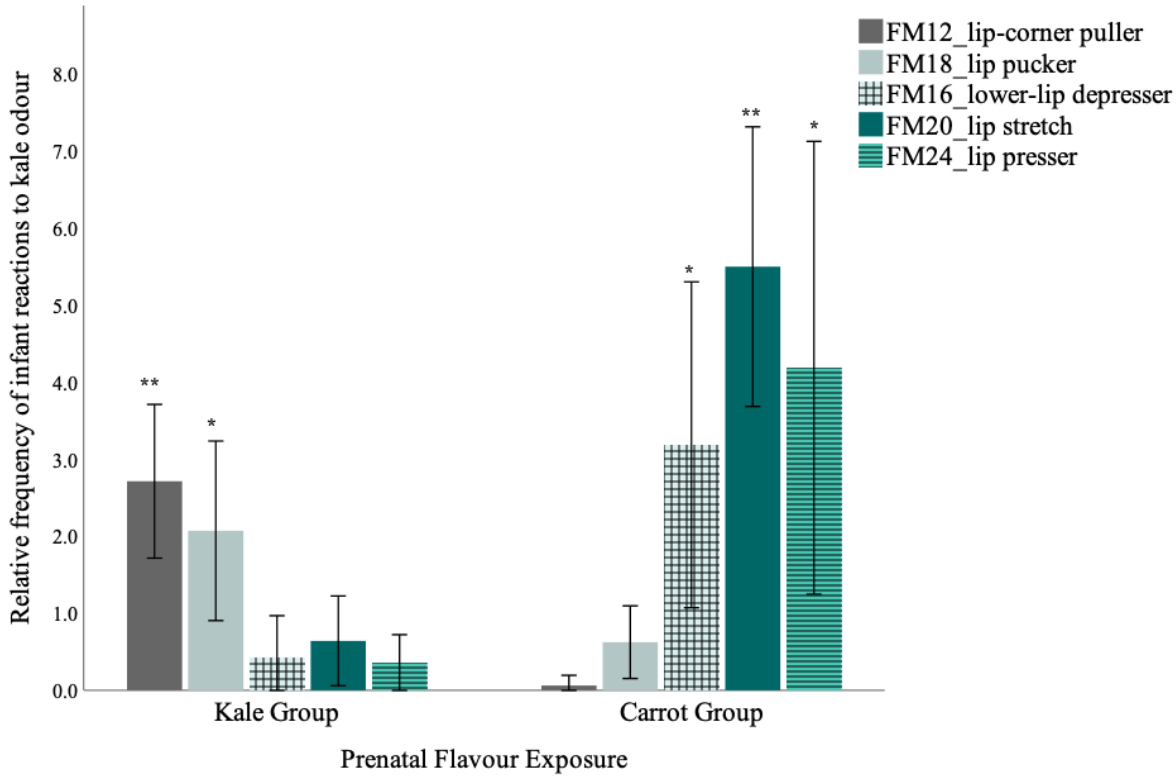


Fig. 5.4. Relative mean frequency of infant discrete facial reactions to kale odour split by prenatal exposure group.

Note: Error bars represent 95% CI, ** $p < .001$ in between-group analyses).

Analyses of facial gestalts

Cry-face reactions. There was a significant main effect of the type of odour on the cry-face gestalt regardless of the prenatal flavour exposure condition, $F(2, 44) = 6.833$, $p = .003$, $n_p^2 = .237$, with higher frequency to kale odour ($M = .7572$, $SD = .5354$) than to carrot ($M = .4621$, $SD = .501$) and control odour ($M = .5852$, $SD = .4490$). There was also a significant interaction between the type of odour and the prenatal flavour exposure condition of the neonate, $F(2, 44) = 63.058$, $p < .001=1$, $n_p^2 = .741$.

The group of neonates who were exposed to carrot in utero ($M = 1.2126$, $SE = .2294$) reacted to kale odour with higher frequency of cry-face gestalt compared to the group neonates who were exposed to kale in utero ($M = .2548$, $SE = .0511$), $t(28) = -12.317$, $p < .001$, $d = .212$. In contrast, kale group neonates ($M = .8312$, $SE = .113$) showed higher frequency of cry-face gestalt to carrot odour in comparison to the group neonates who were exposed to carrot prenatally ($M = .0521$, $SE = .0142$), $t(29) = -7.536$, $p < .001$, $d = .286$. There was no significant difference between the groups when the control odour was presented.

Laughter face reactions. There was a significant main effect of the type of odour on the laughter-face gestalt regardless of the prenatal flavour exposure condition, $F(2, 44) = 9.626$, $p < .001$, $n_p^2 = .304$, with higher frequency to carrot odour ($M = .8918$, $SD = .408$) then to kale ($M = .6513$, $SD = .507$) and control odour ($M = .5366$, $SD = .4755$). There was also a significant interaction between the type of odour and the prenatal flavour exposure condition of the neonate, $F(2, 44) = 23.491$, $p < .001=1$, $n_p^2 = .516$.

Carrot-exposed neonates prenatally ($M = 1.077$, $SE = .0582$) showed a higher frequency of laughter-face gestalt to carrot odour compared to kale-exposed neonates ($M = .6070$, $SE = .1123$), $t(29) = -3.911$, $p < .001$, $d = .333$. But, kale-exposed neonates ($M = .9349$, $SE = .1130$) showed a higher frequency of laughter-face gestalt to kale odour

compared to carrot-exposed neonates (M = .343, SE = .0719), $t(28) = 4.531$, $p < .001$, d = .3568. There was no significant difference between the groups when the control odour was presented.

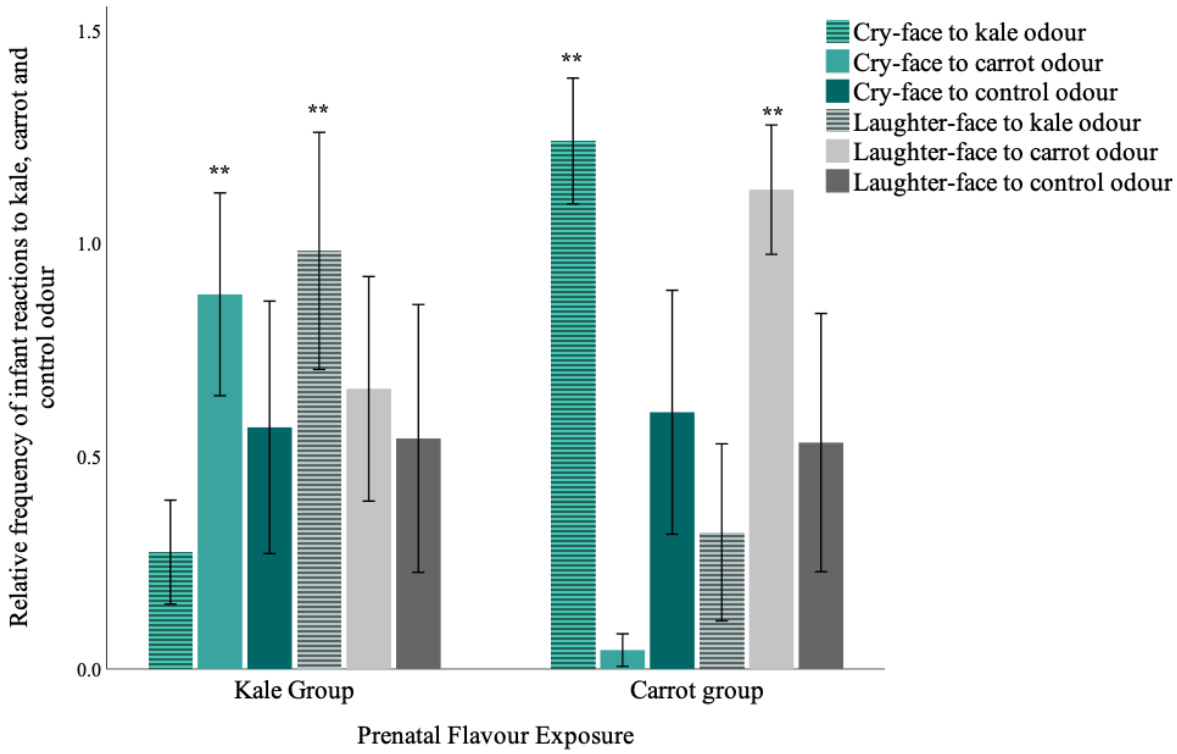


Fig. 5.5. Relative mean frequency of infant facial reactions (gestalts) to kale, carrot and control odours split by prenatal exposure group.

Note: Error bars represent 95% CI, ** $p < .001$ in between-group analyses).

Longitudinal facial responses to odours from fetus to new-born: Within-group comparisons

Facial responses to carrot flavour in carrot-exposed infants. Infants whose mothers consumed carrot in the last trimester of pregnancy showed laughter-face responses to carrot flavour with an increased frequency per minute from prenatal to postnatal measurement, $F(2, 28) = 58.55$, $p < .001$, $n_p^2 = .807$. Although there was no significant difference between 32

weeks and 36 weeks' gestation, contrast revealed a significant increase between 32 weeks (M = .34, SE = .048) and postnatal measurements (M = 1.093, SE = .065), $F(1, 14) = 80.85$, $p < .001$, $n_p^2 = .852$, and a significant increase between 36 weeks (M = .37, SE = .076) and postnatal stage, $F(1, 14) = 99.582$, $p < .001$, $n_p^2 = .877$ (see Fig. 5.6, top panel).

In terms of cry-face reactions to carrot flavour, there was a significant decrease in the frequency of cry-face gestalt from the prenatal to the postnatal period, $F(2, 28) = 8.782$, $p = .001$, $n_p^2 = .385$. There was no significant difference between 32 weeks and 36 weeks, but there was a significant decrease between 32 weeks (M = .150, SE = .029) and the postnatal period (M = 0.59, SE = .015), $F(14) = 13.991$, $p = .002$, $n_p^2 = .500$; and significant decrease between 36 weeks (M = .219, SE = .049, p = .004) to postnatal period (see Fig. 5.6, bottom panel).

