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A Light in the Dark;
an investigation of fetal visual perception

A thesis

submitted in fulfilment

of the requirements for the degree

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“The senses transform the coursing chaos of the world into perceptions and experiences—things we can react to and act upon. They allow biology to tame physics. They turn stimuli into information. They pull relevance from randomness, and weave meaning from miscellany.”

- Ed Yong

Abstract

How does a fetus engage with their visual world? Prenatal developmental trajectories are primarily unexplored, with fetal visual perception the most poorly understood of all sensory systems. In the third trimester, fetuses not only possess the ability to process perceptual information, but recent research suggests that enough light penetrates the womb to enable a visual experience. Understanding fetal visual capabilities has the potential to reshape our knowledge of both visual and psychological developmental trajectories. This thesis sits at the intersection of developmental psychology, medical imaging, and vision science. The overall aim of this work is to assess fetal visual function through novel experimental paradigms. We employed 2D ultrasound to visualize the fetal lens and index eye movements in response to light stimuli presented in utero. Tracking fetal eye movements in response to visual stimuli beyond light / no light has never been previously attempted. Three separate experiments explore central elements of fetal visual perception and cognition, including differential fetal responding to temporal and nasal visual fields, anticipatory gaze, and fetal sensitivity to agentive motion cues. The results of these studies demonstrate that the fetus actively engages with their visual environment exhibiting a preference for stimuli presented in their temporal visual field and the ability to perform anticipatory visual gaze, specifically that fetuses can use an auditory cue to anticipate the appearance of a light stimulus. These experiments and new methods offer new key insights into early sensory and psychological development.

Note on Formatting

This is a thesis by publication and consists of four separate articles.

Chapters 4, 5 and 6, the three experimental studies, are formatted in the *Springer Nature* style. The review article in **Chapter 2** and all other Chapters are formatted in *APA* style.

The references for each article (**Chapters 2, 4, 5 and 6**) are presented within the chapter text. The complete references for all other sections are located at the end of the thesis in the chapter titled *References for Chapters One, Three, and Seven*

The details of the publications are as follows:

Chapter Two:

Leov, J., Isaac, Z., Dunn, K., & Reid, V.M (2024). *The Role of Light in Prenatal Visual Development* Manuscript in preparation for submission to *Developmental Review*

Chapter Four:

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Chapter Five:

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Chapter Six

Leov, J., Dunn, K., & Reid, V.M (2024). *Fetal Response to Agentive Motion Cues* Manuscript in preparation.

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Chapter One

General Introduction

1.1 Why study the fetus?

Every aspect of development, from the establishment of the autonomic nervous system and motor function to emerging learning abilities and language processing, arises during gestation. Despite this, the significance of the prenatal period was long ignored and held as a “developmental limbo” (Hopkins & Johnson, 2005). Although this view is now largely no longer held, prenatal developmental trajectories are not robustly mapped. This is certainly the case with respect to fetal visual perception (Dunn et al., 2015). How does the fetus engage with the visual world? The prenatal period presents a unique window into the origins of our perceptual, social and cognitive abilities.

Fetal sensory development is positioned at an intrinsic disconnect at the core of developmental psychology. Newborns are equipped with a remarkable array of social and cognitive sensitivities (Johnson et al., 2008). Neonates have a memory for auditory stimuli previously heard in utero (DeCasper & Spence, 1986; James et al., 2002), a preference for familiar voices and languages (Byers-Heinlein et al., 2010; Coulon et al., 2011; May et al., 2011; Moon et al., 1993). They can make eye contact (Pitti et al., 2013), have a preference for faces over other visual stimuli (Morton & Johnson, 1991) and have a rudimentary understanding of numeracy (Coubart et al., 2014). It is undisputed that neonates exhibit a complex repertoire of cognitive and social abilities. But when and how does this psychological functionality arise?

Historically, neonatal abilities have been taken as evidence for innate origins or rapid imprinting (DeCasper & Spence, 1986). This belief is often informed by an underlying assumption that prenatal sensory systems have negligible activation across gestation (Graven & Browne, 2008) or that the fetus is held in an unconscious state, incapable of engaging with the external environment (Lagercrantz, 2014; Padilla & Lagercrantz, 2020). Ascribing to the theory of innate origins, however, discounts the potential influence of the prenatal environment. The proposition that these abilities spontaneously appear at birth does not align with our lack of knowledge about prenatal development. An alternative possibility is that these abilities have their foundations prenatally. There is evidence of continuity between neonatal and prenatal sensory perception (André et al., 2018) and there are no movements present in newborns that are not recorded in fetuses (Stanojevic, 2008). Arguably, the uterine environment must play a role in shaping sensory development, but the exact nature of the relationship is still unclear (André et al., 2018). This underscores the critical need to understand the fetal period, as birth may not be the beginning but simply a continuation of developmental trajectories that began in utero (DiPietro et al., 2015).

Although crucial to understanding human psychology, the prenatal period has received limited attention when contrasted with other epochs during development. A primary reason for this is that accessing the fetus is difficult. Situated within the uterus, the fetus is ensconced in amniotic fluid, placenta, and maternal anatomy including muscle, skin, bone and internal organs. Unsurprisingly, this complicates both the delivery of stimuli and the recording of fetal responses; with behavioral and brain-imaging techniques commonly used postnatally not easily adapted to the prenatal environment. The inherent physical complexities of conducting research in utero have hampered attempts to move the field forward (Dunn et al., 2015). The prenatal period is also characterized by rapid and dynamic development with the

implication that behaviour and therefore behavioral measures at 22 weeks gestation are not appropriate for 33 weeks gestation (Hopkins & Johnson, 2005). Despite these significant challenges, novel paradigms and technological advances have allowed for innovative approaches to navigate the technical barriers involved with measuring fetal responses (Dunn et al., 2015).

Of the sensory modalities the auditory domain has been a prominent focus of prenatal research, largely due to the ease of presenting auditory stimuli to the fetus. Sound is transmitted through the maternal abdomen to the uterine environment, providing a clear pathway to assess fetal auditory processing (Dunn et al., 2015). Using a high amplitude sucking paradigm, the seminal work of DeCasper et al.(1986) demonstrated that newborns have a preference for stories heard previously in the womb. Building on this, subsequent studies have shown that fetuses can differentiate between familiar and unfamiliar voices (Kisilevsky et al., 2003), discriminate vowels (Zimmer et al., 1993), and are sensitive to the reversal of learned consonant-vowel order (Lecanuet et al., 1987). Further, prenatal research is not limited to simple perceptual processing. Fetal auditory discrimination has been taken as evidence for language learning (Draganova et al., 2007), short-term memory capacities (Huotilainen et al., 2004), and speech perception (Draganova et al., 2005). Recent studies using an auditory oddball paradigm have shown that fetuses can demonstrate signs of hierarchical rule learning and the ability to form memory traces for complex auditory stimuli. The authors interpreted these results as evidence for the capacity for conscious processing before birth (Moser et al., 2021). This work illustrates that investigating the fetus can provide valuable insights into both low-level perceptual development (i.e. basic auditory processing) and higher-level cognitive functions, including early forms of learning and memory.

The evidence thus far indicates that fetuses have a clear capacity for sensory processing. In contrast to auditory perception, however, there is significantly less research on visual capacities (Dunn et al., 2015). Even though science has moved on from the erroneous assumption that newborns are blind (Aslin, 2012) the challenges associated with studying prenatal vision, such as limited access and the difficulty of delivering visual stimuli in utero, has the consequence that research on fetal visual capacities has been underexplored and research to investigate how fetuses perceive and process visual information is complex. Research has relied on visual paradigms to understand early development in the preverbal infant, using eye-tracking, preferential looking, and habituation techniques to infer cognitive and perceptual capacities (Aslin, 2012). Two implications of this are: 1. These paradigms can be co-opted to fetal work, and 2. Comparisons between prenatal and postnatal capacities could be made if paradigms were moderately similar. Despite the obstacles, understanding fetal visual development remains important, as it may provide deeper insights into early cognitive and sensory processing.

1.2 The Current Thesis

The aim of this thesis is to broadly investigate fetal visual perception, guided by the central question: how does a fetus engage with the visual world, and what can that tell us about visual perception and cognition prior to birth? It consists of one review article and three experimental studies. The thesis is organised as follows:

Chapter 2 contains the first article titled “The Role of Light in Prenatal Development.” This is a comprehensive review of the potential effects of light in the prenatal environment. This article first outlines the development of the fetal visual system across gestation and reviews the current models of light penetration to the human maternal womb. It then continues with a discussion on the role that light could play in both sensory and psychological development of the fetus. The

foundational literature for the subsequent experimental studies is examined, centring on what we know regarding visual development across gestation and the opportunities that arise for radically furthering our understanding of visual capacities of the third trimester fetus. This review therefore addresses the first step in the thesis' central proposition by demonstrating that foetuses have the capacity to engage with and process visual stimuli.

Chapter 3 provides an introduction to and rationale for the experimental studies, integrating the key insights from the literature with the experimental methods, building a cohesive link between theoretical perspectives and the empirical work.

Chapter 4 is the first of the three experimental studies and explores the initial visuospatial parameters of the fetal visual field. Specifically, it tests whether fetuses preferentially orientate to light stimuli in their temporal visual field.

Chapter 5 – the second of the experimental studies, moves beyond perception abilities and investigates if fetuses can perform anticipatory gaze. Specifically, it tests whether fetuses can use an auditory cue to anticipate the appearance of a light stimulus.

Chapter 6 – the final of the experimental studies, this study examines fetal sensitivity to spatio-temporal cues that correlate with adult interpretations of agentive motion. This work tests the hypothesis that fetuses will preferentially attend to a motion sequence that contains low level cues of agentive motion.

The thesis concludes with **Chapter 7** containing an integrative discussion of the contribution of the thesis to the literature. It also explores limitations and future directions for this field of study.

Chapter Two

The Role of Light in Prenatal Visual Development

Abstract

The uterus is a rich sensory environment. Only recently has fetal vision been considered within this framework. Importantly, models of exogenous light penetration to the uterus indicate that it is not dark during fetal development. Given the evidence for light in the womb, what does this mean for the development of the visual system across gestation? First, we examine the evolving capabilities of the fetal visual system and review the models of light in the maternal womb. This work shows that there is sufficient light in the uterus to enable a visual experience by the fetus during the third trimester. We then examine the role that light could play in shaping prenatal visual development. Importantly, animal models suggest that light during gestation is essential for the formation of the visual system. There is also evidence that prenatal visual experience can influence postnatal visual capacities in animals and humans. Finally, the question of how light can shape sensory development will be explored, with the possibility that the fetal body and limb movements could serve as a fundamental stimulus within the uterus. As such, cross-modal processing, embodiment, and multisensory development are likely topics that require reappraisal, given the implications of light in the uterus. Understanding light is essential for conceptualizing not just the prenatal visual system but almost all aspects of early human development.

2.1 Introduction

The developing fetus is immersed in a rich sensory domain. It is undisputed that the uterine environment plays a key role in forming sensory systems, with external auditory and gustatory stimulation a fundamental component of prenatal development (Graven & Browne, 2008). One essential environmental factor that has largely been overlooked during gestation is the role of light. The prevailing consensus is that the womb is a dark place; thus, prenatal visual development occurs in the absence of exogenous stimulation. The role of light and potential visual experience in utero has consequently not been considered. Recent modelling has proposed an alternative perspective whereby the uterus is not a dark place (Del Giudice, 2011; Isaac et al., in press). This challenges traditional assumptions of uterine development and compels us to consider the foundations of the human visual system.