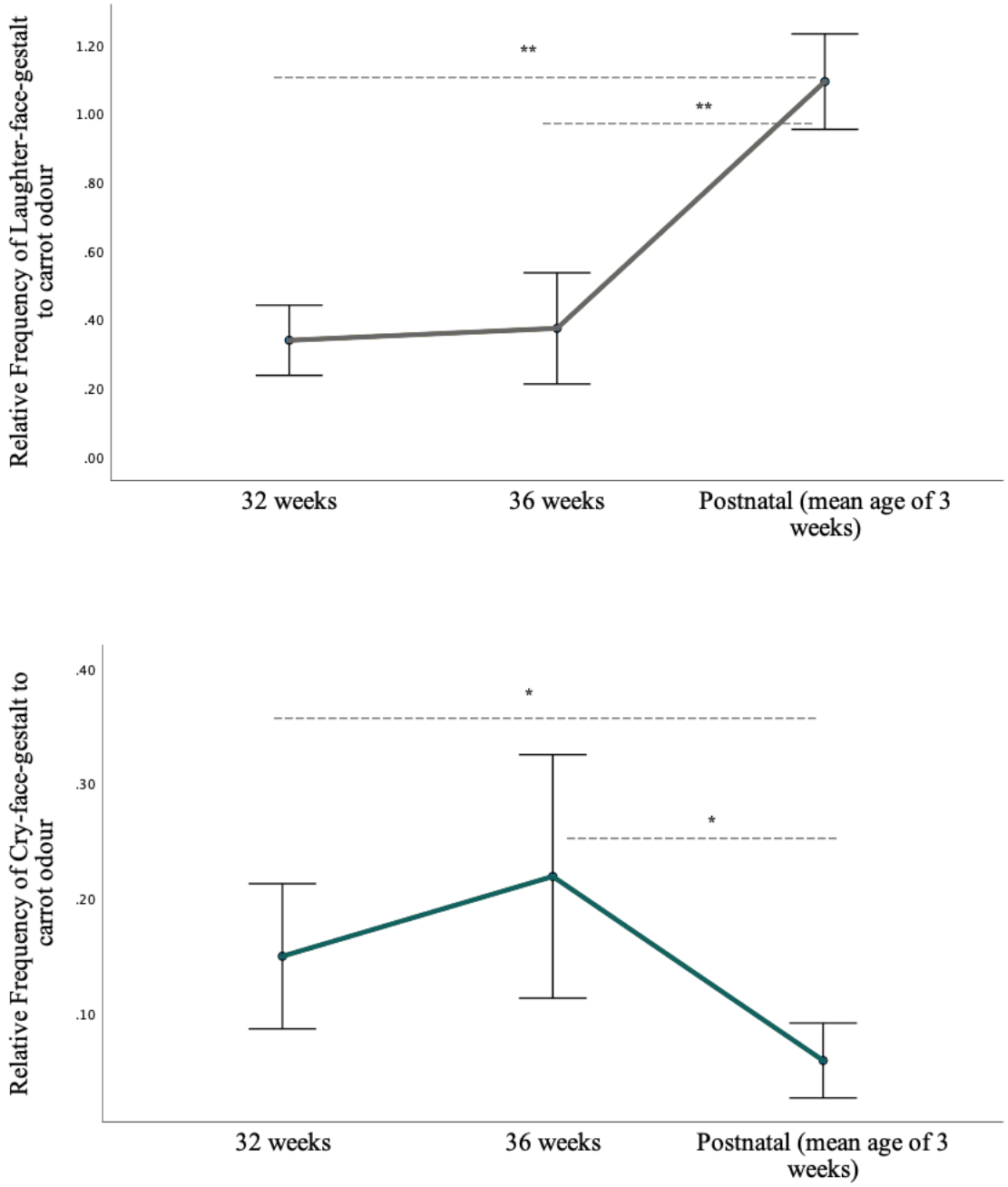


Fig. 5.6. Relative mean frequency of laughter-face-gestalt (top panel) and cry-face gestalts (bottom panel) reactions to carrot odour in the carrot group of neonates split by age.

Note: Error bars represent 95% CI, ** $p < .001$ in within-group analyses).

Facial responses to kale flavour in kale-exposed infants. Infants whose mothers consumed kale in the last trimester of pregnancy showed laughter-face responses to kale flavour with an increased frequency per minute from prenatal to postnatal measurement, $F(2, 26) = 49.771$, $p < .001$, $n_p^2 = .793$. There was no significant difference between 32 and 36-weeks measurements. However, there was a significant increase between 32 weeks ($M = .018$, $SE = .010$), and the postnatal stage ($M = .94$, $SE = .113$), $F(1, 13) = 68.788$, $p < .001$, $n_p^2 = .841$, and a significant increase between 36 weeks ($M = .12$, $SE = .068$) and postnatal stage, $F(1, 13) = 50.094$, $p < .001$, $n_p^2 = .794$ (see Fig. 5.7, top panel).

In terms of cry-face reactions to kale flavour, there was a significant decrease in the frequency of cry-face gestalt from the prenatal to the postnatal period, $F(2, 26) = 18.327$, $p < .001$, $n_p^2 = .585$. There was no significant difference between 32 weeks and 36 weeks, but there was a significant decrease between 32 weeks ($M = .465$, $SE = .063$) and the postnatal period ($M = .255$, $SE = .051$), $F(1, 13) = 18.943$, $p < .001$, $n_p^2 = .593$; and a significant decrease between 36 weeks ($M = .629$, $SE = .080$) to the postnatal period, $F(1, 13) = 24.758$, $p < .001$, $n_p^2 = .656$ (see Fig. 5.7, bottom panel).

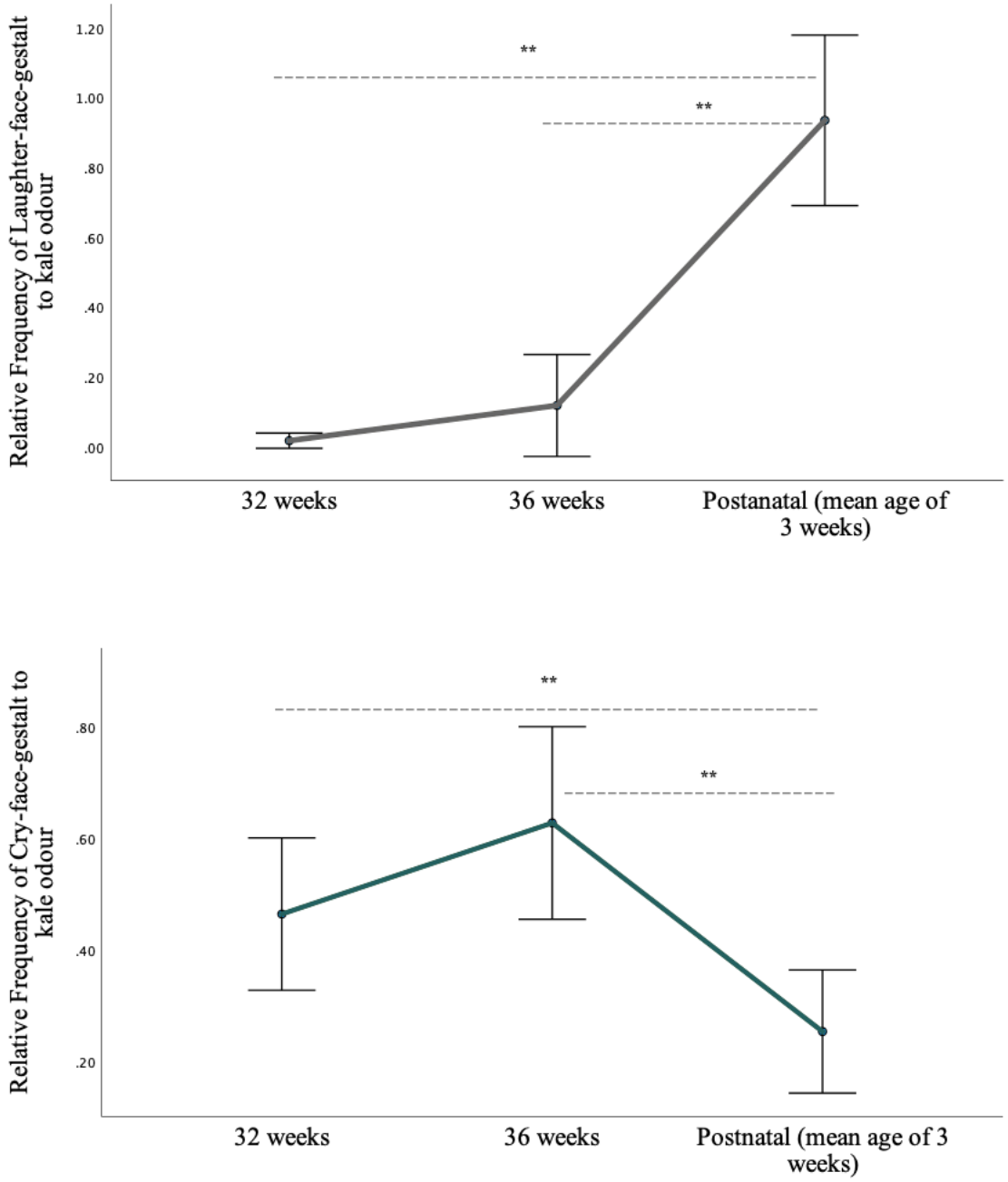


Fig. 5.7. Relative mean frequency of laughter-face reactions (top panel) and cry-face reactions (bottom panel) to kale odour in the kale group split by age.

Note: Error bars represent 95% CI, ** $p < .001$, * $p < .05$ in within-group analyses.

Discussion

In this study, neonatal odour-elicited responses to maternal ingestion of carrot or kale capsules during the last three weeks of pregnancy were investigated. Supporting our hypothesis, overall results showed that neonatal facial reactions to kale, carrot or control odours varied as a function of repetitive stimulation with the odour cues transferred by maternal consumption. When presented with the vegetable odour (kale or carrot) which they had experienced in utero, neonates exhibited more laughter-face gestalts; in contrast, they showed more cry-face gestalts when presented with the unfamiliar vegetable odour. Finally, following repeated exposure from 36 weeks for three consecutive weeks, infants (fetuses/ neonates) reacted to the flavour they were exposed to with an increasing frequency of laughter-face gestalts and decreasing frequency of cry-face gestalts from prenatal to postnatal assessments.

Perinatal continuity in sensing odour stimuli and memory

Neonatal reactions observed in this study in both flavour groups provide evidence that exposing a kale or carrot flavour (around a total of 5 g) in the last three weeks of pregnancy was sufficient for fetuses to practice and memorise the odour cues passed the placenta. These results corroborate with previous studies that have shown that exposure to similar dosages of flavours in the third trimester of pregnancy is effective for a fetus to learn about specific flavours and that these memories of odours might persist postnatally for a few hours, days, months and potentially years after birth (Hepper, 1995; Hepper et al., 2013; Schaal et al., 2000; Wagner et al., 2019). In line with studies reporting short-term effects (Hepper et al., 1995; Schaal et al., 2001), the effect of prenatal exposure occurred in this study within between two and four weeks. The results also supported previous evidence on the transition of perception of chemicals from aquatic (intrauterine/amniotic fluid) to airborne forms

(extrauterine), suggesting the perinatal continuity of sensing stimuli from prenatal to postnatal life (Hepper 1995; Marlier et al., 1998 a, b; Schaal et al., 2000).

Neonatal facial reaction is specific to flavour exposure in utero

The anatomical specificity of new-born facial reactions found in this study is consistent with prior studies investigating flavour-elicited reactions postnatally (Mennella et al., 2001; Schaal et al., 2000). When neonates were presented with the kale odour, those infants who had been exposed to kale prenatally reacted with a higher frequency of lip-corner-puller (FM12), lip-pucker (FM18) and the combination of movements resulting in a laughter-face gestalt in comparison to the group of neonates who were exposed to carrot prenatally. In contrast, carrot group neonates who were exposed to carrot flavour prenatally reacted to kale odour stimulus with a higher frequency of upper-lip raiser (FM10), lower-lip depressor (FM16), lip stretch (FM20), lip presser (FM24) and cry-face gestalts.

Carrot odour presentation resulted in greater lip corner puller (FM12), lip pucker (FM18) as well as the combinations generating laughter-face gestalts in the carrot group exposed to carrot in the last three weeks of pregnancy. In contrast, neonates who were exposed to kale during pregnancy reacted to carrot odour with a higher frequency of lower-lip depressor (FM16), lip stretch (FM20), lip presser (FM24), and the combination of movements contributing to a cry-face gestalt.

Given that laughter-face reactions resemble positive hedonic reactions and cry-face reactions resemble negative hedonic reactions postnatally (Berridge, 2000; Mennella et al., 2001; Schaal et al., 2000; Steiner et al., 2001), the findings of the present study suggest that neonates prefer the odour of vegetables which they were exposed in the last trimester of pregnancy.

Repeated prenatal flavour exposures heighten odour-liking responses in neonates

The results indicated that, with repeated exposure to a certain odour (kale or carrot) from 36 weeks' gestation onwards, an increase in the frequency of laughter face reactions and a decrease in the frequency of cry-face reactions could be observed from prenatal to postnatal period. Although kale group fetuses prenatally reacted to the kale flavour with cry-face gestalts at 32 and 36 weeks, the results suggested that these initial responses can be modified into laughter-face gestalts by repeated exposure. This result expands on the findings of a previous study (Wagner et al., 2019) showing the effects of repeated exposure on liking responses to green vegetable odour and provided the first longitudinal experimental evidence in humans.

The results of this study suggest that introducing various flavours starting the prenatal period can attenuate innate flavour preferences by increasing their familiarity with different tastes and odours and may ultimately facilitate acceptance of healthy green vegetables such as kale, and broccoli which are usually unpalatable for children (Beauchamp & Mennella, 2009; 2011; Cooke &Wardle, 2005; Mennella & Bobowski, 2015).

Limitations and future directions

Individual differences in taste sensitivity (Cont et al., 2019; Feeney, 2011) and maternal awareness of the experimental conditions are some of the limitations of this longitudinal study. Similarly, new-borns may have seen the shades of the vegetable powder because the type of the powder affected the colour of the cotton part of the swab. However, since behavioural data coders are blinded to the condition, these are unlikely to affect results. Furthermore, the maternal consumption after 36 weeks was based on maternal self-report. Future research should aim to obtain an objective measure of vegetable consumption, such as using mobile apps to measure capsule intake (May et al., 2022). This study shows that the memory traces of prenatal experiences with certain flavours persist at least until the first

month after birth. However, the results cannot be extrapolated to longer time periods or to different flavours. Future studies need to include a long-term follow-up spanning childhood and possibly into adulthood to investigate the chemosensory longevity of prenatal exposure.

Conclusion

This is the first longitudinal prospective study that assessed fetal and neonatal responsiveness to kale or carrot odour experienced in the womb at three different time points, ranging from the third trimester of pregnancy (32 and 36-weeks' gestation) to the first postnatal month. Overall, our results provide human experimental evidence that neonates reacted to a familiar vegetable odour, which they experienced prenatally, with a laughter-face gestalt, but they reacted to an unfamiliar vegetable odour with more cry-face gestalt. Also, after repeated prenatal flavour exposure in both groups from the prenatal to the postnatal period, laughter-face responses to odour increased whereas cry-face responses decreased. These findings have important implications for our understanding of perinatal continuity in stimuli perception and memory from fetal to neonatal life.

Chapter 6

General Discussion

The studies presented in this thesis have investigated the effects of prenatal flavour exposure on fetal and infant behaviours, as well as the longitudinal developmental trend of these behaviours from fetal to neonatal life. This final chapter provides an overview of key findings of each study, highlights how these findings contribute to the existing literature, outlines the implications of these findings and directions for future research. Lastly, this chapter discusses the limitations of the current work.

Summary of findings

Chapter 3 reported the first direct evidence of human fetal facial responses to flavours passed from the mother's diet by using advanced 4D ultrasound imaging. Facial reactions from 99 fetuses were analysed in terms of their timing, specificity, and complexity from 32 to 36 weeks. The seminal consequences are that: i) fetal responses were recorded non-invasively in utero relying on fine-grained quantitative analysis of fetal facial response. The study established that late-gestation fetuses react selectively to two contrasting flavours (non-bitter vs. bitter) that reach them around 30 mins after the pregnant mother's ingestion of specific flavours. ii) These responses took the form of structurally differentiated "gestalts" or facial reactions (see images in Chapter 3). Fetuses exposed to carrot flavour showed "laughter-face" reactions more frequently, while those exposed to kale flavour showed more frequent "cry-face" reactions in comparison to each other as well as to the control group fetuses. iii) Based on previous data on neonatal responsiveness (Hepper 1995; Mennella et al., 2001; Schaal et al., 2001; Wagner et al., 2019), these responses can be interpreted in terms of acceptance and rejection of certain flavours. Finally, iv) the longitudinal follow-up (32 to 36 gestational

weeks) of the fetuses indicated that the flavour-elicited facial responsiveness develops in complexity, suggesting joined effects of neuromuscular maturation and experience.

Following the prenatal study, Chapter 4 (investigating 260 infants from pregnancy until the first year of life - the systematic review and meta-analysis) and Chapter 5 (investigating 32 neonates from 32 weeks until the first postnatal month) established that flavour exposure in the last trimester of pregnancy heightens preference for the matching taste/odour in the short term after birth (from day 1 until the age of 6 months). Neonates between two-four weeks old (Chapter 5) reacted to flavours to which they had been exposed in utero with a higher frequency of laughter-face reactions whereas they showed more cry-face reactions to the flavours they had not experienced prenatally. The important finding from the longitudinal analysis (Chapter 5) showed that the initial response to flavour in utero (after two exposures at 32 and 36 weeks) was altered into a qualitatively different pattern after 12 consecutive exposures from 36 weeks' gestation until birth, irrespective of the type of flavour. Neonates showed a higher frequency of laughter-face response and a lower frequency of cry-face response to odours after repeated exposure compared to their initial response in utero. This yielded cutting-edge evidence regarding i) the transfer of flavour compounds from the maternal diet to the fetal compartment ii) prenatal maturation of human reactions to flavours in the amniotic fluid including a positive maturational predisposition for liking bitter vegetable flavours iii) the degree of exposure to vegetable flavours in utero can facilitate postnatal reactions iv) the timing, the degree and the type of flavour intrauterine exposure in determining the efficacy of exposure.

Contributions to the literature: Timeliness, Novelty, and Impact

Although earlier studies (e.g., Marlier et al., 1998a, 1998b; Mennella et al., 2001; Schaal et al., 2000) already demonstrated that fetuses can detect and memorize flavour and

odour cues in their prenatal environment through tests conducted a posteriori in neonates or infants, this thesis is the very first longitudinal work comparing the fetal chemoreceptive responses measured in utero after the mothers' consumption of flavours and neonatal responses to these prenatally repetitive experienced flavours. Results of this study also are the very first to be produced with such a level of reliability in the behavioural analysis of undisturbed, healthy fetuses responding to a low-intensity stimulus, comparable to those received in normal uterine life, after maternal flavour consumption. The study has thus novel and important contributions to the literature and has real-world implications.

The findings showed indirect evidence that flavour compounds that are ingested by the mother can rapidly find their way into the bloodstream and subsequently into the amniotic fluid that surrounds the fetus. So far, we have little knowledge about the transplacental kinetics of flavours ingested by mothers, but this relatively short transfer time is compatible with the pharmacokinetic data on drugs in the human materno-fetal system (for a review, see Schoretsanitis et al., 2021; Tse et al., 2022). This finding also supports experiments measuring sensory analysis of the amniotic fluid itself and of new-born body odours whose amniotic fluid was still present in their bodies. The presence of volatile flavours, such as fenugreek and garlic, was detected in the odour of new-born babies and in the odour of amniotic fluid just after birth and after 45 mins of ingestion, respectively (Hauser et al., 1985; Mennella et al., 1995). However, pungent odours like cumin scents that are passed from the mother's meal might perhaps persist in the amniotic fluid for a few days (Hauser et al., 1985). Although pregnant women in the prenatal stage of this thesis were instructed to avoid consuming any meals, including target flavours (kale and carrot), and not to eat during the full hour leading up to the scan, the possibility that other flavours were present in the amniotic fluid could not be ruled out.

Furthermore, fetuses involved in this study might be exposed to other flavours temporarily due to the complex composition of the amniotic fluid (Underwood et al., 2005) during the three weeks of the flavour exposure period. In healthy, non-diabetic pregnancies, the amniotic fluid composition is under homeostatic control linked to fetal urination and swallowing, with decreases in sodium and chloride concentrations and increasing urea and creatinine concentrations in the second half of pregnancy (Malhotra & Deka, 2004). Changes in maternal hydration induce only *temporary changes* in osmolality; normal intakes of water, sugar and salt in the maternal diet are unlikely to significantly affect the sweet or salty taste of the amniotic fluid (Underwood et al., 2005).

The findings of the current work also corroborate what has been discovered about the period that it takes for flavour compounds to transfer from the mother's diet into breastmilk, which shares numerous chemical compositions with amniotic fluid and reflects on the maternal dietary habits (Mennella et al., 1991; Hausner et al., 2010; Stafford et al., 1976; Underwood et al., 2005). For example, a study using an animal model showed that the detection time for flavours in cow milk after they were presented to the lungs was 15 minutes, but the rumen presentation required at least 30 minutes to process chemicals, suggesting that the digestive route requires a longer time than the respiratory route (Dougherty et al., 1962). Similarly, chemical analysis of milk content from human studies revealed that ethanol compounds showed an apex at 30 mins to an hour after ingestion, which may be a consequence of the direct absorption of these compounds into the vascular system (Beauchamp & Mennella, 2011).

Building on the results of these studies on the transfer of flavour compounds to milk fluid, together with the thesis findings, it can be argued that flavour molecules may reach the fetal gustatory or olfactory systems through a hematogenic route, bypassing the steps of fetal

metabolism and flavour collection in the amniotic fluid. Flavours carried in the bloodstream can indeed activate olfaction in adult rats and their olfactory learning can be engaged by odorants diffusing from the capillaries that irrigate chemosensory mucosae (Maruniak et al., 1983a, b). Likewise, odorants or tastants may stimulate the fetuses' odour or taste receptors when they diffuse from the capillaries that contact the olfactory sensory neurons or taste buds. Furthermore, once flavourants have crossed the placenta, both pathways of chemostimuli to the chemosensory sites may function sequentially, the vascular route being briefer than the amniotic route.

Additionally, regarding the amount of flavour experienced in the womb, this study showed that around 400 mg of flavour exposure on one occasion resulted in fetal responses to flavours and a total of ~ 5 g of flavour exposure during the last three weeks of pregnancy resulted in facial responses by infants to the matching odour. Our findings corroborated the results of previous studies on infant responses after repetitive maternal consumption of anise (a total of ~ 700 mg during the last 15 days of pregnancy, Schaal et al., 2000) and carrot juice (a total of ~ 3600 mL during the last three weeks of pregnancy, Mennella et al., 2001).

The current study showed that kale and carrot compounds passed to the fetal compartment after 30 mins of maternal ingestion of around 400mg of a vegetable capsule. However, the results from the current work cannot be extrapolated to different types of flavours because food flavour molecules are metabolised in fairly unique patterns due to their diverse chemical compositions (Jeleń, 2012). To fully understand the mechanisms of flavour intake, transmission, circulation, and excretion in amniotic fluid, further studies are needed, including chemical analyses of the extent of flavour transfer using different flavour compounds (e.g., mushroom–umami taste) from maternal consumption to amniotic fluid.

Furthermore, the specificity and complexity of fetal facial reactions found in this study offer us a window into our understanding of the development and maturation of the central nervous system, chemosensory system, and facial morphology (AboEllail & Hata, 2017; Ceriani et al., 2015; Hata et al., 2010, 2015; Forestell & Mennella, 2015; Schaal, 2016).