This review explores the known connections between light and prenatal development in utero. First, we examine the capabilities of the fetal visual system across gestation and the models of light in the maternal womb. This is followed by a discussion of the likely role of light in shaping prenatal development and the significance of an illuminated uterus for perceptual and cognitive growth in utero. Through so doing, we are able to determine how and why light is an intrinsic environmental component for the developing human fetus.

2.2 The Fetal Visual System: from Components to Visual Capacities

Understanding fetal visual capabilities is one pathway via which we can understand the role of light in utero. Without an understanding of the anatomical structures of the visual system required for the interpretation of photons, any subsequent exploration of the role of light in prenatal development would be moot. Assessing fetal vision in utero poses inherent challenges with researchers often relying on animal models to

make working assumptions about the prenatal visual system and its development (Hendrickson et al., 2012; Kolb, 2003). Knowledge of the specific mechanisms that drive the development of the visual system in utero is an area of intense research activity in the biological sciences. But what are the capabilities of the fetal visual system? Here we outline the anatomical and physiological development of the visual system during pregnancy.

2.2.1 Visual system development during gestation

Though the visual system is generally regarded as the last of the sensory systems to mature, it is functional before birth (Lecanuet & Schaal, 1996). At 24 weeks gestation, the essential components of fetal vision are present, with significant and swift anatomical growth related to vision occurring in the first and second trimesters (Glass, 2002; Hendrickson et al., 2012; Hendrickson & Drucker, 1992). Biologically, the eye begins as an outgrowth of the brain in the embryonic period (Remington & Goodwin, 2021). Human eye development begins at around three weeks gestation, with the invagination of the optic grooves (Ludwig et al., 2023). At four weeks gestation, the optic grooves transform into optic vesicles, followed by optic cup formation at five weeks (Mann, 1928). The optic cup comprises an inner layer of the neural retina and an outer layer of the pigmented retina that will unite and form the optic stalk (Ludwig et al., 2023). Consistent linear growth is observed in the diameter and axial length of the fetal eye until midgestation (Ehlers et al., 1968; Hendrickson, 2016). Ocular vascularisation is a synchronous process that begins at five weeks gestation (Lutty & McLeod, 2018). Evidence of the eyelid structure begins in week nine of gestation; at week 20, eyelids are formed but fused. It is not until the third trimester, at 32 gestational age (GA), that the eyelids are typically separated and nearly fully developed (Tawfik et al., 2016), with blinking observed from 31 weeks GA (Petrikovsky et al., 2003). Embryonic eye development commences in early gestation, and by the third

trimester, the foundational ocular structures have primarily taken shape. The intricate developmental process involves both formation and maturation of multiple critical components essential for visual perception. The sequential progression of these critical structures provides insight into their fundamental growth throughout prenatal development.

One of the most critical structures of the visual system is the retina, with the consequence that it has been a primary focus of prenatal visual research. A light-sensitive tissue located at the back of the eyeball, the retina is responsible for transforming photons of light into images (Remington & Goodwin, 2021). The retina arises from the optic grooves originating as ectodermal and neural crest embryonic cells (Remington & Goodwin, 2021). By eight weeks gestation, the precursor retinal layers have developed (Bovolenta et al., 2010). Histological samples demonstrate that from 12 weeks, the fetal retina is partially functional (Hendrickson & Drucker, 1992). Unsurprisingly, clear anatomical differences exist between the fetal and adult eyes. Noticeably, before birth, the central retina is structurally immature, whilst the peripheral retina is more developmentally advanced (Abramov et al., 1982; Hendrickson et al., 2012; Hendrickson & Drucker, 1992; Vajzovic et al., 2012). Imaging of premature newborns and infants demonstrates that the central retina (including the fovea) continues to develop post-birth (Maldonado et al., 2011; Vajzovic et al., 2012). The development of specific components of the retina is discussed in more detail below.

Two distinct layers within the retina are the inner plexiform layer (IPL) and the outer plexiform layer (OPL). The layers contain complex neural networks and synaptic connections (Haines & Mihailoff, 2018). As the name suggests, the outer plexiform layer (OPL) is located on the retina's outer (Remington & Goodwin, 2021) and contains the synaptic connections between photoreceptors and horizontal cells. The

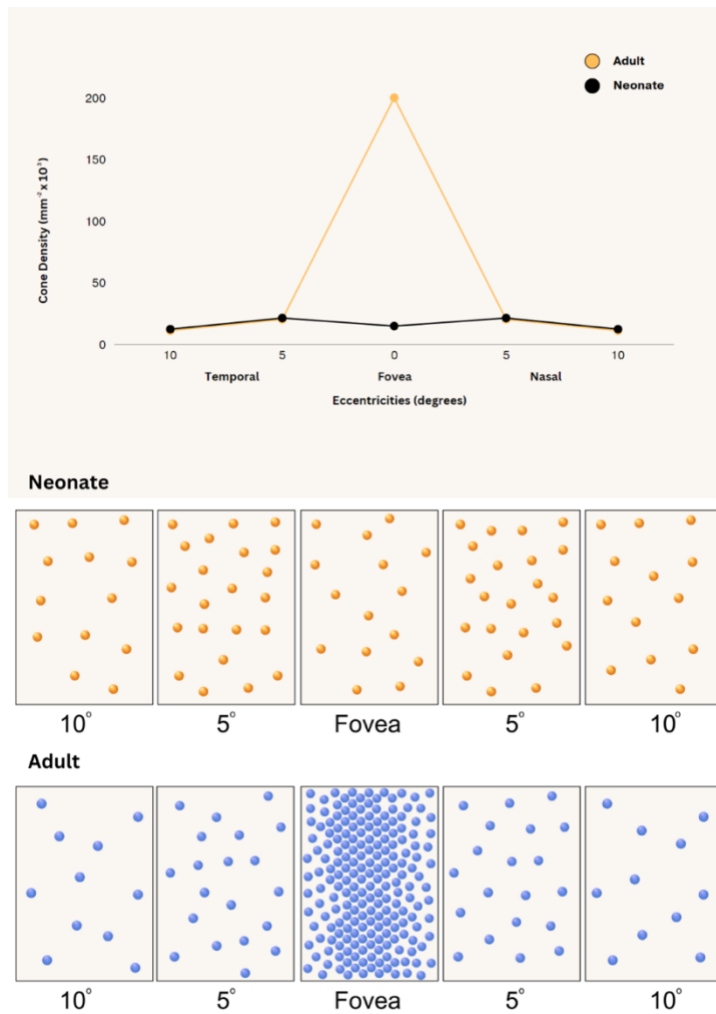
inner plexiform layer is located in the innermost layers of the retina and contains the synaptic contacts between bipolar, amacrine and ganglion cells (Remington & Goodwin, 2021). The fetal development of these two components of the retina is not uniform, with the inner plexiform layer (IPL) developing rapidly, starting in week eight of gestation and reaching full retinal coverage at mid-gestation (Hendrickson, 2016). In contrast, the outer plexiform layer (OPL) has a slow growth trajectory for the first 20 weeks of gestation but undergoes rapid extension to reach the peripheral edge at week 30 (Hendrickson, 2016). The development of the OPL is accompanied by the later maturation of the photoreceptors (Hendrickson, 2016). Clear changes happen in retinal layers throughout gestation but what is unknown is what precisely this reflects in terms of visual functionality and downstream implications for perception.

The central part of the retina is the fovea. It is highly specialised and responsible for high visual acuity and colour vision (Kolb, 2003). In adult humans, the fovea is characterised by the highest density of cone photoreceptors in the retina and a relative absence of rod photoreceptors (Remington & Goodwin, 2021). Morphologically the fovea is identified as a pit or depression with displaced inner retinal layers (Remington & Goodwin, 2021). The fovea is present from ten weeks gestation (Hendrickson & Drucker, 1992; Linberg & Fisher, 1990; Provis et al., 1985; Xiao & Hendrickson, 2000). The fovea pit begins to develop in mid-gestation at around week 24 (Hendrickson et al., 2012; Hendrickson & Yuodelis, 1984) but is not fully mature until after birth (Hendrickson et al., 2012; Vajzovic et al., 2012). The outward displacement of the inner retinal layers to form the foveal pit produces the inward displacement of photoreceptors post birth to increase cone density (Diaz-Araya & Provis, 1992; Hendrickson & Yuodelis, 1984; Yuodelis & Hendrickson, 1986). Foveal inner and outer segments are shorter (more immature) than the peripheral retina for weeks after birth (Abramov et al., 1982; Maldonado et al., 2011; Vajzovic et al., 2012). When

combined with the development and distribution of the photoreceptors, the maturation of the fovea suggests that during the prenatal period, the peripheral vision of the fetus might be more developmentally advanced than central vision.

Located within the retina are the photoreceptors. These are specialised cells that convert light into electrical signals, which are then transmitted to the brain for visual processing (Remington & Goodwin, 2021). The two main types of photoreceptor cells in the retina are cones and rods. Cones are responsible for colour vision and acuity in bright light, while rods are sensitive to dim light and responsible for vision in low light (Rodieck, 1998). Photoreceptors are organised in specific spatial mosaics for optimal visual functioning (Curcio et al., 1991; Osterberg, 1935). In the adult human eye, cones are most dense at the fovea; outside the central retina, rods outnumber cones twenty to one (Curcio & Hendrickson, 1991), with rods reaching peak density at the edge of the optic disc (Curcio et al., 1990; Hendrickson et al., 2008; Osterberg, 1935) see Figure 2.1.

Figure 2.1 Cone distribution in the adult and neonate central retina



Note. Cone density calculations from Candy et al., (1998).

A key component of vision located in photoreceptors are opsins. Opsins are the family of g-protein-coupled receptors responsible for detecting environmental light; they are photosensitive and activated by photons of light (Lamb, 2022; Shichida & Matsuyama, 2009). Visual opsins in the rods and cones of the retina are the start of the visual cascade that enables the decoding of light information and the construction of visual images. These include L-cone opsin, M-cone opsin, S-cone opsin and Rhodopsin (Lamb, 2022). Photoreceptors can first be identified in the fovea from around ten

weeks gestation (Hendrickson et al., 2008; Linberg & Fisher, 1990). Cones are first recognised at the fovea at ten weeks by the presence of S opsin, with the first cones identifiable at week 15 (Xiao & Hendrickson, 2000). Cones become more densely packed at the fovea during the formation of the foveal depression. This is due to the phenomenon known as cone migration. Migration of the cones occurs early in development, beginning at 13 weeks; before the formation of the foveal depression (Diaz-Araya & Provis, 1992) and continues post-birth into infancy (Maldonado et al., 2011). There is also a centrifugal migration of cells in the inner retinal layer away from the centre (Diaz-Araya & Provis, 1992; Hendrickson & Kupfer, 1976; Mann, 1928; Yuodelis & Hendrickson, 1986).

Rod generation begins in week 11 of gestation, and by week 15, rod maturation begins with the appearance of rhodopsin (Hendrickson et al., 2008). Rod density increases in the area adjacent to the foveal cone mosaic across gestation and into infancy. A distinctive aspect of the fetal retina is that in late gestation (week 35-37), photoreceptors located at the central retina (fovea) are notably immature compared to those located in the periphery (Abramov et al., 1982; Cornish et al., 2004; Hendrickson et al., 2012; Hendrickson & Drucker, 1992). Consequently, it is hypothesised that the fetal parafovea (periphery) might have better resolution and contrast sensitivity than the fetal fovea in the prenatal period (Hendrickson et al., 2012; Maldonado et al., 2011).