The occurrence of the different facial movements can suggest that the fetus might shorten or prolong stimulus seeking as a function of the type of stimulus. The different facial movements might express the literal "openness" of oronasal orifices to the environment. Some of the movements that occurred in the kale-exposed group such as lip presser (FM24) - which typically presses the lips against each other and closes the mouth-, and lip stretch (FM20) - which stretches the lips causing the lips to be thinner-, might be interpreted as suppressing momentarily the input of amniotic fluid conveying "unwanted" stimuli. On the other hand, one of the movements that emerged in the carrot-exposed group, lip pucker (FM18) - which pulls the corner of the mouth towards the centre causing the lips to protrude forward- can be thought of as practising – in amniotic fluid - the preliminary movement necessary for breastfeeding or bottle-feeding (Reissland et al, 2012, 2016). Furthermore, data from the current study showed that this facial movement (FM18) appeared on the faces of 36-week-old fetuses, but not at younger gestational ages. Considering the emergence of FM18 on the new-born face to the familiar vegetable odour during the postnatal assessment, it can be argued that fetuses begin to practice such feeding movements in the last month of pregnancy and onwards.

Additionally, the findings provide innovative data on the frequency of flavour-induced facial action, but also on their organization. Different facial configurations of movements, cry face gestalt in response to kale flavour and laughter face gestalt in response

to carrot flavour, indicated that a non-random association between the given sensation and a particular facial muscle regulation has arisen. For example, the cry face reaction might be readable as an aversion and the laughter face reaction as an attraction, supporting the biological predisposition of flavour preferences (Mennella, 2005, 2014; Schaal et al., 2000; Steiner et al., 2001). Neuroimaging methods suited for fetal life (i.e., fetal functional magnetic resonance imaging – fMRI) can infer beyond sensory detection to identify emotional valence or learning processes (Domínguez, 2011; Ji et al., 2022).

Fetal responses may not only be structurally differentiated on average, but also in temporal/ sequential terms. One flavour stimulus might evoke facial movements more quickly than the other, and these facial movements may occur for a longer duration for one flavour stimulus than the other. Besides, individual differences arising from genetic differences in taste sensitivity, especially for bitter taste might also affect the flavour sensitivity of fetuses (Feeney, 2001). It would therefore be of great interest to map the onset of different facial movements for each individual.

In addition to the anatomical differences to generate laughter-face and cry-face gestalts, the developmental complexity observed in the cry-face reactions in response to kale flavour, but not observed in the laughter-face reactions in response to carrot flavour might indicate the vital and immediate role of seeking out sweetness postnatally for human growth (Drewnowski et al., 2012). Avoidance of bitter compounds, which is also an innate reaction (Bartoshuk & Beauchamp, 1994), may develop a little later. It is therefore of interest to investigate the earliest development of functions that are most key to survival. Given that gustatory and olfactory skills are functional at this time (Schaal et al., 2016), a further study involving a longitudinal cohort starting from 24 weeks gestation would allow addressing this issue.

In sum, these results allow us to suggest that the fetus is highly active toward its chemical environment which is shaped by maternal dietary aromas. Therefore, it is possible to argue that fetuses are not protected from maternal food choices, a situation exposing them inescapably to the environmental regimen mediated by the mother's body. This study with given flavours may call to research in other domains of materno- fetal transfer of ingested or inhaled compounds, such as addictive compounds (nicotine, drugs, alcohol), and their possible long-term effects relating to the concept of "developmental origins of health and disease" (Barker, 2002). Given the widespread public interest shown in the prenatal study (Chapter 3) since it is published, the outcome of this study is believed to have the potential to increase pregnant mothers' awareness of the influence of their own food intake on their unborn babies.

The result from the postnatal study indicated that manipulation of prenatal flavour exposure of kale and carrot during the last three weeks of pregnancy facilities neonatal preferences for the matching odour. This finding supports and extends previous research that prenatal flavour exposure affects postnatal odour-liking reactions (for a review, see Chapter 4).

The findings of the current work also corroborate previous studies investigating the effects of postnatal exposure such as during breastfeeding or complementary feeding (Birch et al., 1998; Forestell & Mennella, 2007; Remy et al., 2013; Sullivan & Birch, 1994). For example, one study showed that infants at the beginning of the complementary feeding period (four to seven months old) were more likely to accept a novel green vegetable after 10 exposures (Sullivan & Birch, 1994). Despite the possibility that postnatal flavour learning via breastfeeding and formula feeding in the first few years of life can increase flavour acceptance (Barends et al., 2019; Spahn et al., 2019; Ventura, 2017), it might be still

challenging to modify flavour preferences to accustomed to the bitter taste compounds in green vegetables after birth because of the innate avoidance reactions. The results of the current work suggest that flavour learning can start even before birth.

Additionally, the findings from the longitudinal study showed that examining manipulation of fetal exposure to bitter-tasting vegetable flavours through the third trimester of pregnancy attenuates the initial (at the fetal stage) cry-face responses and heightens the initial laughter-face responses to the matching odour postnatally. This finding is consistent with the hypotheses that were derived from animal models. Most of the animal research led to the conclusion that repeated prenatal exposure to flavours like garlic, orange, ethanol, or cumin can reverse potential aversion reactions to the target flavour (Chotro & Molina, 1991; Hudson et al., 1999; Nolte & Mason, 1995; Semke et al., 1995). The findings provide direct evidence that repeated flavour exposure facilities the plasticity of preferences acquired in utero, suggesting the existence of perinatal continuity in memory.

Despite the health benefits, especially for the prevention of cardiovascular disease and cancer, most children in the United Kingdom and Europe do not consume the recommended servings or a variety of vegetables (Ahern et al., 2019; Manchali et al., 2012; NHS Digital, 2020; Wang et al., 2014; Yngve et al., 2005). A substantial change in consumer behaviour is required to meet the goal of a food intake of a “5-a-day”, which would result in a considerable increase in life expectancy and a reduction in the "environmental footprint" of food-related behaviours (Eustachio Colombo et al., 2021). The low degree of enjoyment and liking for vegetables, especially those with bitter qualities, is a big hurdle to this (Krølner et al., 2011; Rasmussen et al., 2006). Given that parents use their infants' facial expressions to judge their enjoyment and frequently stop feeding them when they display negative facial

expressions (Forestell & Mennella, 2012), increasing the new-born's facial responses to acceptance and minimising the facial reactions of rejection at weaning (Monnery-Patris et al., 2015) through prenatal exposure may enhance the probability of parental exposure to vegetables.

By providing the earliest evidence of such reactions, it can be argued that exposing pregnant women to bitter vegetables is a potentially plausible way to improve lifelong health and drive healthy choices in populations widely concerned by the obesity pandemic by facilitating the process of infant vegetable acceptance. To address this issue, longitudinal follow-ups covering the period of at least the first 1000 days, which is a critical period to develop long-term eating habits (Nicklaus et al., 2019), are needed.

Strengths and limitations

The limitations of each study are discussed in detail in each relevant section; therefore, this section is a brief reflection on the overarching strengths and limitations of the work in this thesis.

A key methodological strength of this thesis is that an objective coding scheme was applied across the studies allowing for equivalent measurements at prenatal and postnatal periods of observation. Objective descriptions of facial movements allow this research to be replicated by different research teams and compared with different populations. Furthermore, the main coder (Beyza Ustun) and the reliability coders in the prenatal and postnatal part of the thesis were qualified to analyse facial movements (certified FACS scorers and/or qualified FOMS coders). Excellent intra and inter-reliability scores in all stages were achieved. All sonographers were highly qualified to perform 4D ultrasound scans and were blinded to the study aims as well as the condition of the participants.

Despite their strengths, the studies reported in this thesis have some limitations. Due to the COVID-19 outbreak in March 2020, all face-to-face participant testing was stopped for four months, making data collection impossible, and thus this leads an unexpected attrition rate between the phases. From July 2020 onwards, because the clinics have a clear COVID-19 policy, permission was granted to resume ultrasound scans with applying restrictions on the maximum number of scans per week. However, home or laboratory visits were still not allowed. Therefore, a contingency plan was developed to continue the postnatal stage of the research safely during the pandemic. Without compromising the quality of the research, the postnatal assessment was switched from in-person home visits to virtual home visits via the online recordings of the experiment conducted by the parents.

Due to the limited budget and to cover the missed period because of the Covid-19 pandemic, the project was designed to use archived scans for the control group because there was a good match in eligibility criteria and in measures across groups. The demographics and measures for maternal mental health were the same in the archived data as in the newly collected data. The collection of ultrasound scans in the archived data followed the same protocol as the prenatal study. Using archived control scans allowed us to increase the sample size in our experimental groups and therefore to reach the required sample size to obtain a study power. Despite the advantages, assessing archived data for the control group led to the limitation of the research, namely the potential impact of uncollected data (i.e., maternal vegetable consumption).

Due to the colour of the capsules and mothers' reluctance to participate in the study without being aware of the specific flavour they were being asked to consume, mothers could not be blinded to the flavour condition. Future research could use the blinding method commonly used in pharmacological studies, such as (over-encapsulation), a technique that hides the contents of the capsule by covering it with an opaque capsule shell (Wan et al.,

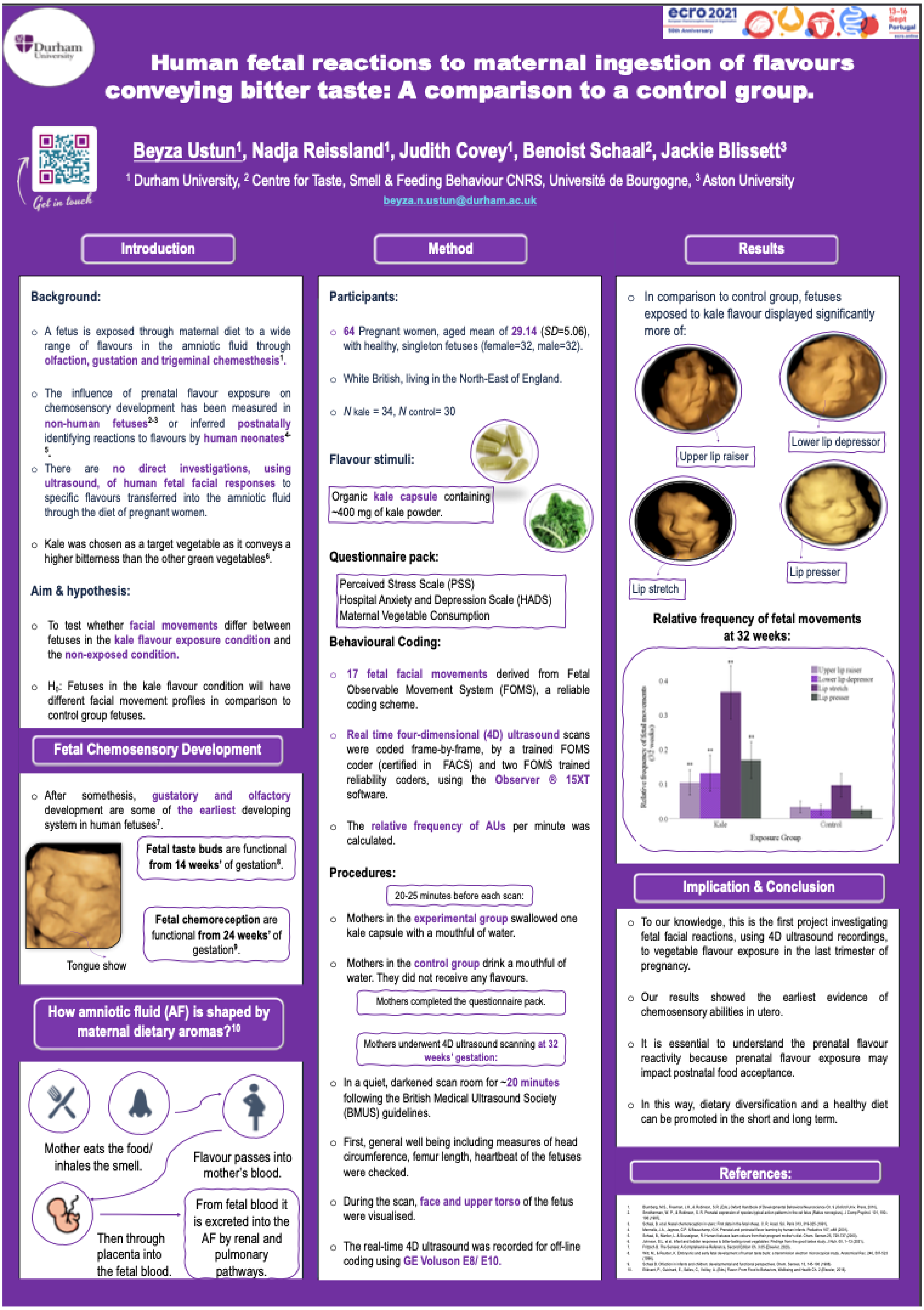
2013). In addition, the sample of pregnant mothers included in the study was all Caucasians based in the northeast of England, more work is needed to investigate the fetal receptivity to cultural chemistry conveyed by the mother's environmental choices and constraints in terms of the impact of different cuisines and flavour preferences across cultures. For example, examining how fetuses respond to bitter flavours in cultures where extra spicy or extra bitter foods play a big role in a country's culinary patterns would be great of interest.