The innervation of the retina to the relevant cortical structures begins early in gestation. The establishment of neuronal connections in the visual system undergoes various stages. By week seven, optical axons have reached the lateral geniculate nucleus (LGN), a structure in the thalamus that processes visual information (Cooper, 1945; Gilbert, 1935), with synapses forming between the two structures at around 13-14 weeks (Khan et al., 1994). Around week 20, synapses start forming in the occipital subplate, a region in the developing brain associated with visual processing. At the same time, the afferent

connections from the LGN become segregated (Hevner, 2000). Finally, by week 23, synapses begin to form in the cortical plate, which is involved in higher-order visual processing (Hevner, 2000). These developmental milestones outlined in this paragraph capture the beginnings of the intricate wiring and maturation of the visual system in the fetus. The visual pathway from the retina to the visual cortex is complete at this time.

The major eye structures are in place from 24 weeks. While the visual system is still immature, with the development and organization of the anatomical architecture extending well into the postnatal period, the fetal visual system is anatomically functional in the third trimester, and the fetus has the capacity for a visual experience. The maturation process of the constituent components of the visual system means that the final trimester has been the focus of work exploring vision.

2.2.2 Visual research in utero

Although presenting visual stimuli in utero and recording fetal responses is complex, formative research has attempted to overcome the technical barriers associated with these activities (Dunn et al., 2015). Observational studies have presented light flashes to the fetus and recorded physiological responses. Peleg & Goldman, (1980) and Kiuchi et al., (2000) detailed increased eye movements and heart rate in response to a visual stimulus (a photographic flash) and vibroacoustic stimulation. Fetal magnetoencephalography (fMEG) has demonstrated fetal cortical activation in response to visual stimuli (Eswaran et al., 2002, 2004, 2005; Matuz et al., 2012; Sheridan et al., 2008). Similarly, a fetal magnetic resonance imaging (fMRI) study reported increased activity in the frontal cortex in response to the presentation of a constant light source to the fetus in utero (Fulford et al., 2003). Fetuses even demonstrate evidence of habituation to a visual stimulus, with decreased visually evoked responses to light flashes (Matuz et al., 2012; Sheridan et al., 2008).

Eye movements play a critical role in postnatal studies of visual processing (Fisher et al., 2017). Movement of the eye in response to visual information can also be documented in utero. The lens of the fetal eye is easily distinguished as a white circle within the hypoechoic eyeball using 2D ultrasound (Inoue et al., 1986). 2D ultrasound imaging can focus on the acoustic reflection of the eye lens and can record how it moves within the orbital socket (Horimoto et al., 1993). Eye movements are detected in the fetus at 14 weeks (Birnholtz, 1981; Horimoto et al., 1993). At 26 weeks, the eyelids can be partially open, and at 31 weeks GA, the pupil can dilate and detect light (Kiuchi et al., 2000; Tawfik et al., 2016). The amount of eye movements steadily increases throughout later gestation (Birnholtz, 1981; Horimoto et al., 1993; Inoue et al., 1986; Okawa et al., 2017). Studies of fetal eye movements have been largely observational and focus on indexing patterns of eye movements and their relationship to broad indicators of neurological development, including REM cycles and sleep (Birnholtz, 1981; Horimoto et al., 1993; Okawa et al., 2017). Birnholtz et al. (1981) classified four types of movements relating to the motion pattern of the lens. Type I and II eye movements are analogous to slow eye movements while Type III and IV correspond to rapid eye movements (Birnholtz, 1981). Different eye movement patterns aligned with specific gestation ages, with more Type III and IV movements seen in later gestation. Further, eye movements have been recorded in response to visual information. When presented with light, the eye movements of fetuses aged 34 weeks also correspond to activation in visual and central brain regions, suggesting an active visuomotor system prior to birth (Schopf et al., 2014). When coupled with the ability to process perceptual information in the third trimester (DeCasper & Fifer, 1980; Witt & Reutter, 1996; Zoia et al., 2007), this work shows that the fetus can engage with the visual environment.

Experimental studies have also expanded the understanding of fetal visual perception. Donovan et al., (2020) recorded eye movements before, during, and after the delivery of moving light stimuli. Results showed significantly increased eye movement during and after the presented light stimulus. These findings indicate that fetal oculomotor behavior is not random and is informed by the uterine environment.

Reid et al., (2017) simultaneously investigated face-processing capabilities in the fetus and explored a new method of delivering visual stimuli to the fetus in utero. Grounded in the established neonatal preference for face-like stimuli (Fantz, 1963; Morton & Johnson, 1991), the researchers presented two separate light arrays to the fetus, face-like stimuli and the same stimuli inverted. Fetal head movements were concurrently recorded via a 4D ultrasound. Fetuses demonstrated significantly more head turns towards the face-like stimuli when contrasted with the inverted stimuli. Given the lack of knowledge surrounding fetal behavior, the authors also examined whether the sample would turn away from the light derived stimuli, as it may have been aversive. Very few such responses were seen. These results suggested that the preference for the face-like stimulus seen in neonates is present prenatally and that visual preferences are present prior to birth.

The human fetus has a functional visual system capable of visual experience in the third trimester. It is evident across various measures, from fetal heart rate (Kiuchi et al., 2000; Peleg & Goldman, 1980) to fMEG (Eswaran et al., 2002, 2004, 2005; Matuz et al., 2012; Sheridan et al., 2008), that fetuses are responsive to external visual stimuli. Taken together, these studies are evidence of prenatal perceptual visual capabilities in the third trimester. Observational and experimental research has demonstrated that fetuses respond to visual stimuli in utero across various behavioral indicators showing that the visual system is operational at this time during gestation.

However, the question remains, what visual experience does a typically developing fetus have in utero? The overriding assumption has been that the fetus receives no exogenous stimulation to the visual system. Despite the fetus having the biological components required for vision, it has been conjectured that the light levels within the womb were too low to actualise a visual experience. A persistent idea in developmental research is that neither light nor visual experience is a requirement for prenatal visual development. This sentiment continues to be present in modern literature (e.g., Graven & Browne, (2008); Streri & de Hevia, (2023)), where it is stated that the visual system develops without external stimulation and is only functional after birth. The possibility of prenatal visual perception has consequently been largely ignored or dismissed (Glass, 2002; Lecanuet & Schaal, 1996; Myowa-Yamakoshi & Takeshita, 2006).

Can we conclude that visual development in utero occurs without light and, by proxy, there is no visual experience during gestation? Animal models with mice show that light is necessary for normal eye development in utero (Rao et al., 2013). What evidence do we have that the uterus is dark? Early modelling by Del Giudice (2011) challenged this assumption, concluding that the light levels within the uterine environment were much higher than initially presumed. In the final two months of pregnancy, the Del Giudice model suggests that the uterine environment is illuminated above the threshold for fetuses to attend to and orient towards visual stimuli within the uterus. A uterus with a sufficiently high ambient light level raises multiple questions. What does the visual landscape of the uterine environment consist of? What visual experience could the fetus realistically have, and what might this tell us about fetal visual development and light in the womb? In section two, we explore light levels in utero and the implications for fetal development.

2.3 Light in the Uterus

Initial work on how much light passes through tissue to the uterus was based on animal models. This work suggested that sufficient light enters the uterine environment to allow for external visual stimulation (Jacques et al., 1987). The uterus might not be a dark place. Jacques et al., (2000) used mathematical modelling to simulate the intensity of light reaching the fetal brain in order to understand how photons interact with human tissue. More recent work by Del Giudice (2011) explored the potential of human visual experience prior to birth by using quantitative modelling to assess the visual environment of the human fetus during the final trimester. The conclusion, that fetuses could experience sufficient illumination for a visual experience, challenges prior assumptions of the uterine environment. In the following section, we explore the current state of our understanding of this environment and its illuminance, and we propose several directions to expand on the previous work.

2.3.1 Early Models of Light in the Uterus

An extensive body of literature within the field of tissue optics focuses on applying mathematical modelling techniques to light propagation through human tissue. Analytic approaches (focusing on the transport equation and diffusion approximation) were common before the widespread adoption of computational modelling techniques such as Monte Carlo (MC) methods (Ishimaru, 1978; Van Gemert et al., 1987; Yoon et al., 1987).

Much research has been devoted to understanding the interactions between light and tissue; however, few attempts have explored the amount of light reaching the uterus and what that would mean in terms of illumination for the fetus. The first experimental investigation into the levels of light reaching the fetus was conducted via surgically implanted optical fibers in animals (Jacques et al., 1987). The light was detected by the fibers in the uterus after light was applied to the skin. This work showed that certain

experimental conditions could lead to an illuminated uterine cavity in animals. This result offered clear implications for an extension to the human fetus.

Due to the difficulties in gathering experimental data for transmitting light stimuli toward the human fetus, computational modelling of tissue optics has been used to explore this issue. Jacques et al., (2000) developed a model-based investigation of light in the womb, exploring photon transport through maternal tissue to the fetal brain. The focus was on the possibility of detecting fetal distress prior to birth with simulated results providing early quantitative predictions of photon incidence upon the fetus. This work also demonstrated the feasibility of light reaching the human fetus.

Little work has focused on quantifying the illuminance of the womb from external light sources. Despite the long-held assumption of a dark uterine environment, Del Giudice, (2011) provided an early attempt at modelling light transmission to the uterus. Combining experimental and modelling approaches, the work provided a qualitative model of illuminance in the uterine cavity compared to the external environment's illuminance. The transmission coefficients for samples of deceased avian tissue (with varying thicknesses) and clothing (with varying colours) were determined by physically measuring the illuminance present above a light source with and without the samples obstructing the source. Next, multiple linear regression methods were used to determine a collective regression equation for the abdominal wall through the values determined via avian tissue sampling. The computed equation incorporated factors representing the impact of clothing ($c = 1.00$ for bare skin, 0.50 for one layer of light cloth, and 0.05 for one layer of dark, heavy cloth), the total thickness of the abdominal wall (t), and the muscle/fat ratio of the abdominal wall.

The approach employed by Del Giudice (2011) was similar to those used by Fulford et al. (2003) to determine an avian tissue proxy for human tissue. The latter's work focused on using results to guide the measurement of fetal brain activity in response

to visual stimuli utilizing fMRI. It also excluded the impact of adipose tissue in calculations. The calculated uterine illumination levels in Del Giudice (2011) were comparable to those measured by Jacques et al. (1987) – even though the latter’s work used rats and guinea pigs as experimental subjects – indicating potential extension to the human fetus. Crucially, the conclusion of Del Giudice (2011) was to suggest that many human fetuses develop in an environment that would enable a visual experience. Del Giudice’s (2011) work to accurately measure uterine illumination should be handled with care, however, due to the limited nature of the model employed and the use of avian tissue. Light has been shown through simulations to penetrate human maternal abdominal tissue and reach the uterus (Jacques et al., 2000), but the exact uterine illumination levels are not yet determined.

2.3.2 Limitations in Recent Modelling

Despite the profound implications of the conclusions drawn by Del Giudice (2011), there are several clear limitations to this first foray into quantifying human uterine light levels. First, the model implicitly assumes uniformity in the maternal tissue. We know, however, that there is anatomical variation in tissue density around the uterus which should be considered when determining the levels of light reaching the fetus. For example, tissue density varies across individuals, and this variation is also evident across the body during the later stages of gestation. We cannot assume uniformity in tissue density, with the depth of the anterior abdominal wall reduced due to the growth of the fetus. In contrast, tissue nearer to the spine remains relatively dense. Taking these factors into account could enable a wider application of the results. In addition to the static nature of the model, it is also incapable of readily accounting for any variance in tissue density from a temporal perspective - i.e., across the gestation period – nor is the positioning of the fetus able to be accounted for, despite the changing location of the fetal eyes within the womb over gestation as the fetus typically orients

in different positions in the womb. This is a common limitation of mathematical models beyond that of Del Giudice: the works present a snapshot of the uterine environment, but the anatomical surroundings experience large scale changes across the course of gestation. Incorporating these factors would enable a more accurate and dynamic picture of the levels of uterine illumination.

Building on the assumption of tissue uniformity, the initial model cannot readily account for the inhomogeneity of the tissue with increasing depth. For instance, skin tissue comprises multiple layers, e.g. epidermis, dermis, and subcutaneous tissue, each with varying optical properties (Nasouri et al., 2014; Nishidate et al., 2004). Light attenuation is dependent on the properties of the mediums through which it propagates. Despite this, Del Giudice's (2011) model assumes a homogeneous averaging of the tissue and does not account for any layer-dependent light diffusion. Although the approximation of skin tissue as a uniform, homogenous medium provides an initial estimate for the transmission of light, the practical extensions of this estimation are constrained. Any modelling of mobile light sources, for instance, would introduce variations across the tissue that need to be accounted for, such as changes in networks of blood vessels. Similarly, there needs to be an assessment of skin tone on light transmission.

Further, Del Giudice. (2011) does not consider any effects due to amniotic fluid. Clearly, there would be some impact on light transmission to the fetus from the presence of this fluid due to its scattering nature. For example, the transmission from maternal tissue to amniotic fluid would introduce refraction and reflection to light propagation, thereby influencing the levels of light reaching the fetus. The light levels reaching the fetus, as determined by a model without amniotic fluid consideration, will vary noticeably from those with amniotic fluid (Gunther et al., 2021).

Despite these issues, Del Giudice's (2011) model provides an excellent starting point to examine the illuminance of the uterus over pregnancy. It also provides the basis for developing other models to determine more precise levels of uterine illuminance.

2.3.3 Modelling Approaches to How Light Moves Through Tissue

Modelling light typically simulates it as the propagation of many photons. When travelling through tissue, light undergoes attenuation due to absorption and scattering. Absorption occurs within the tissue due to photon interactions with chromophores, with the photon being absorbed in an all-or-nothing event if its frequency matches that associated with the chromophore's energy transition (Sandell & Zhu, 2011). Similarly, photon scattering occurs most strongly from structures with sizes comparable to the photon's wavelength – skin, at the histological level, has many such structures – which leads to the scattering of light as it travels through tissue. Each layer can be treated as a distinct medium for modelling, with each of these mediums having their own associated absorption and scattering parameters. It is possible to account for these photon-tissue interactions via computational modelling to accurately represent the light propagation from a physical perspective. Typically, two main approaches are employed in modelling the propagation of photons through tissue (i.e., tissue optics): diffusion theory approximations and, more recently, Monte Carlo (MC) methods. Here we explore the usage of models to simulate the movement of light through tissue and outline the underlying components and parameters involved.

A prior modelling method involved estimating the diffusion approximation to light and solving the resultant diffusion equations by analytic or numerical means. Even though diffusion approximation can provide results in agreement with those found empirically, it has several limitations. Its validity in tissue is primarily for situations where the light has been highly scattered (Welch & Van Gemert, 2011) or where the probability of scattering events is much greater than absorption events (Duderstadt &

Hamilton, 1976). Models of diffusion approximation do not converge in cases where there is high significance for absorption alone, as well as scattering processes. Diffusion theory approximation can also fail when photon path lengths are too small (Graaff et al., 1993). Further, not all wavelengths provide reliable results (Patterson et al., 1991). For these reasons, modelling research within tissue optics has moved towards what is currently referred to as the “gold standard” (Periyasamy & Pramanik, 2017): simulation via Monte Carlo methods.

The term “Monte Carlo method” refers to a broad class of mathematical and computational techniques used to predict the outcomes of an uncertain event by generating numerical results via repeated random sampling. More generally, the MC method can provide numerical solutions to problems involving the evolution over time of object-object interactions (Seco & Verhaegen, 2013). Through so doing, randomness can approximate the outcomes of complex but deterministic aspects of a problem. This is readily applicable to the case of photon propagation through tissue via the assumption of photons acting as ballistic particles repeatedly interacting with tissue. The first MC model to assess human tissue was implemented by Wilson & Adam, (1983). In this work, a computational model was developed to simulate the propagation of light through homogeneous tissues under different absorption and scattering ratios. Light attenuation was assumed to result from a combination of absorption and isotropic scattering, the latter of which enabled a much-simplified model alongside the assumed homogeneity of tissue. This early work was challenged due to the nature of scattering - isotropic vs forward-scattering (Jacques et al., 1987) whereby most scattering events in tissue result in photons scattering with an angle of less than 90 degrees from their initial propagation direction.

Jacques et al (1987) demonstrated that how light interfaced with human tissue could be well-described by an analytic expression initially used to describe the scattering of

light by clouds of interstellar dust (Henyey & Greenstein, 1941). This function enabled variation from back-scattering tissue to isotropic scattering to forward-scattering tissue by the adjustment of a single parameter: the anisotropy. Typically, the anisotropy value of biological tissues is ~ 0.9 , indicating highly forward-scattering material (Tuchin, 2007). This was incorporated into a photon propagation model through biological tissue by (Keijzer et al., (1989). This model introduced a standard sequence for photon behaviour through tissue (Jacques, 2022). Specifically, a “Hop” stage where the photon moves along its trajectory, a “Drop” stage where the photon interacts with the tissue at its new position in a manner that involves a proportion of estimated absorption, and a “Spin” stage where the photon is scattered into a new direction. Each MC model must take into account absorption and scattering due to interactions with the medium through which the photon passes.

The refractive index is the final optical parameter required to specify the tissue medium beyond the three-step process described thus far. This defines the change in the local speed of light as it propagates between medium and consequently, the amount of “bending” experienced by photon trajectories across mediums. By incorporating this parameter, the propagation effects of photons traversing multiple mediums can be taken into account.

From this description, it is possible to see that the process of MC modelling for photon propagation in tissue is relatively clear: photons are initialised; they are launched with a calculated step size calculated via appropriately distributed random numbers; at the end of this step size (for single layer tissues), they experience some absorption; and they experience a scattering process. This continues until the “weight” of the photon is reduced (absorbed) below a designated threshold. If the medium involves multiple layers with differing parameters, we can then use the refractive indices via further

mathematical inclusions to model and incorporate reflection at the boundaries between tissue layers.

2.3.4 Directions for further research on light modelling

Significant work in this area is required. Rather than simply simulating light transmission to the fetus from a temporally stagnant view, the literature currently lacks a mathematical model of light in the uterus that incorporates changes across gestation. Such a model could map how the amount of light reaching the fetal eye through dynamically dispersive (living) tissue changes as pregnancy progresses, which would involve investigating not only the changes due to tissue density fluctuation but also those brought about by the variable orientations and positions taken by the fetus.

Similarly, we have readily apparent tools in place via the described Monte Carlo models to understand how individually targeted light stimuli appear to the fetus. The possibility to utilise moving stimuli opens up many exciting avenues for research into the fetal visual system. This is an area in which computational modelling efforts can enable a clearer understanding of the limitations present when designing light-based stimuli for experimental work with the fetus. For example, a greater understanding of the distances at which light sources can be placed from each other before beginning to appear as one light source can be determined. As a further example, exploring the fetal visual system through experimental arrangements involving motion of light may now be possible given informed simulations of the appearance of the light source for the fetus.

A further extension to the current modelling work involves exploring the illumination of the uterus provided by natural light during pregnancy. The visual experience provided to the fetus by ambient natural light on a daily basis has not been determined to date. This includes our understanding of the actual typical intensity of daylight encountered by the fetus.

2.3.5 Summary: What do Models Tell Us About Light in the Uterus?

Light penetrates through maternal tissue, illuminating the uterus. The fetus is not in a dark environment throughout gestation although the degree of illumination that the fetus experiences is not fully determined. Even though we know that the womb is not dark, what is its illuminance across gestation? Research examining how light moves through tissues and to the uterus is limited. Those papers investigating similar scenarios do so with different conditions, alternative means of modelling, one layer assumptions of tissue, or limited extension to the temporal and dynamic nature of change during pregnancy. The modelling of Jacques et al., (2000) and Del Giudice (2011) provides an excellent foundational point from which to examine light levels in the womb. The MC models previously outlined would unquestionably improve our understanding of the uterine environment and the developing fetal visual system. A key question must be asked that moves beyond modelling light levels in the uterus: what role does light play in development across gestation?

2.4 The Role of Light in Prenatal Development

It has been established that the uterine environment is illuminated. This provokes the question of what role prenatal light exposure may play in developmental outcomes. A persistent assumption present in an array of fields has been informed by the belief that the uterus was dark. In this framework, light, and therefore visual stimulation, is not a requirement for normative visual or physical development in the prenatal period. The knowledge that fetal development occurs in the presence of photic stimulation opens up new avenues for exploring and understanding the earliest developmental processes on a physiological and behavioral level. The research highlighted here shows that light exposure in utero is an environmental factor that requires careful consideration.

2.4.1 Opsins in the Retina and Beyond

Opsins provide fundamental insights into the role of light in utero. Opsins are the family of g-protein-coupled receptors responsible for detecting environmental light; they are photosensitive and activated by photons of light (Shichida & Matsuyama, 2009). Operating in parallel to visual opsins are non-visual or atypical opsins. A relatively recent discovery, atypical opsins are photoresponsive but not directly involved in visual perception (Tarttelin et al., 2003). The atypical opsins include RGR-opsin, Opsin 3 (Opn3, panopsin), Opsin 4 (Opn4 or melanopsin) and Opsin 5 (Opn5, neuropsin) (Blackshaw & Snyder, 1999; Provencio et al., 1998; Tarttelin et al., 2003). Relatively little is known about the exact function of the atypical opsins and the function of light detection outside the visual system; however, Opn4 has been found to play key roles in circadian processes, DNA repair and cell cycle regulation (Freedman et al., 1999; Lucas et al., 2003; Xue et al., 2011).

Atypical opsins are expressed from six to eight weeks gestation, much earlier than the rod and cone opsins that are present in the fetal eye tissue later in development (Bibb et al., 2001). The presence of atypical opsins in early gestation suggests that they may play a role in light-mediated functions in utero (Tarttelin et al., 2003). Understanding the role of atypical opsins in prenatal development is vital for understanding how light exposure during this period can impact upon development. Novel research on Opn4 (Rao et al., 2013) and Opn3 (Davies et al., 2021) suggest light exposure in utero is important for visual and nervous system development. Vascular patterning, including hyaloid regression (removal of established blood vessels), is a vital aspect of eye development in the mouse that occurs postnatally. Rao et al. (2013) demonstrated that Opn4 prenatally mediates a light-dependent pathway in the developing visual system. Researchers found that mice gestated in a dark environment did not exhibit typical hyaloid regression, indicating that light exposure was required to initiate this process.

These results were replicated in knockout *Opn4*^{-/-} mice raised in normal light conditions indicating that the photosensitive *Opn4* mediates the pathway for this development (Rao et al., 2013). Interestingly, there was also a critical light-response period for stimulating hyaloid regression around day 16 in the mouse gestational period. These results suggest that a prenatal light response is a critical trigger for specific retinal and vascular development in the mouse eye.

Work with opsins shows that light exposure directly to the developing fetus, not the gestating mother, is critical for the vascular development of the eye. Knockout *Opn4* mice (*Opn4*^{-/-}) gestated in wild-type females demonstrated persistent hyaloid vasculature. In contrast, wild-type pups gestated in enucleated female mice under normal lighting conditions demonstrated typical hyaloid regression. This work shows that the direct light exposure of the mouse fetus - not the mother - determines typical visual development. With humans, the *Opn4* light-mediated pathways in the fetus are currently unknown; however, the presence of *Opn4* early in human gestation suggests that light levels experienced throughout pregnancy may play a similar role in specific prenatal developmental processes.