Thesis conclusions

This thesis provides the reliable visualization and measurement of the fetal neural pathways work from the reception of flavours to oro-facial effectors and that the mother's chemo-environment is conveyed transplacentally to affect fetal behaviour. This mother-to-fetus transfer was effective with minute quantities of food and measurable quasi-concurrently with maternal ingestion – a point never tested before. Therefore, the results suggest that the fetus is chemically cued to maternal activities, and these initial responses in utero can be modified by prenatal exposure leading to an increased preference for certain flavours. As outlined throughout this final chapter, this thesis has potential impacts on the fields of sensory science, developmental psychobiology and beyond, and offers a substantive basis for the development and testing of real-world interventions with the capacity to impact human health.

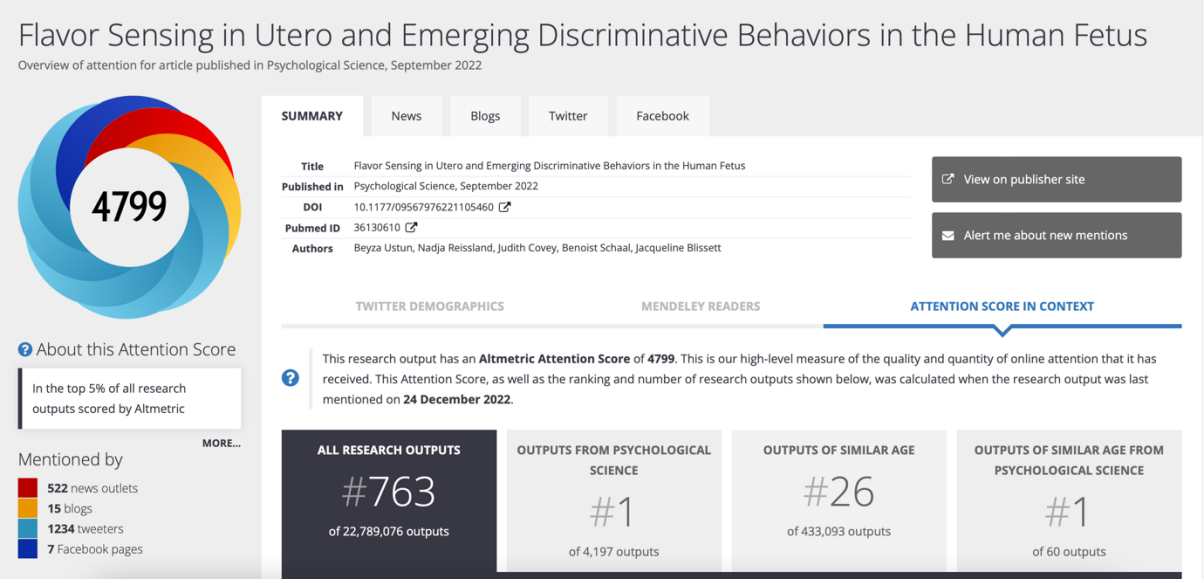
Appendices

Appendix 1: Poster presented at ECRO 2021, Portugal

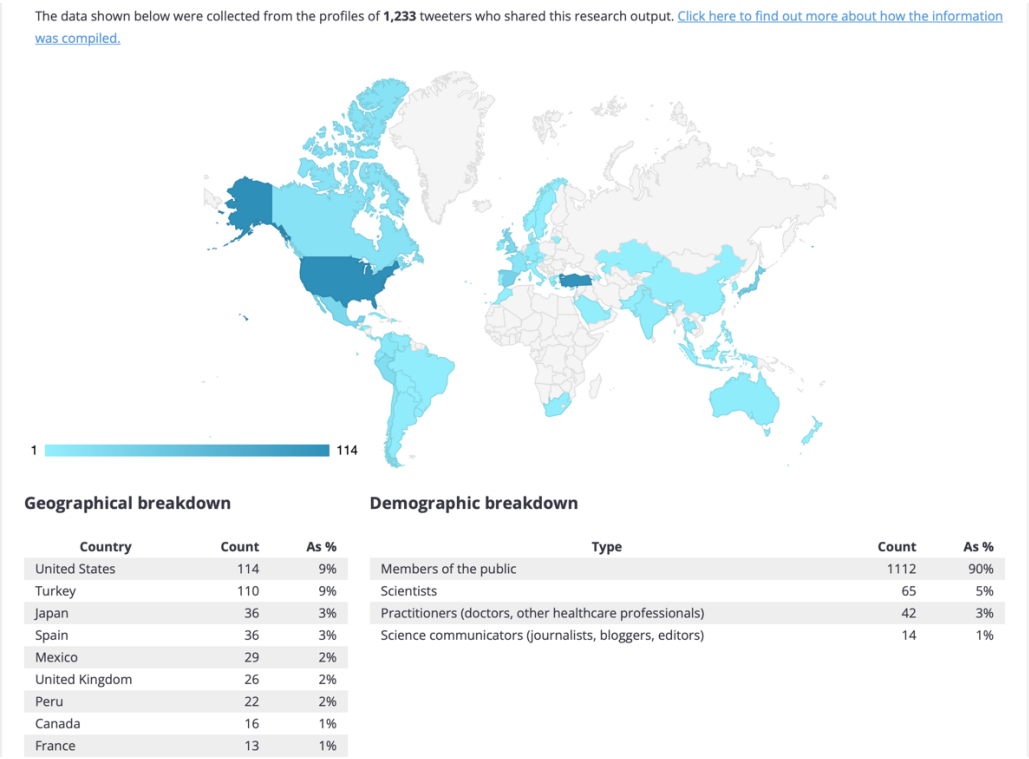


Appendix 2: Press releases for this thesis

Attention score by Altmetric

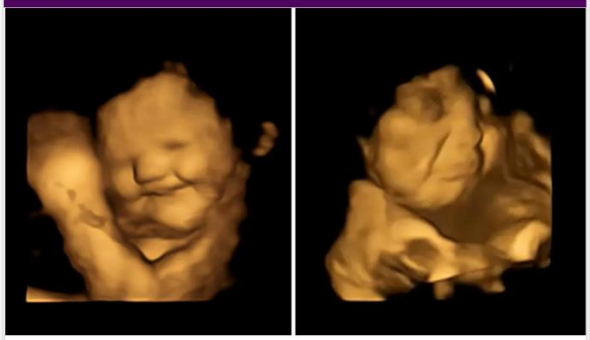


Twitter Demographics



Selected press releases:

The Times

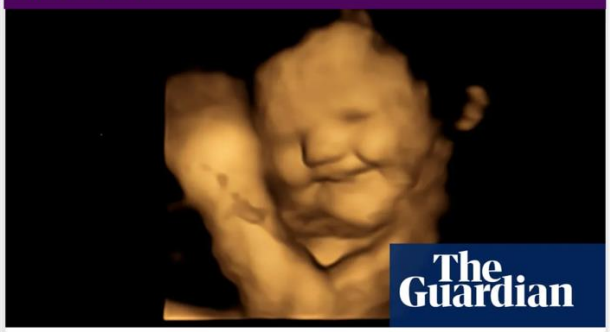


Babies in womb quail at tasting kale, Durham University researchers find

Scientists have captured the facial expressions of unborn babies as they taste food for the first time, finding that they smile when they encounter carrots and

thetimes.co.uk / 22 Sep

The Guardian

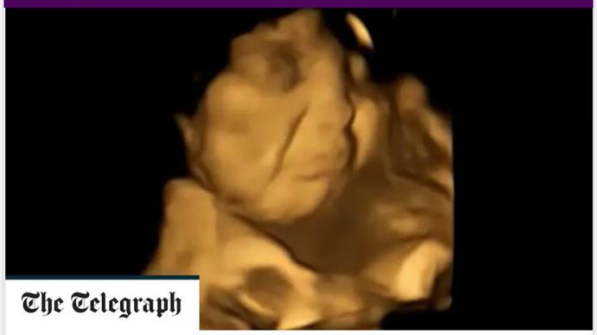


Taste of kale makes unborn babies grimace, finds research

First study to look at facial responses of foetuses to tastes shows crying expression twice as likely for kale than carrot

the Guardian / 22 Sep

The Telegraph

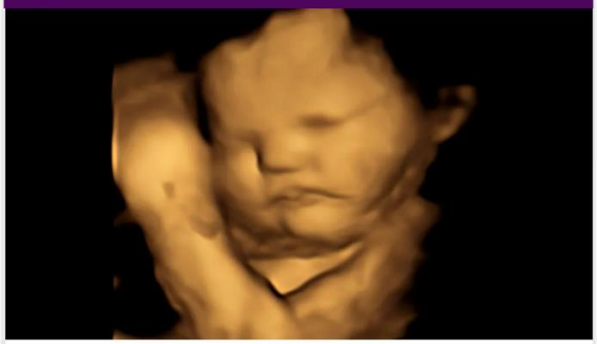


Kale 'makes unborn babies cry - but carrots make them laugh'

Groundbreaking research could be the first step towards establishing healthy eating habits after weaning

The Telegraph / 22 Sep

Sky News

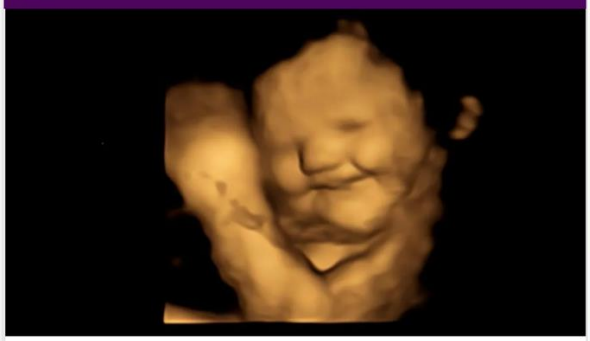


Babies in the womb 'smile for carrots and cry at greens', study suggests

Researchers believe unborn babies can experience the flavour of their mother's food by inhaling or swallowing amniotic fluid in the womb.