A further atypical opsin that could illuminate light-dependent processes in utero is *Opn3*. Researchers used fluorescent protein markers to map the temporal and spatial location of *Opn3* during mouse embryogenesis (Davies et al., 2021). *Opn3* expression was prolific and not exclusive to the retina. It was also detected widely throughout the developing cortex, including the cranial and spinal nerve ganglia, sensory organs, brainstem, cerebellum, hypothalamus and thalamus. The widespread distribution of *Opn3* in gestation suggests that light-dependent developmental processes may exist in the prenatal period in mice. This work, while at an early stage, shows that atypical opsins can provide insights into the effects of prenatal light on development. Research on the mechanisms of atypical opsins is currently grounded in mouse models (Davies

et al., 2021; Rao et al., 2013) and clearly demonstrates that light is an important factor in prenatal development. Further work exploring these mechanisms in both animal and human models will provide a strong starting point for understanding the role of light-dependent pathways during fetal development.

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2.4.2 Prenatal Light Exposure and Postnatal Functioning in Avian Models

Complimenting the research on atypical opsins in mammalian models is an abundance of studies on the effects of embryonic light experiences in bird species. Avian research consistently demonstrates the vital role of prenatal light exposure in postnatal outcomes. A notable example is the hemispheric lateralisation of the avian brain as a direct result of environmental light in the prenatal period (Rogers, 1997). A chicken embryo's left eye is occluded from visual stimulation due to the position of its body and yolk sac; in contrast, the chick's right eye receives direct light stimulation (Rogers, 1997). Light exposure to the right retina of the chick embryo produces structural asymmetries that result in hemispheric lateralisation observed at a neural and behavioral level in the newborn chick (Deng & Rogers, 2002; Rogers, 1982; Rogers & Deng, 1999; Skiba et al., 2002). This lateralisation is triggered by prenatal visual stimulation, with dark incubated chicks demonstrating no hemispheric lateralization (Dharmaretnam & Rogers, 2005; Rogers, 1997). Hemispheric asymmetry can be reversed by exposing the opposite eye (left) or both eyes to prenatal visual stimulation (Casey & Karpinski, 1999; Manns & Güntürkün, 1997, 1999; Rogers, 1997).

Environmental light in early and late embryonic development has triggered different mechanisms that result in asymmetric functioning in the avian brain. Prenatal light exposure in early gestation can influence lateralisation before the visual system is functional (Chiandetti et al., 2013). For this period in development, the proposed mechanism is not the same as the retinal path for light exposure in late gestation. It is proposed that prenatal light exposure (or lack thereof) influences gene expression in photosensitive areas of the anatomy (Chiandetti et al., 2013; Chiandetti & Vallortigara, 2019). The research on hemispheric lateralisation in the avian brain demonstrates that multiple periods and mechanisms are responsive to light stimulation across embryonic development. Importantly, it is not only light stimulation but the timing of that light exposure that has implications for cortical development.

Light exposure in the embryonic phase has impacts beyond avian neural development. Manipulating environmental light in the prenatal period can affect avian gait (Belnap & Lickliter, 2019; Sindhurakar & Bradley, 2010), body composition (Rozenboim et al., 2004; Sindhurakar & Bradley, 2012), motor development (Belnap & Lickliter, 2017; Bradley & Jahng, 2003; Sindhurakar & Bradley, 2012), incubation length (Lauber, 1975; Siegel et al., 1969) and visual motor-specialisation (Sindhurakar & Bradley, 2012). Evidently, light exposure in gestation is a critical environmental factor for a wide range of avian developmental outcomes. Overall, avian models have contributed significantly to our understanding of the effects of prenatal light and highlight the importance of exploring this area of research.

The work explored thus far on atypical opsins is centred on non-human models. Obvious ethical issues prevent many of these studies, and those from avian populations, from being replicated with human samples. With humans, a body of observational epidemiological research indicates prenatal light exposure has implications for postnatal outcomes.

2.4.3 Season of Birth

Season of birth (SOB) is a natural experiment that can provide insights into candidate environmental factors for postnatal function in humans. Season and month of birth provide direct support for intrauterine exposures (independent of genetic effects) that may impact later developmental outcomes. Light exposure fluctuates regularly across the year, while other environmental and genetic factors remain relatively stable. Differential outcomes depending on SOB could indicate that light levels affect specific developmental processes. One area of work explores myopia (a common visual impairment (Remington & Goodwin, 2021)) and SOB. Retrospective cross-sectional research in both Israeli (Mandel et al., 2008) and Australian (McMahon et al., 2009) samples demonstrated that the season of birth was associated with an increased risk of severe myopia. Children born in the summer months are at the most risk. Mandel et al. (2008) found that there was also a significant correlation between ambient light in the prenatal period and the risk of myopia. The researchers proposed that the mechanism for this relationship was the amount of ambient light an individual experienced in early postnatal development. Under this model, children born in the summer months experience higher ambient light levels, affecting eye development and increasing risk of myopia. This was challenged by McMahon et al. (2009), who found no association between high myopia and neonatal ambient light exposure. If significant consideration is given to ambient light exposure postnatally, could the ambient light experience in the prenatal period also offer a possible explanation? Individuals born in different seasons may have experienced different ambient light levels during gestation. Ambient light levels vary across the seasons and other factors, including the type of clothing worn during pregnancy (heavier winter clothing occludes light to the uterus) and the amount of time spent outside in direct sunlight (potentially higher in the summer months). Undoubtedly many other factors could mediate the risk factor for

severe myopia and SOB. However, as evidenced in the light-dependent pathways of *Opn4* (Rao et al., 2013) and the visual system, consideration could be given to exploring the role of light in the developmental process throughout the prenatal period and how that impacts visual system development.

Season of birth is not limited to increased risk of myopia. In fact, SOB is linked to varied conditions, including schizophrenia and bipolar disorder (Torrey et al., 1996, 1997), risk of suicide (Chotai et al., 2003; Rock et al., 2006), cognitive function (Kucera et al., 2021), depression (Torrey et al., 1997), anorexia (Disanto et al., 2011; Jongbloet et al., 2005) personality disorder (Kendell et al., 2002; Videbech et al., 1974), panic disorder (Castrogiovanni et al., 1999), autism (Stevens et al., 2000), weight (Hemati et al., 2021) and emotional and behavioral regulation (Asano et al., 2016). The mechanisms surrounding the relationship between SOB and specific health outcomes are widely debated; however, one clear conclusion from this research is that environmental conditions in utero influence outcomes. Light experience in utero is a factor that requires exploration. Ambient light levels can vary depending on the specific seasons across gestation. Light experienced in utero at specific critical times could influence light-dependent processes that, in turn, have significant effects on development ranging from visual sensory systems to long-term mental health.

2.4.4 Fetal Circadian Rhythms and Prenatal Light Exposure

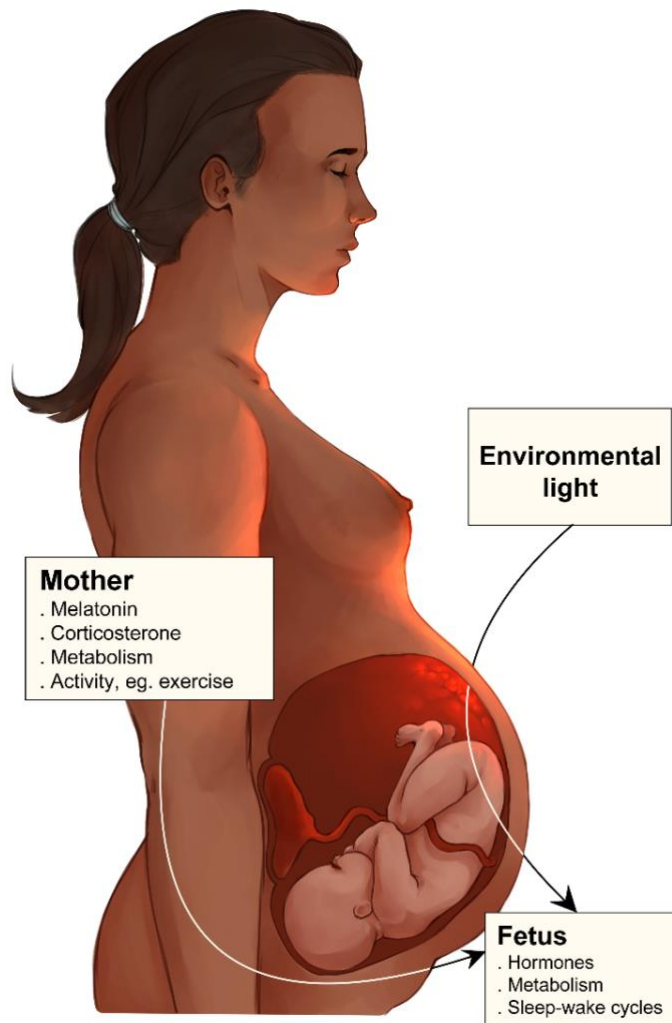
Fetal circadian rhythms are another avenue of investigation for prenatal light exposure. Circadian rhythms are 24-hour cycles ubiquitous across physiological processes in the human body (Reppert & Weaver, 2002). Circadian rhythms allow humans to maximise responding to their environment (light-dark cycle), including when to sleep, move and eat. Disrupted circadian cycles are associated with various brain disorders and impaired functioning (Logan & McClung, 2019). The circadian system is controlled by the suprachiasmatic nuclei (SCN), often referred to as “the master clock,” and is

influenced by complex interactions of both internal intrinsic and environmental stimuli (Reppert & Weaver, 2002). Fetal circadian rhythms are of interest because light is the most important environmental signal for entraining circadian rhythms in the adult human (Wright et al., 2001, 2013).

The development of mammalian circadian rhythms begins in the fetal period (Mirmiran et al., 1990, 1992; Serón-Ferré et al., 2001). Human fetuses demonstrate evidence of circadian rhythms in the third trimester that is synced to the maternal day-night cycle (Lunshof et al., 1998; Serón-Ferré et al., 2001). An unknown feature of the fetal circadian clock is entrainment. Models concerning fetal circadian rhythms propose that they are initiated by maternal factors, including the mother's light exposure, feeding, body and hormone release (Logan & McClung, 2019; Seron-Ferre et al., 2007; Serón-Ferré et al., 2012). In this model, the fetus is not responding directly to external environmental factors; instead, their observed circadian rhythms respond to maternal signalling. Fetal responding is undoubtedly intrinsically linked to the maternal environment; however, models of fetal circadian rhythms are based on the premise that the fetus is in a dark environment and, therefore, there is no light-based environmental cue that directly interfaces with the fetus.

A potential parallel mechanism for entraining fetal circadian rhythms is the fetus directly responding to light experienced in utero (see Figure 2.2). Given that the uterine environment is not dark, there is the possibility for the fetus to experience differential light levels across the light-dark cycle. This enables light to serve as a direct environmental cue to the fetus and the fetal SCN. Under this new proposed scenario, maternal factors may not wholly mediate fetal circadian rhythms but instead these rhythms are also influenced by the light levels the fetus experiences in utero.

Figure 2.2 *Entrainment of fetal circadian rhythms.*



Note. Illustrating two potentially separate pathways that could act on the fetus. The first is a direct light path of environmental light through the uterus. The second pathway is an indirect route through the mother's circadian response that acts on the fetus.

Understanding the nuances of fetal circadian cycles, including the role of direct light experience in utero, is essential for comprehending the genesis of circadian rhythms and the effect that early circadian rhythms have downstream for development. It is already known that disruptions to maternal circadian cycles can have adverse postnatal outcomes. For example, chronic circadian disruption in utero is associated with

behavioral deficits in mice, including increased social avoidance and hyperactivity (Smarr et al., 2017). Exposing pregnant rats to a reversal of the day-night cycle also alters gene expression in the fetal liver (Varcoe et al., 2013). Further, the offspring of non-human primates who experience constant light exposure during pregnancy present dysregulated melatonin and body temperature rhythms (Matsumoto et al., 1991; Serón-Ferré et al., 2013; Torres-Farfan et al., 2004). In humans, pregnant shift workers who experience disrupted circadian rhythms are at increased risk of miscarriage, low birth weight and premature delivery (Aspholm et al., 1999; Bisanti et al., 1996; Mahoney, 2010). Maternal light exposure throughout pregnancy clearly has far-reaching effects; understanding circadian cycles and the fetus's direct role in responding to light is vital for our understanding of these mechanisms.

In summary, light is an important environmental factor that must be considered when understanding various mechanisms throughout prenatal development. Prenatal light exposure can have wide-ranging effects on development, from neural structures to behavioral outcomes. These factors go well beyond the visual sensory system. Studies on atypical opsins, avian models, the season of birth and circadian rhythms provide the foundation for investigating the impact of prenatal light exposure on development. When considered together, this diverse range of research deriving from biology, anatomy, and obstetrics highlights the importance of investigating the effect of light during pregnancy.

Developmental processes throughout the prenatal period present a large number of unknowns. Light exposure has not typically been considered as a factor that could influence development. Further research in this area may have important implications for designing appropriate lighting environments or light therapies during pregnancy. It may help to mitigate the effects of light-related disorders, including adverse outcomes associated with the season of birth and disrupted circadian rhythms. Light is a crucial

environmental factor that must be considered to comprehensively understand the mechanisms of prenatal development.

2.5 The Role of Light in Perceptual and Sensory Development

How does a fetus engage with their visual world? Sufficient illumination in the womb challenges the long-held notion that the newborn is devoid of a prenatal visual experience. The previous section explored the role light could play in shaping fetal anatomical and physiological structures. This section explores the possibilities that emerge for perceptual and cognitive development if the fetus has visual stimulation in the third trimester. Could prenatal visual experiences better equip newborns to make sense of James' "blooming buzzing confusion" than previously considered?

Fetal sensory development also lies at the heart of a fundamental debate in developmental psychology, centered around the origins of perceptual and cognitive abilities. Newborns are equipped with a remarkable array of social and cognitive sensitivities (Johnson et al., 2008). These neonatal abilities have been taken as evidence for the innate origins of cognitive or perceptual processing or rapid imprinting (DeCasper & Spence, 1986). However, ascribing to the theory of innate origins discounts the influence of the prenatal environment. An alternative possibility is that these abilities have their foundations prenatally. Birth is not the beginning but simply a continuation of developmental trajectories that began in utero (DiPietro et al., 2015).

The prenatal period was long considered a "developmental limbo" (Hopkins & Johnson, 2005). Although this view is now largely disregarded, the inherent physical challenges of conducting research in utero have stalled attempts to move the field forward (Dunn et al., 2015). What is undisputed is that neonates possess a complex repertoire of cognitive and social abilities. Newborns have a memory for auditory stimuli that they have heard in utero (DeCasper & Spence, 1986; James et al., 2002)

and a preference for familiar voices and languages (Byers-Heinlein et al., 2010; Coulon et al., 2011; May et al., 2011; Moon et al., 1993). Neonates hold eye contact with others (Pitti et al., 2013), have a preference for faces when contrasted with other visual stimuli (Morton & Johnson, 1991) and possess a rudimentary understanding of numeracy (Coubart et al., 2014).

Despite research indicating perceptual abilities in utero, one view in the literature is that the fetus is in stasis and lacks “awareness” within the prenatal period (Lagercrantz, 2016). Padilla and Lagercrantz (2020) argue that the fetus does not meet the criteria for consciousness and is, therefore, unlikely to be aware of the uterine environment or itself. The suggestion that neonatal abilities spontaneously appear autonomously at birth or result from active sleep learning (Padilla & Lagercrantz, 2020) is remiss when so little is known about prenatal development. Further, there is evidence of continuity between neonatal and prenatal sensory perception (Almli et al., 2001; André et al., 2018; Holst et al., 2005). An fMEG study comparing fetal and neonate responses to tones indicated a continual decrease in response times as age increased, with no significant jump between the pre and post-natal periods (Draganova et al., 2007). Leg movements in sleep states have continuity across the fetal to neonate trajectory (Almli et al., 2001), and there are no self-produced movements present in the newborn that is not also present in the fetus (Stanojevic, 2008). Arguably, the uterine environment plays a role in shaping sensory development, but the exact nature of this relationship is still unclear (André et al., 2018).

Prenatal visual research provides clear evidence of fetal visual capabilities (Dunn et al., 2015). Observational studies have utilised fetal magnetoencephalography (Eswaran et al., 2002, 2004, 2005; Matuz et al., 2012; Sheridan et al., 2008), fetal magnetic resonance imaging (Fulford et al., 2003) and ultrasound (Kiuchi et al., 2000) to demonstrate physiological and behavioral responses to generalised light stimuli (photic flash). The

experimental research of Reid et al., (2017) established a fetal preference for the face-like stimulus. This research also demonstrated an integrated visuomotor response and selective visual attention, suggesting that the fetus is an active participant in its uterine environment.

Prenatal studies exploring visual responding have presented stimuli (face-like stimuli and generalised light flashes) that are not representative of what the fetus could be reasonably expected to encounter in the third trimester. It could also be argued that the experimental stimuli (photic flashes or specific face-like stimuli) serve to arouse the fetus. If the fetus is generally in an unaware sleep state (Padilla & Lagercrantz, 2020), then the results from arousing visual stimulation are not representative of typical prenatal visual responses. yet to dismiss prenatal visual capabilities outright is concerning, and further exploration of visual development is warranted. The role of visual experience in the developing fetus is an open empirical question and presents exciting possibilities to understand the earliest development of cognitive function and social learning.

An important consideration is what visual stimuli the fetus receives in utero. Even though there might be sufficient light to enable a visual experience, the constraints of the uterus mean the fetus has limited access to visual stimuli. Adequate light in the womb could enable the fetus to access one fundamental visual stimulus: their own body. The fetus is not stationary (Kurjak et al., 2003; Kurjak et al., 2005; Zoia et al., 2007). Flexing their fingers, moving limbs and changing positions allow ample opportunity to serve as a self-produced primary visual stimulus. Observing their body and receiving visuomotor feedback on their movements has implications for visual and multisensory development in the fetus.

2.5.1 Multimodal sensory systems

The sensory systems are not siloed, acting in isolation from each other. Adult experiences are the result of integration across sensory modalities. Each sensory system provides unique information that, taken together, builds the whole experience (Bremner et al., 2012). Multisensory perception is advantageous in economy of learning and, while appearing effortless, results from complex neurological processes (Calvert et al., 2004). Prenatal visual experiences could allow for the development of multisensory systems incorporating visual perception. The literature on newborn multisensory development provides insight into what we could reasonably expect to see in the fetus regarding multi-sensory processing.

Multi-sensory processing is evident in newborns. Streri & Gentaz (2003) demonstrated that newborns could reassign shape information from touch to the visual modality with a cross-modal transfer task. Cross-modal processing is evidence of links between sensory systems and multisensory development because it requires coordinating sensory information across modalities (Brenmer et al., 2012). It has been concluded that cross-modal processing arises independently of experience in the neonate, refuting the philosophical and empirical hypotheses that cross-modal processing results from experiential learning (Bremner et al., 2012; Streri & Gentaz, 2003). This is a reasonable conclusion; however, it is dependent on the premise that the neonate enters the world visually naive. In light of a new understanding of the visual experience in the uterus, could there be an alternative hypothesis for neonate cross-modal processing? If the fetus is receiving visual feedback from their actions and tactile exploration in utero, it is plausible to consider that rudimentary experiential learning occurs between the sensory modalities. Visual and tactile experiences become linked as the fetus observes specific experiences giving rise to newborn cross-modal processing capacities. There

is evidence of cross-modal processing in auditory and visual modes in the fetus (Matuz et al., 2012).

Neonates' multi-modal processing is not limited to visual-tactile pairing. Newborns demonstrate explicit audio-visual integration; they associate objects with sounds (Morrongiello et al., 1998; Slater et al., 1997), can pair visual arrays with abstract numerical sounds (Izard et al., 2009), and combine audio-visual learning and face-recognition (Guellai et al., 2011). Evidence thus far fails to support the notion that newborns' multisensory abilities are innate or appear only at birth. Instead, prenatal experiences of visual, tactile and auditory stimuli allow the earliest interactions of multimodal processing, thus, preparing the fetus to deal with the overwhelming sensory array they must make sense of at birth. Multisensory perception is required to successfully navigate and manipulate our external environment (Bremner et al., 2012; Gallagher, 2006). Multisensory representations of the body give rise to "embodied cognition" (Gallagher, 2006). An embodied representation of where one's body is in space enables one to have a fundamental reference point to both the self and other objects in both time and space. As fetuses investigate the body through sensory modalities, the first rudimentary body schema could arise.

A structure that could also be interconnected with fetal visual development is the mirror neuron system (Del Giudice et al., 2009). Mirror neurons are acknowledged as a potential critical substrate for action processing, imitation and social learning, although the exact mechanism of mirror neurons is debated (Heyes & Catmur, 2022; Heyes, 2010; Rizzolatti et al., 2001). Mirror neurons have the unique property of activating when an individual performs an action or when an individual observes another performing a similar action (Gazzola & Keysers, 2009; Iacoboni et al., 1999). Although how the mirror neuron system develops is still unknown, there is a consensus that both sensory and motor experience (observing and executing actions) play a role

in facilitating their development (Heyes, 2010). With sufficient light, a fetus in utero could repeatedly observe motion patterns, such as the hand opening and closing. Frequently performing and simultaneously observing this action lays the foundation for the synaptic path of this visuomotor program (Del Giudice et al., 2009; Heyes, 2010). The ability to have an integrated sensory experience could facilitate mirror neuron development (Del Giudice et al., 2009). Before birth, a simple foundation for the mirror neuron system has been laid down through the simple associative pairing of the visual and motor experience.

Speculation is required to determine the visual experiences that the fetus has in utero. At the simplest level, there are fluctuating light levels in the uterus throughout gestation and as the mother moves through different light environments during her day. The architecture of the maternal anatomy may also provide specific patterns of light to the fetus in relation to the positioning of specific internal organs; for example, the light passing through the maternal ribs could result in a specific striation pattern. Even though the visual stimuli are likely simplistic, particularly when compared to the intensity and breadth of the visual world postnatally, it is clear that the earliest opportunities for visual learning could occur prenatally.

Basic visual experiences in utero have the potential to drive, in an experience-dependent manner the development of more sophisticated cognitive abilities in the fetus (Catmur et al., 2009). For example, the fetus moves their hand in front of their face which occludes light or changes the light levels. The fetus has the potential to learn contingencies (if-then) that hand position can change the visual experience. A simple understanding of light/no light and contingent factors can support more sophisticated cognitive abilities, for example a visual anticipation. The fetus associates a sensory experience, change in maternal position (i.e. moving from supine to standing when getting out of bed) with changes in light levels. The understanding of this

contingency directs anticipation behaviour with the fetus directing visual attention or changing positioning in anticipation of this change in light levels.