Sky News / 20 Sep

CNN



Fetuses smile for carrots but grimace over kale, study suggests

While it is known that some children are not huge fans of greens, a new study suggests that such dietary preferences could come about before they're even born.

CNN / 22 Sep

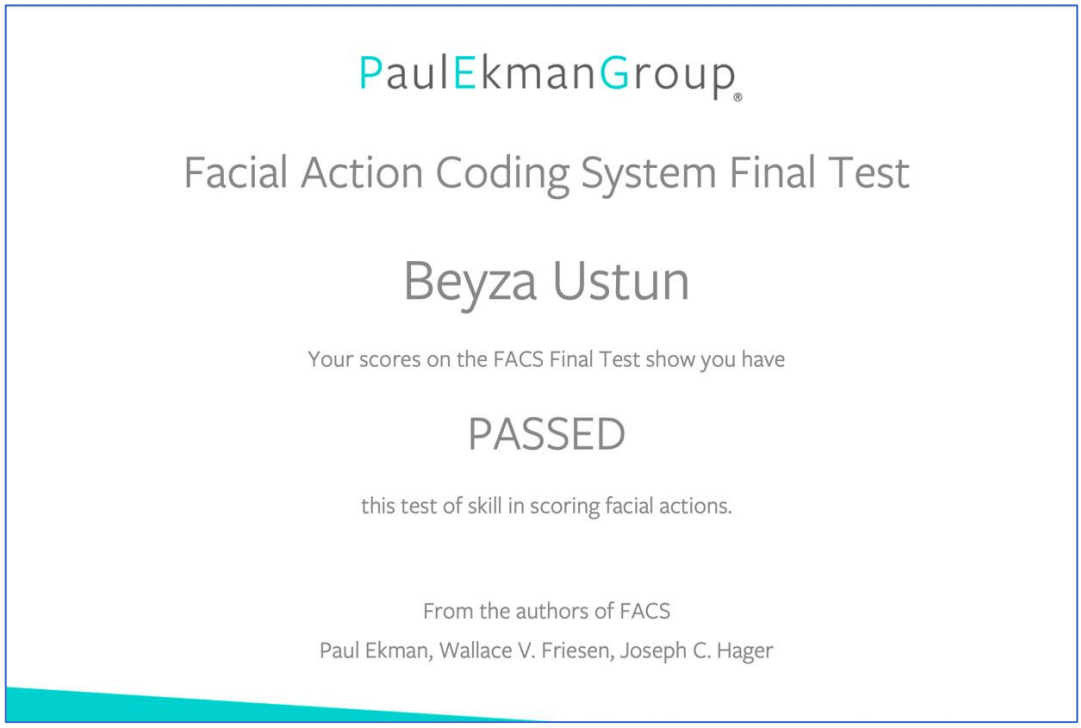
Psychology Today

Babies Can Taste Their Mothers' Food in the Womb

New research shows fetuses smile or frown in response to different tastes.

Psychology Today

Appendix 3: Awards and certificates



PaulEkmanGroup®

Facial Action Coding System Final Test

Beyza Ustun

Your scores on the FACS Final Test show you have

PASSED

this test of skill in scoring facial actions.

From the authors of FACS

Paul Ekman, Wallace V. Friesen, Joseph C. Hager

Durham University
Wolfson Research Institute for Health and Wellbeing

Wolfson Research Institute for Health and Wellbeing

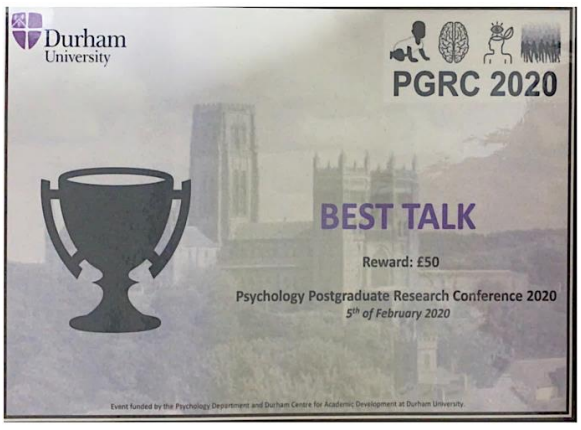
This is to certify that

Beyza Nur Ustun

was awarded Best Presentation at the Fifth Annual Wolfson Research Institute for Health and Wellbeing ECR Conference, 21st June 2022

Awarded by

Professor Amanda Ellison
Executive Director
Wolfson Research Institute for Health and Wellbeing



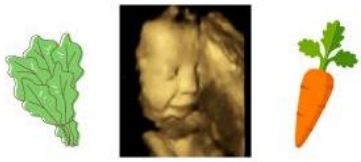
Researcher Development Programme

2022 Three Minute Thesis (3MT) Final winners announcement

What a great competition we had this year. Fantastic presentations from seven amazing researchers. The judges deliberated, cogitated and debated but, after a difficult discussion, selected a winner and two runners-up. <drum roll>

Our 2022 Three Minute Thesis (3MT) winner is ...
Beyza Nur Ustun (Psychology)
with her talk, "Does what the mother eats during pregnancy affect infant food preferences?". Beyza scoops the **£400 winners prize**.

Appendix 4: Information sheet, privacy notice and consent form



Prenatal Flavour Experience

Durham University

Psychology Department
Durham University
Science Laboratories
South Road
DURHAM
DH1 3LE
Tel: +44(0)191 3343240
www.dur.ac.uk/psychology

Fetal and infant behaviours in response to vegetable flavours in the womb

Information Leaflet for Parents

Before you decide whether to take part, please read the following information.

We would like to invite you to take part in a study looking at fetal movements before and after birth in reaction to two vegetable flavours, kale and carrot. Your participation will help us to learn about the facial reactions of your baby when exposed to vegetable flavours in the womb.

What is the purpose of this study?

The purpose of this study is to establish if the unborn baby will react with different facial reactions when exposed to kale and carrot. We do this by using 4D ultrasound scans. In addition, we are assessing if the new-borns will react differently to the smell of the vegetable which they experienced prenatally.

What will we ask you to do?

We will invite 100 mothers who are approximately 20-30 weeks pregnant. We ask mothers who have had their 20-week anomaly scan showing a healthy baby. You will be asked to swallow a vegan/vegetarian organic capsule containing either Kale or Carrot powder at 32 and 36 weeks gestation around 20 minutes before the ultrasound scans. The scanning will last approximately 20-25 minutes. During the scan we will show you the baby's face and point out to you the baby's movements. You can see the unborn baby on the screen. Sometimes, when the baby is in the wrong position (e.g. hiding the face behind an arm) you cannot see much. At other times, you can see mouth movements or even the baby sucking his or her thumb. You will be lying on your back as you did in your 12 and 20-week scans. An experienced sonographer will perform the ultrasound scan in order to see the face of your baby. From 36 weeks gestation for 3 consecutive weeks, at least 4 times per week, you will also be asked to take one vegetable capsule either kale or carrot in a day. You will also be asked to complete a checklist regarding the time and date of taking the capsules. Before the ultrasound scans and follow up, we will ask you to fill out questionnaires on food frequency, perceived stress, anxiety and depression in order to see whether this might have an effect on how the baby moves. These will take about 5-10 minutes each. We will visit you at your home within the first two-three weeks after birth. In this session, your baby will take a smell test to see whether they react differently to the smell of vegetable which they experienced prenatally. For the purpose of the study, we will also request a few demographic information, and birth outcomes

including delivery date, gestation at delivery, sex of the baby, Apgar scores, head circumference and the general health of the baby.

If you choose to take part, you will be involved in this study for two months in the latter part of your pregnancy and just after birth. We will scan your unborn baby using the ultrasound scanners in one of three ultrasound clinics (Window to the Womb in Chester-Le-Street, Darlington or Middlesbrough). Although we cannot reimburse your travel expenses, you will get a copy of your 4D ultrasound scans.

Is it safe to take kale or carrot capsules in pregnancy?

Kale and carrot are high in vitamins and minerals, good for you and for your baby. Kale is known as non-allergic for most people, whereas consuming more than the required amount of carrot might cause some allergic reactions. When a person eats a specific food very often, allergic symptoms might be developed in any food.

The capsules (~400mg) are a food supplement which contains either kale or carrot powder with a capsule shell of Hydroxypropyl Methylcellulose. These supplements are organic and suitable for vegetarians and vegans. The capsules are considered to be harmless for humans and can be taken during pregnancy. Before using the capsules, you will be asked about any allergies you might have and you will be asked to consult your healthcare provider.

What happens if you take any other vegetable supplements or give up taking kale or carrot capsules during the study?

You will be randomly assigned to kale or carrot groups. During the study, you are free to take other kinds of supplements. But we will ask you to not exceed the recommended dose (one capsule a day) for kale or carrot capsule during the study.

If you give up taking either kale or carrot capsule after you have been assigned to the group, you will be excluded from the rest of the study.

What happens if you are allergic to kale or carrot?

Before you take part in the study, we will ask you whether you have any known allergies to kale and carrot. If you are allergic to you will be excluded from the study.

Although you might not have had any allergies to kale or carrot, if you notice any allergic symptoms (itchy tongue, mouth, ears or throat), please stop taking the vegetable capsules and see your GP.

What is the smell preference test?

Within the first two-three weeks after the birth of your baby, we will visit you at your home. Your baby will be in a comfortable baby bouncer which is suitable for new-borns. Your baby will be presented with a series of odour stimuli (kale, carrot or water). Your baby will be videotaped during the experiment (3-5 minutes) to measure facial responses.

What are reasons why you might not be able to participate in the study?

We want to establish the normal range of movements that the unborn baby shows. In the unlikely event that you develop any complications (e.g., gestational diabetes) during the pregnancy, you will not be able to continue in the study. As you are asked to take kale or carrot capsule in the study, if you have any allergies to kale and carrot, you will not be able to take part in the study.

What happens if we find an anomaly?

The scan is **not** intended to look for problems with your baby. If there are any concerns during the non-medical scans, you would be referred to your usual doctor for follow up.

Confidentiality:

Details about how we will ensure your data is kept confidential is contained in the attached privacy notice.

What happens if you no longer want to participate?

It is your decision to take part in this study. You are free to withdraw from the study without giving a reason at any time without penalty or loss of benefits until your data is fully anonymised in our database by destroying the codes that link to your identity. We will fully anonymise your data 10 years after testing data. If you request before your data is fully anonymised, we will destroy your consent form and the codes that link to your identity.

Results of this study

All participants get a summary of the results on request, but we will NOT share any individual scores regarding questionnaire data, fetal or infant results. The questionnaires or fetal scans are not diagnostic. We aim to publish the study. We may show pictures of the scan in publications and media releases. The picture will not include any information that would allow identification of you or your baby.

Questions or concerns

Please read the attached privacy notice and consent form. If you have any questions or concerns, please contact Beyza Ustun or N. Reissland.

Before signing the consent form, you will have the opportunity to ask any questions and address any concerns.

We thank you for your time and if you would like any more information please feel free to ask.