A visual experience in the third trimester could shape sensory development in the fetus in ways that have not been previously considered. Newborns have a repertoire of competencies that allow them to build more complex abilities, and the foundations of these abilities could be formed in utero. Understanding the earliest sensory perception iterations provides valuable insight into the working of the visual system and the cognitive scaffolding required for higher social and cognitive processes. Considering the potential for visual experiences in the fetus opens up exciting avenues for future sensory and perceptual research.

2.6 Overall Conclusion

Evidence strongly supports the notion light is as a compelling environmental variable that merits investigation across the prenatal period. With a functional visual from 24 weeks gestation (Glass, 2002; Hendrickson et al., 2012; Hendrickson & Drucker, 1992) and neurological (Dunn et al., 2015) and behavioral (Donovan et al., 2020) evidence of perceptual abilities in the third trimester, the fetus has the prerequisites to engage with, and learn from, the visual world. Models of light in utero make it clear that the fetus is not shrouded in darkness. Even though the exact illumination of the uterus across gestation is yet to be determined, it is clear that the fetus, at least in the third trimester, is in an illuminated environment (Del Giudice, 2011; Isaac et al., in press). Further, prenatal light exposure is known to have wide-ranging effects on development, from neural structures to behavioral outcomes, that transcend the visual sensory system. Studies on atypical opsins, avian models, the season of birth and circadian rhythms provide the foundation for investigating the impact of prenatal light exposure on development. When considered together, this diverse body of research underscores the imperative of studying the effect of light on prenatal development.

Integrating light into current theories of prenatal development introduces unexplored dimensions and potential therapeutic applications. The intricate interplay between light and fetal maturation underscores the complexity of human development prior to birth. The presence of light in the uterus holds profound implications for sensory and perceptual research, offering further insight into the foundational processes of higher cognitive functions. Quite simply, light, as an environmental factor, is likely to play a substantial role in prenatal development.

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Chapter Three

Experimental Background and Context

The preceding literature review details the development of the fetal visual system and the role of light in the uterine environment. It highlights that the fetal visual system is functional prior to birth. At 24 weeks gestation the essential components of fetal vision are present (Hendrickson & Drucker, 1992; Hendrickson et al., 2012). Eye movements in fetuses aged 34 weeks correspond to activation in the visual and central cerebral areas, suggesting an active visuo-motor system in the third trimester (Schopf et al., 2014). When coupled with the ability to process perceptual information in the third trimester (Decasper & Fifer, 1980; Witt & Reutter, 1996; Zoia et al., 2007) fetuses have the underpinning biological functionality required to engage with their visual environment.

Even though the third trimester fetus has the anatomical elements required for vision, it was previously assumed that the light levels within the womb were too low to facilitate a visual experience. As a result, the possibility of prenatal visual perception was largely dismissed (Glass, 2002; Lecanuet & Schaal, 1996; Myowa-Yamakoshi & Takeshita, 2006). Modelling by Del Giudice (2011) challenged this assumption, positing that the light levels within the uterine environment were much higher than originally presumed. In the final two months of pregnancy, the uterine environment is sufficiently illuminated to allow fetuses to attend to and orientate towards visual stimuli (Del Giudice, 2011; Isaac et al., in press). The fetal visual system is operational and adequate light levels – typically equivalent to vision under a full moon - suggest that visual experiences occur prenatally.

Previous studies have relied upon preterm infant responses to understand prenatal visual development. From the age of 32 weeks, premature infants can perform fixating and tracking (Dubowitz et al., 1980; Morante et al., 2008; Romeo et al., 2012). While informative, clear limitations exist when drawing conclusions from infants born prematurely. First, preterm infants are at an increased risk for poor neurodevelopmental outcomes as a function of the factors that have resulted in prematurity (Behrman & Butler, 2007). Second, premature infants clearly experience different environmental input from infants at term. To understand fetal visual perception, research needs to be conducted prenatally rather than with a premature infant sample.

Even though presenting stimuli in utero and recording fetal responses is complex, formative research has attempted to overcome these technical barriers (Dunn et al., 2015). As detailed in **Chapter 2**, observational studies have presented flashes of light to the fetus and have recorded physiological responses. Fetal magnetoencephalography (fMEG) work has demonstrated fetal cortical activation in response to visual stimuli (Eswaran et al., 2002, 2004a, 2005; Matuz et al., 2012; Sheridan et al., 2008). Similarly, a fetal magnetic resonance imaging (fMRI) study reported increased activity in the frontal cortex in response to a constant light source (Fulford et al., 2003). Kiuchi et al. (2000), detailed increased eye movements and heart rate in response to a visual stimulus (a photographic flash) and a vibroacoustic stimulation. Taken together, these studies are evidence of neonatal visual capabilities. It is important to note that the research detailed above used generalised light stimuli (photic flash) with none delivering stimuli that has contained the percept of an image or a shape. Newborns have clear preferences for specific visual stimuli, is this sensitivity reflected in the fetus? There is a clear need for an experimental approach,

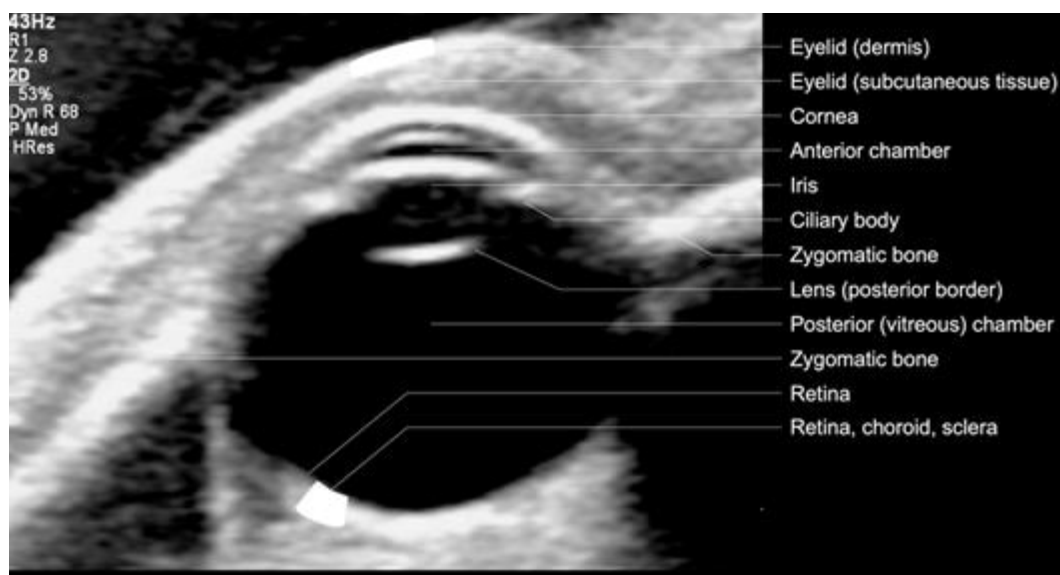
where fetal behaviour is measured before, during and after a visual stimulus to assess active engagement with the environment.

Recent research has championed an experimental approach to understanding fetal capabilities. Reid et al. (2017) simultaneously investigated face-processing capabilities in the fetus and explored the feasibility of a new method for presenting visual stimuli in utero. Grounded in the established neonatal preference for face like stimuli, this experiment presented two separate light arrays to the fetus: a face-like stimulus and the same stimulus inverted. Fetal head movements were recorded during these presentations via 4D ultrasound. Fetuses demonstrated significantly more head turns towards the face-like stimuli. These results suggested that a preference for the face-like stimulus as seen in neonates is also present prenatally. Importantly it also demonstrated that it is possible to present a simple pattern of visual stimuli to the fetus. This new methodological paradigm significantly opened the field to further explore fetal visual capabilities.

As we see in Reid et al (2017), visual responses offer more than insights into visual perception; they can also serve as a window into discriminative ability and visual preferences. Visual behaviors have played a pivotal role in understanding all aspects of infant development. From the early work of Fantz, (1958, 1963) to the present day, visual behaviours in the infant have been instrumental in understanding a raft of processes including memory, perception, body schema and language processing (Aslin, 2007). Behavioural measures of visual attention are foundational to understanding the non-verbal infant. Could this framework of methods be applied to the fetus too? Examining the earliest manifestations of visual perception provides crucial insights into how visual stimuli are initially processed, including the underlying drivers of visual attention.

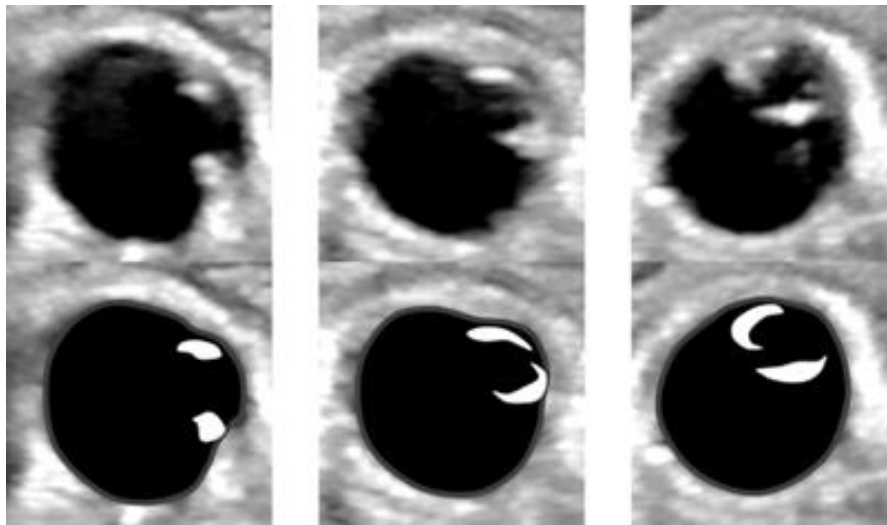
In Reid et al. (2017), head movements were taken as a measure of visual attention. The rationale of that study was that head turns towards or away from the stimuli would not be random if the visual system was being stimulated by the stimulus. It did not, however, measure eye movements directly. Eye movements play a critical role in studies of visual processing postnatally. For this reason, a direct set of measures investigating fetal eye movements in response to visual stimuli could provide a detailed picture of fetal visual capacities. We must ask the question of whether fetal eye movements can be indexed in an ethically appropriate manner. Certainly, detailed anatomy of the fetal eye can be visualised in utero: the lens of the fetal eye can be distinguished as a white circle within the hypoechoic eyeball during ultrasound (Inoue et al., 1986). 2D ultrasound imaging can focus on the acoustic reflection of the eye lens (Figure 3.1) and record in real time how the lens moves within the orbital socket (Figure 3.2). This provides a clear measure of eye movements, and thus, visual attention.

Figure 3.1 2D ultrasound of the fetal eye



Note. The anatomical detail of the fetal eye in the transverse section.

Figure 3.2 *Visualisation of the lens in the transverse plane during fetal eye movements.*



Note. Ultrasound images (top) with corresponding diagrammatic representation (bottom).

Bots et al (1981) first reported fetal eye movements in 2D ultrasound. Subsequently, Birnholz (1981) conducted a review of fetal eye movements, developing a classification system which consists of four types of fetal eye movements. The four types were: type 1, a single movement with a slower return; type two, a prolonged single eye movement; type three, multiple complex, brisk movements and type four, repetitive movements analogous to REM eye movements. Birnholz(1981) noted that the frequency of the four types of movement differed as a function of gestational age, providing a framework for understanding oculomotor activity. This work, despite being foundational to understanding eye movements, is constrained by the quality of the ultrasound technology used. A contemporary re-evaluation using modern ultrasound would likely yield a more nuanced understanding of prenatal eye movements.