Contact details:

Beyza Ustun (PhD researcher)
University of Durham
Email : beyza.n.ustun@durham.ac.uk
Tel: 0191 3343278

Dr Nadja Reissland (Associate Professor)
University of Durham
Email: n.n.reissland@durham.ac.uk

Privacy Notice

PART 2 – TAILORED PRIVACY NOTICE

This section of the Privacy Notice provides you with the privacy information that you need to know before you provide personal data to the University for the particular purpose(s) stated below.

Project Title: Prenatal fetal movements and postnatal facial expressions in response to vegetable taste.

Type(s) of personal data collected and held by the researcher and method of collection:

We will ask you to sign a consent form to confirm your willingness to take part in this study. If you choose to take part, your unborn baby will undergo 4D ultrasound scans twice, once at 32 weeks gestation and once at 36 weeks gestation and we will visit you and your baby at your home within the first month after the birth of your baby. Your baby will be video-recorded during the preference test. Before the ultrasound scans and follow-up, we will ask you to fill out questionnaires on vegetable frequency, whether you are stressed and how positive/negative your mood is now in order to see whether this might have an effect on how the baby moves. We will also ask you for information about your delivery (e.g. Apgar scores; health report of the birth), and demographics (e.g. age, ethnicity, level of education). You will be allocated an anonymous number and we will keep your personal data secure in a locked research room (The Fetal and Neonatal Research Room) and on password-protected hard drives and computers. A copy of the original scan and video will be stored in a locked filing cabinet in that locked research room.

Lawful Basis

The legal basis for which the personal data that we collect and processed will be under Durham University's 'public task', which includes conducting research. Further information can be found here: *https://www.dur.ac.uk/ig/dp/lawfulbases/public/*

How personal data is stored:

All personal data will be held securely and strictly confidential to the research team. The electronic form of the data will be stored on a password-protected computer and hard drive, and any hardcopies and CDs will be kept in locked storage in the Fetal and Neonatal Research Room. Personal data will not be available to anyone outside the research team. We may show pictures and video clips of the scan in publications, media releases but the picture will not include any information that would allow identification of you or your baby.

How personal data is processed:

The signed consent form provides evidence of your consent to take part in this study, which is an ethical requirement. We keep a record of the anonymous code that has been assigned to you to enable us to connect the data we collect from you at different phases of this study and any future research that we conduct that you consent to take part in.

Withdrawal of data

You can request withdrawal of your data until it has been fully anonymised *in our database by destroying the codes that link to identity*. Once this has happened it will not be possible to identify you from any of the data we hold.

Who the researcher shares personal data with:

Personal data will not be shared with anyone outside the research team. Anonymised, non-identifiable data may be used in publications, media releases, reports, presentations, web pages and other research outputs. At the end of the project, anonymised data (not-identifiable) may be archived and shared with others for legitimate research purposes.

How long personal data is held by the researcher?

All research data and records needed to validate the research findings will be stored for 10 years for the purpose of potential follow up studies. After this period, any personal information will be fully anonymised by destroying *the codes that link to identity.*

Changes to this privacy notice:

As this project will be running over several years, we will review this noticed annually. Should we make any changes, we will inform you via your preferred contact method. After the fully anonymization of the project data, we will no longer review this notice.

How to object to the processing of your personal data for this project:

If you have any concerns regarding the processing of your personal data, or you wish to withdraw your personal data from the project, contact Beyza Ustun-PhD researcher, using the following contact details.

Further information:

Please contact Beyza Ustun (PhD researcher) at beyza.n.ustun@durham.ac.uk or Dr Nadja Reissland (Associate Professor) at n.n.reissland@durham.ac.uk.

Consent Form

This form is to confirm that you understand the purposes of the project, what is involved and that you are happy to take part. Please initial the box to indicate your agreement:

	Initial
I confirm that I have read and understand the information sheet dated [/ /] and the privacy notice for the above project.	
I have had sufficient time to consider the information and ask any questions I might have, and I am satisfied with the answers I have been given.	
I understand who will have access to personal data provided, how the data will be stored and what will happen to the data at the end of the project.	
I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason.	
I give my consent for videos of the scan to be made of my unborn baby.	
I give my consent for videos of the smell preference test to be made of my baby after birth.	
I give my consent for material to be shown for research and teaching purposes, used in publications, journals, textbooks and media releases.	
I understand that the scans are only used for research purposes and cannot be used to identify any specific conditions.	
I agree to provide delivery details of my baby (Due date, gestation at delivery, sex of the baby, Apgar scores, head circumference, health of baby).	
I understand that data will be anonymous, and no names will appear in any published results.	
I confirm that I have no known allergies to kale.	
I confirm that I have no known allergies to carrot.	
I agree to take kale or carrot capsules during the study.	
I agree to be seen 3 times (at 32 weeks and 36 weeks gestation and within the first two-three weeks after the birth of my baby).	

I can review the material by arrangement with the University of Durham, Psychology department (Beyza Ustun email: beyza.n.ustun@durham.ac.uk, Dr. N. Reissland, Email: n.n.reissland@durham.ac.uk).	
I confirm that I have had my 20 week anomaly scan and my baby has been determined to be healthy.	
I confirm I have consulted my healthcare provider to take kale or carrot capsules at a specified time.	
I agree to take part in the above project.	

Participant's Signature: _____Date_____

(NAME IN BLOCK LETTERS) _____

Researcher's Signature _____Date_____

(NAME IN BLOCK LETTERS) _____
_____

Address: _____

_____

E-mail: _____

Appendix 5: Hospital Anxiety and Depression Scale (HADS)

Below are 14 statements of how you feel in the past week. There are no "right" or "wrong" answers. Please give an immediate response which comes closest to how you have been feeling in the past week.

1. I feel tense or 'wound up'

a) Most of the time
b) A lot of the time
c) From time to time
d) Not at all

2. I still enjoy the things I used to enjoy:

a) Definitely as much
b) Not quite as much
c) Only a little bit
d) Hardly at all

3. I get a sort of frightened feeling as if something awful is about to happen:

a) Very definitely and quite badly
b) Yes, but not too bad
c) A little bit, but it doesn't worry me
d) Not at all

4. I can laugh and see the funny side of things:

a) As much as I always could
b) Not quite so much now
c) Definitely not so much now
d) Not at all

5. Worrying thoughts go through my mind:

a) A great deal of the time
b) A lot of the time
c) From time to time, but not too often
d) Only occasionally

6. I feel cheerful:

a) Not at all
b) Not often
c) Sometimes
d) Most of the time

7. I can sit at ease and feel relaxed:

a) Definitely
b) Usually
c) Not often
d) Not at all

8. I feel as if I am slowed down:

a) Nearly all the time
b) Very often
c) Sometimes
d) Not at all

9. I get sort of frightened feeling like ‘butterflies’ in my stomach:

a) Not at all
b) Occasionally
c) Quite often
d) Very often

10. I have lost interest in my appearance:

a) Definitely
b) I don’t take as much care as I should
c) I may not take as much care
d) I take as much care as ever

11. I feel restless, as I have to be on the move:

a) Very much indeed
b) Quite a lot
c) Not very much
d) Not at all

12. I look forward with enjoyment to things:

a) As much as I ever did
b) Rather less than I used to
c) Definitely less than I used to
d) Hardly at all

13. I get sudden feelings of panic:

a) Very often indeed
b) Quite often
c) Not very often
d) Not at all

14. I can enjoy a good book or a TV programme:

a) Often
b) Sometimes
c) Not often
d) Very seldom

Appendix 6: Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts during the last month. In each case, you will be asked to indicate by highlighting how often you felt or thought a certain way.

0 = Never 1= Almost never 2 = Sometimes 3 = Fairly often 4 = Very often

1. In the last month, how often have you been upset because of something that happened unexpectedly? **0 1 2 3 4**

2. In the last month, how often have you felt that you were unable to control the important things in your life?.....**0 1 2 3 4**

3. In the last month, how often have you felt nervous and "stressed"? **0 1 2 3 4**

4. In the last month, how often have you felt confident about your ability to handle your personal problems? **0 1 2 3 4**

5. In the last month, how often have you felt that everything was going your way?..... **0 1 2 3 4**

6. In the last month, how often have you found that you could not cope with all the things that you had to do? **0 1 2 3 4**

7. In the last month, how often have you been able to control irritations in your life?..... **0 1 2 3 4**

8. In the last month, how often have you felt that you were on top of things?.....**0 1 2 3 4**

9. In the last month, how often have you been angered because of things that were outside of your control? **0 1 2 3 4**

10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?..... **0 1 2 3 4**

Appendix 7: Participant checklist to record capsule consumption

		DATE	TIME
FIRST WEEK	**Day 1:** One capsule		
	Day 2: One capsule		
	Day 3: One capsule		
	Day 4: One capsule		
	Day 5: One capsule		
	Day 6: One capsule		
	Day 7: One capsule		
SECOND WEEK	**Day 1:** One capsule		
	Day 2: One capsule		
	Day 3: One capsule		
	Day 4: One capsule		
	Day 5: One capsule		
	Day 6: One capsule		
	Day 7: One capsule		
THIRD WEEK	**Day 1:** One capsule		
	Day 2: One capsule		
	Day 3: One capsule		
	Day 4: One capsule		
	Day 5: One capsule		
	Day 6: One capsule		
	Day 7: One capsule		

Appendix 8: Instructions for parents regarding the postnatal assessment

Dear ……,
Congratulations on your baby! You are now in the follow-up session. In this session, we ask you to conduct a quick smell test (around 10 mins) to see your baby's smell preferences.

What do you need?
- Follow-up package and instruction sheets we provided.
- A camera or phone / laptop camera
- A bouncer (if you do not have a bouncer, you can conduct the experiment on a sofa but you need to make sure that the face of your baby is visible in the camera)
- Zoom or Teams account (if you don't have an account, please set up before the session)

What is inside of the follow-up package?
- Three pairs of gloves
- Three cotton buds
- Organic Kale Powder
- Organic Carrot Powder

Test environment and your baby's condition:
- Your baby should be awake during the experiment.
- Before you present the smell, please make sure that your baby's face is neutral (not crying or laughing). If your baby is crying anytime, you can stop the experiment until your baby is calm. Once your baby is content again, you can put the baby on the bouncer and continue to the experiment.
- The experiment needs to be conducted in a silent room. Please make sure that your TV is off and there is nothing to distract your baby (e.g. a person, an animal or a toy) in the room. You can ask your partner to accompany you during the experiment.
- Please wear different gloves while preparing the smell and during the experiment.
- The camera will be positioned against a background and your baby sits in a bouncer facing a camera.

Before the experiment:
- Please feed your baby ***at least 30 minutes prior*** to the experiment.
- ***Prepare the vegetable smell*** using the kale/carrot powder and cotton buds following the instruction sheet called how to prepare the smell stimulus.
- There is an ***instructional video*** explaining how to conduct the smell test. Please watch the video carefully before you start the experiment and feel free to ask any questions.

ORDER OF THE STIMULI

- Present the first stimulus **WATER** using a cotton swab for *20 seconds*
- Wait for 60 seconds (no stimuli)
- Present the second stimulus for **KALE** *20 seconds*
- Wait for 60 seconds (no stimuli)
- Present the third stimulus for **CARROT** *20 seconds*
- Wait for ***60 seconds*** (no stimuli)
- Present the first stimulus **KALE** for *20 seconds*
- Wait for *60 seconds* (no stimuli)

- Present the second stimulus **WATER** for *20 seconds*
- Wait for *60 seconds* (no stimuli)
- Present the third stimulus **CARROT** for 20seconds
- Wait for *60 seconds* (no stimuli)

This is the end of the smell test.

How to prepare the smell using kale and carrot powder?

You will receive two boxes; one includes kale powder and the other one includes carrot powder: Follow the same instructions for kale and carrot powder

Smell Preparation:

1. Dip the cotton swab into the water, then take it out.
2. Dip the same cotton swab into the powder and roll the swab.

3. After you remove the cotton swab, it should look like this:

Appendix 9: Covariates in the prenatal study

Fetal sex differences in the dataset:

Chi-Square Test

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	.018	2	.991
N of Valid Cases	99		

*Group * Sex Cross tabulation*

Exposure group		Female	Male	Total
Kale	Count	17	17	34
	Expected Count	16.8	17.2	34.0
Carrot	Count	17	18	35
	Expected Count	17.3	17.7	35.0
Control	Count	15	15	30
	Expected Count	14.8	15.2	30.0
Total	Count	49	50	99
	Expected Count	49.0	50.0	99.0

Predictor fetal and maternal variables in relation to fetal movements:

Variable			95% Confidence Interval for Mean	
	F	Sig. (Between groups)	Lower	Upper
Maternal Age	2.698	.072	28.914	30.921
Maternal pre-pregnancy BMI	.319	.728	25.268	26.469
Gestational Age at Birth (in weeks)	2.517	.086	39.275	39.693
Birth Weight (in gram)	.405	.668	3295.73	3452.541
Head Circumference (in cm) at 20 weeks*	6.781	**.002***	166.123	169.304

*included as a covariate.

Independent sample t-test on the frequency of maternal vegetable consumption:

	F	Sig.	t	df	Two-Sided p value	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
Vegetable consumption at 32 weeks								Lower	Upper
Kale	1.910	.172	.458	67	.649	.068	.149	-.229	.365
Carrot	.079	.780	-.054	67	.957	-.010	.185	-.380	.360
Overall bitter	.545	.463	.517	67	.607	.294	.569	-.841	1.429
Overall non-bitter	.748	.390	.011	67	.991	.005	.431	-.855	.865
Vegetable consumption at 36 weeks									
Kale	3.400	.070	2.156	67	.035	.513	.238	.038	.988
Carrot	1.927	.170	-.660	67	.511	-.152	.231	-.613	.309
Overall bitter	1.142	.289	.598	67	.552	.271	.452	-.632	1.174
Overall non-bitter	.029	.864	-.721	67	.473	-.305	.422	-1.147	.538

Appendix 10: Correlations between maternal mental health scores and FMs in the prenatal study

At 32 weeks:

Fetal Movements		Anxiety (HADS) 32 weeks	Depression (HADS) 32 weeks	Stress (PSS) 32 weeks
FM1-Inner-brow-raiser 32 weeks	Pearsons Correlations	.075	.152	.070
	Sig. (2-tailed)	.464	.138	.494
	N	97	97	97
FM2-Outer-brow-raiser 32 weeks	Pearsons Correlations	.112	.015	-.003
	Sig. (2-tailed)	.275	.881	.977
	N	97	97	97
FM4-Brow-lowerer 32 weeks	Pearsons Correlations	-.005	.110	-.008
	Sig. (2-tailed)	.960	.281	.938
	N	97	97	97
FM6-Cheek-raiser 32 weeks	Pearsons Correlations	.043	-.001	-.067
	Sig. (2-tailed)	.679	.989	.513
	N	97	97	97
FM9–Nose-wrinkle 32 weeks	Pearsons Correlations	-.032	-.033	-.074
	Sig. (2-tailed)	.756	.751	.470
	N	97	97	97
FM10–Upper-lip-raiser 32 weeks	Pearsons Correlations	.111	.028	.036
	Sig. (2-tailed)	.280	.782	.723
	N	97	97	97
FM11–Nasolabial-furrow 32 weeks	Pearsons Correlations	.114	.092	.060
	Sig. (2-tailed)	.267	.372	.559
	N	97	97	97
FM12–Lip-corner-puller 32 weeks	Pearsons Correlations	-.098	.002	-.069
	Sig. (2-tailed)	.338	.985	.502
	N	97	97	97
FM16–Lower-lip-depressor 32 weeks	Pearsons Correlations	.092	.011	.066
	Sig. (2-tailed)	.368	.912	.519
	N	97	97	97
FM18–Lip-pucker 32 weeks	Pearsons Correlations	-.180	-.118	-.146
	Sig. (2-tailed)	.078	.251	.154
	N	97	97	97
FM19–Tongue-show 32 weeks	Pearsons Correlations	-.154	-.049	-.155
	Sig. (2-tailed)	.133	.634	.128
	N	97	97	97
FM20–Lip-stretch 32 weeks	Pearsons Correlations	.193	.014	.058
	Sig. (2-tailed)	.058	.892	.574
	N	97	97	97

FM24–Lip-presser 32 weeks	Pearsons Correlations	.080	.036	.051
	Sig. (2-tailed)	.438	.724	.622
	N	97	97	97
FM25–Lips-parting 32 weeks	Pearsons Correlations	-.152	-.037	-.136
	Sig. (2-tailed)	.137	.720	.183
	N	97	97	97
FM26–Jaw-drop 32 weeks	Pearsons Correlations	-.099	-.065	-.109
	Sig. (2-tailed)	.332	.526	.289
	N	97	97	97
FM27-Mouth-stretch 32 weeks	Pearsons Correlations	-.151	-.014	-.097
	Sig. (2-tailed)	.141	.891	.344
	N	97	97	97
FM28-Lip-suck 32 weeks	Pearsons Correlations	-.033	-.043	-.083
	Sig. (2-tailed)	.750	.679	.417
	N	97	97	97

At 36 weeks:

Fetal Movements		Anxiety (HADS) 36 weeks	Depression (HADS) 36 weeks	**Stress (PSS) 36 weeks**
FM1-Inner-brow-raiser 36 weeks	Pearsons Correlations	-.086	-.170	-.080
	Sig. (2-tailed)	.440	.124	.472
	N	83	83	83
FM2-Outer-brow-raiser 36 weeks	Pearsons Correlations	.053	.004	-.021
	Sig. (2-tailed)	.634	.968	.853
	N	83	83	83
FM4-Brow-lowerer 36 weeks	Pearsons Correlations	-.040	-.079	.006
	Sig. (2-tailed)	.723	.477	.958
	N	83	83	83
FM6-Cheek-raiser 36 weeks	Pearsons Correlations	.180	.063	.083
	Sig. (2-tailed)	.103	.574	.457
	N	83	83	83
FM9–Nose-wrinkle 36 weeks	Pearsons Correlations	-.066	-.066	-.123
	Sig. (2-tailed)	.554	.555	.266
	N	83	83	83
FM10–Upper-lip-raiser 36weeks	Pearsons Correlations	-.096	-.185	-.038
	Sig. (2-tailed)	.386	.094	.733
	N	83	83	83
FM11–Nasolabial-furrow 36 weeks	Pearsons Correlations	.102	.024	.165
	Sig. (2-tailed)	.357	.828	.136
	N	83	83	83

FM12–Lip-corner-puller 36 weeks	Pearsons Correlations	.141	-.013	.108
	Sig. (2-tailed)	.202	.906	.331
	N	83	83	83
FM16–Lower-lip-depressor 36 weeks	Pearsons Correlations	.138	.113	**.220***
	Sig. (2-tailed)	.213	.309	**.045**
	N	83	83	83
FM18–Lip-pucker 36 weeks	Pearsons Correlations	.102	-.180	.031
	Sig. (2-tailed)	.360	.104	.779
	N	83	83	83
FM19–Tongue-show 36 weeks	Pearsons Correlations	.132	.189	.108
	Sig. (2-tailed)	.234	.087	.333
	N	83	83	83
FM20–Lip-stretch 36 weeks	Pearsons Correlations	.042	.061	.197
	Sig. (2-tailed)	.706	.587	.074
	N	83	83	83
FM24–Lip-presser 36 weeks	Pearsons Correlations	-.074	-.133	-.026
	Sig. (2-tailed)	.507	.232	.813
	N	83	83	83
FM25–Lips-parting 36 weeks	Pearsons Correlations	.028	-.032	-.014
	Sig. (2-tailed)	.803	.777	.898
	N	83	83	83
FM26–Jaw-drop 36 weeks	Pearsons Correlations	.031	-.007	-.008
	Sig. (2-tailed)	.783	.946	.946
	N	83	83	83
FM27-Mouth-stretch 36 weeks	Pearsons Correlations	.021	.015	-.002
	Sig. (2-tailed)	.848	.894	.983
	N	83	83	83
FM28-Lip-suck 36 weeks	Pearsons Correlations	.128	-.110	.078
	Sig. (2-tailed)	.248	.321	.483
	N	83	83	83

Appendix 11: Correlations between maternal/ infant covariates and fetal/infant reactions

Covariate	Dependent variable	Correlation
Maternal other non-bitter vegetable consumption (32 weeks)	FM1 (32 weeks)	-.387*, p = .029
Maternal other non-bitter vegetable consumption (postnatal stage)	FM16 (postnatal stage)	.418*, p = .042
	FM18 (postnatal stage)	.618**, p = .001
	FM24 (postnatal stage)	.490*, p = .015
	FM25 (postnatal stage)	.417*, p = .043
Maternal anxiety scores (32 weeks)	FM2 (32 weeks)	.379*, p = .032
Maternal anxiety scores (36 weeks)	FM6 (36 weeks)	.434*, p = .016
Maternal anxiety scores (postnatal stage)	FM6 (postnatal stage)	.484*, p = .016
	FM18 (postnatal stage)	.624**, p = .001
	FM24 (postnatal stage)	.454*, p = .026
Maternal stress scores (32 weeks)	FM6 (32 weeks)	-.358*, p = .044
	FM16 (32 weeks)	.463**, p = .008
	FM6 (postnatal stage)	.517**, p = .008
Maternal stress scores (postnatal stage)	FM6 (postnatal stage)	.517**, p = .010
	FM18 (postnatal stage)	.521**, p = .009
	FM24 (postnatal stage)	.490*, p = .015
Maternal depression scores (36 weeks)	FM6 (36 weeks)	.430*, p = .018
Maternal depression scores (postnatal stage)	FM1 (postnatal stage)	.408*, p = .048
	FM18 (postnatal stage)	.478*, p = .018
	FM24 (postnatal stage)	.645**, p < .001
Fetal head-circumference at 20 weeks	FM12 (32 weeks)	-.527**, p = .002
	FM12 (36 weeks)	-.396*, p = .030
	FM16 (32 weeks)	.576**, p < .001
	FM20 (32 weeks)	.582**, p < .001
	FM20 (36 weeks)	.530**, p = .003
	FM24 (32 weeks)	.720**, p < .001
	FM24 (36 weeks)	.372*, p = .043
	Laughter-face gestalt (32 weeks)	-.540**, p < .001
	Cry-face gestalt (32 weeks)	.650**, p < .001
	Cry-face gestalt (36 weeks)	.430*, p = .018
Infant age at testing	FM20 (32 weeks)	t = -2.270, p = .031
	FM24 (32 weeks)	t = -2.652, p = .013

Note. * Correlation is significant at the 0.05 level (two-tailed), ** Correlation is significant at the 0.001 level (two-tailed).

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