Studies of fetal eye movements have been exclusively observational to date, with a focus on indexing patterns of eye movements and their relationship to neurological

development (Birnholz, 1981; Horimoto et al., 1993). One study, however, recorded eye movements before, during and, after specific light stimuli presentations (Donovan et al., 2020). Significantly increased eye movements were seen during and after the presentation of a light stimulus. This study showed that 2D ultrasound can meaningfully index fetal eye movements in response to light stimuli and that fetal eye movements are not random but related to the uterine environment. Tracking eye movements in utero presents a promising experimental approach. By integrating Reid et al. (2017)'s method of presenting visual stimuli while simultaneously measuring eye movements, a novel avenue for exploring fetal visual perception could be established.

3.1 Experimental Research

The following empirical research studies investigate three separate aspects of fetal visual perception. Utilizing the technique developed by Reid et al.(2017), the studies use 2D ultrasound to visualize the fetal lens and record eye movements in response to light stimuli that are projected onto the maternal skin through to the uterine environment. This experimental method is unique; tracking fetal eye movements in response to targeted visual stimuli has never been previously conducted.

The three experimental studies in this thesis explore fetal visual and cognitive capacities. **Chapter 4** investigates whether fetuses preferentially orient to light stimuli in their temporal visual field, examining the initial visuospatial parameters of fetal perception. **Chapter 5** advances this exploration by testing whether fetuses can perform anticipatory gaze, using an auditory cue to predict the appearance of a light stimulus, thus moving beyond basic perceptual abilities. Finally, **Chapter 6** examines fetal sensitivity to spatio-temporal cues, specifically testing whether fetuses preferentially attend to motion sequences containing low-level indicators of agentive

motion. Together, these studies build on existing research to deepen understanding of early fetal perception and cognition.

To support this experimental method, a coding scheme was developed to index fetal eye movements and is detailed in an Eye Movement Coding Manual (appendix A) with specific eye movement coding descriptions detailed in the respective methods sections for each experiment.

The following three studies utilise experimental research drawing on the fields of developmental psychology, medical imaging, and visual perception in order to further our understanding of the nature of prenatal visual capacities. Exploring visual behaviour has implications for comprehending fetal vision and determining developmental processes more broadly. In the next chapter, the first of three empirical studies is reported. The study examines visuo-spatial processing in the fetus, with results indicating that the fetus preferentially orients to visual stimuli present in the temporal visual field.

Chapter Four

Visual Processing of Stimuli in the Human

Fetus: A Peripheral Field Advantage?

Summary

How the fetus interacts with the visual world is still relatively unknown¹. Clear anatomical differences exist between fetal and adult eyes. Noticeably, the central retina is structurally immature during the fetal period, with the peripheral retina relatively more developmentally advanced prior to birth^{2,3}. It has long been known that neonates are more likely to orient to stimuli initially presented to their temporal visual field^{4,5}. The present study explores fetal vision, specifically whether the human fetus will preferentially orient to light stimuli presented to their temporal visual field when contrasted with nasal presentations. Participants include 49 singleton fetuses between 33 and 36 weeks gestation ($M=238.0$ days). A light stimulus was moved in a vertical orientation to the face of the fetus in two separate locations: the temporal and nasal visual fields. 2D ultrasound was used to visualise the fetal lens and record fetal eye movements. A Wilcoxon signed rank test indicated that fetuses demonstrated significantly more eye movements to stimuli in the temporal presentation ($M = 6.55$, $SD = 6.21$) compared to stimuli in the nasal presentation ($M=4.08$, $SD = 4.00$), $Z=2.336$, $W=879.500$, $p=0.02$. These findings provide initial insights into the parameters of fetal visual processing – notably that the predisposition for visual field preferences is present before birth. As the fetus has visual experiences in utero, this work highlights the nascent capacities underpinning visual search behaviours before birth.

4.1 Main

Understanding fetal visual capabilities has the potential to reshape our knowledge of visual perception and developmental trajectories. Newborn visual preferences are taken as evidence for genetic underpinnings of visual development⁶; however, this assumption discounts the potential for environmental influence in the prenatal period. Our current understanding of fetal visual perception in utero is highly limited despite recent modelling work demonstrating that the prenatal environment has greater luminosity than previously thought^{7,8}. This presents a sensory-rich environment for the fetus to engage in and suggests that visual experience begins before birth. How the human fetus interacts with the visual world is still unknown. This includes variation in field sensitivities and visual preferences. Exploration of sensory development in the prenatal period is central to understanding the origins of human development and offers insight into the complex interplay between genetic and environmental contributions to emerging abilities.

The fetal visual system has the anatomical structures present for functional activity by 24 weeks of gestation^{2,3}. Clear structural differences nonetheless exist between the fetal and adult eye. The central retina is substantially immature in the fetus, with the peripheral retina more developmentally advanced prior to birth^{2,3}. The fovea, key to visual acuity and colour vision in human adults⁹ is present from ten weeks gestation^{2,10-12}. The foveal pit begins to develop in mid-gestation at around week 24^{3,13} but does not reach maturity until after birth^{3,14}. The action of outward displacement of the inner retinal layers to form the foveal pit produces the inward displacement of photoreceptors post birth to increase cone density^{13,15,16}. Foveal inner and outer segments are shorter (more immature) than is seen in the peripheral retina for the neonate^{14,17,18}. When combined with the development and distribution of the photoreceptors, the maturation of the fovea suggests that during the prenatal period,

peripheral vision capacities of the fetus may well be more developmentally advanced than central visual space. Eye movements in fetuses aged 34 weeks correspond to activation in visual and central brain regions suggesting an active visuo-motor system prior to birth¹⁹. Coupled with the ability to process perceptual information in the third trimester²⁰⁻²², fetuses have the requirements to engage with their visual environment.

Prior neuroscience work has presented flashes of light to the fetus with magnetoencephalography demonstrating cortical activation in response to visual stimuli²³⁻²⁶. Similarly, fetal magnetic resonance imaging has reported increased activity in the frontal cortex in response to a constant light source²⁷. Further, Kiuchi²⁸, detailed increased eye movements and heart rate in response to a visual stimulus (a photographic flash) and vibroacoustic stimulation. Taken together these studies indicate extant neonatal visual capabilities.

Despite having a functional visual system capable of visual experience in the third trimester, the overriding assumption has been that the fetus receives no exogenous stimulation to the visual system^{6,29}. This was based on the hypothesis that the light levels within the womb were too low to actualize a visual experience. A persistent idea in developmental research is that neither light nor visual experience is a requirement for prenatal visual development²⁹. The possibility of prenatal visual perception has consequently been largely ignored or dismissed^{6,30} and many neonatal visual preferences have thus been interpreted as evidence for genetic origins^{29,30}. More recent work, however, has shown that light levels are present such that fetal vision is feasible^{7,8}.

Visual responses of neonates and infants can provide insights into the expectancies of the fetal visual field. Both the central and peripheral visual fields are functional at birth⁵, however, neonatal behavioural responses to visual stimuli reflect the asymmetric development in the temporal and nasal visual fields⁴. In adults the visual field can be divided into two hemispheres, the temporal and nasal visual fields. From central

fixation the temporal field extends from the centre of the fovea out to 90 degrees towards the temple while the smaller nasal field extends 50 degrees toward the nose⁹. Infants demonstrate clear difference in both sensitivity and reaction time to stimuli in the temporal and nasal visual fields. Neonates more readily orientate to stimuli presented in their temporal visual field, as opposed to their nasal visual field^{4,5,31}. Static light presentations at specific locations across the visual field have demonstrated that infants detect a greater range of stimuli presented in their temporal visual fields^{4,5}. Even though the infant visual field expands with age, sensitivity to stimuli in the temporal field develops more rapidly than in the nasal field⁴. This difference in responding across the two hemifields is proposed to be the result of subcortical processing that is more sensitive to temporal field outputs³². When considered together with the anatomical development of the eye, this suggests that a fetus will be more sensitive to stimuli presented in their temporal visual field.

The parameters and sensitivities of the fetal visual field remain unknown. How does the more advanced peripheral retina and infant's increased sensitivity to the temporal visual field translate into behavioural responses in the fetus? To date, fetal research has only delivered targeted light stimuli to the temporal visual field³³. Despite the physical constraints of delivering visual stimuli in utero, some research has demonstrated that fetuses respond to and engage with light stimuli^{1,33}.

Postnatal assessments of visual field perception have often been conducted monocularly⁴. An individual has one eye covered while the other eye is tested. Monocular testing is more precise as a clear comparison between the temporal and nasal fields can be made^{4,5}. Monocular presentation is not possible with a fetal sample in utero. This raises the issue that stimuli presented in the temporal visual field are almost certainly processed monocularly, whereas, stimuli presented in the nasal field are most likely processed binocularly.

Despite temporal and nasal presentation conditions not being equivalent, Lewis⁴ found that infants at five weeks were more responsive to temporal stimuli even within the region of binocular overlap present in an adult visual field array. Given our knowledge of the anatomy of the fetal eye, we predict that the fetus will be more responsive to stimuli presented to the temporal visual field when compared to the nasal field. The study outlined below aims to address this question by presenting fetuses with light stimuli in their separate visual fields and measuring their eye movements in response. This work will provide the first initial insights into the parameters of fetal visual processing – notably that the predisposition for visual field preference is present before birth. As the fetus has visual experiences in utero, this work highlights the nascent capacities likely driving visual search behaviors before birth. Mapping these capacities provides the foundation for determining postnatal visual trajectories in humans.

4.2 Experiment Summary

Participants included 49 singleton fetuses between 33 and 36 weeks gestation ($M=238.00$ days, $SD=5.76$). 2D ultrasound was used to visualise the fetal lens and record fetal eye movements in the parasagittal plane (profile visualisation).

A light stimulus (dot diode emitting at 650nm) was presented to the fetus in two separate locations; the temporal and nasal conditions (Figure 4.1). The stimuli were projected on the maternal abdomen and moved in a vertical orientation to the face of the fetus. In the nasal condition, the start location of the light stimulus display was the middle of the fetal forehead in the vertical plane of the fetal nose. In the temporal condition, the light stimulus start point was also the middle of the fetal forehead but in the vertical plane of the zygomatic bone of the assessed target eye.

In both conditions the light stimulus was moved vertically at a rate of 10 mm per second for six seconds. Upon reaching the bottom point of the trajectory, the stimulus was then moved in an upward trajectory to the original start point. This sequence was repeated five times for a total of sixty seconds stimulus exposure in each condition. Between stimulus condition presentations there was a 120 second interstimulus interval to enable the fetus to return to the initial resting, or canonical, eye position. The total duration of the experiment, including interstimulus interval, was 300 seconds (five minutes). The condition order of temporal or nasal presentation was counterbalanced across the sample.

Stimuli were presented in an orientation to induce superior and inferior eye movements. Superior movements are when the fetal lens moves towards the infra-orbital foramen (as if the fetus is looking up to the top of their head) and inferior movements are a lens movement to the superior orbital fissure (as if the fetus is looking down their body).

The reasons for the orientation of the stimulus presentation are twofold. First, inducing specific eye movements demonstrates clear sensitivity to the visual stimuli and presents a decreased likelihood of being due to random eye movements. Second, given the constraints of presenting light stimuli to a specific location in utero, this presentation orientation allows for the most precise separation of the two conditions.

The number of eye movements in response to the stimuli was assessed using condition-blind coding of the 2D scans. Fetal eye movements were either coded as inferior (lens movement towards the infra-orbital foramen) or superior (lens movement to the superior orbital fissure) using the Observer XT software³⁴. These two categories of eye movements were then collapsed to give the total eye movements for each condition.

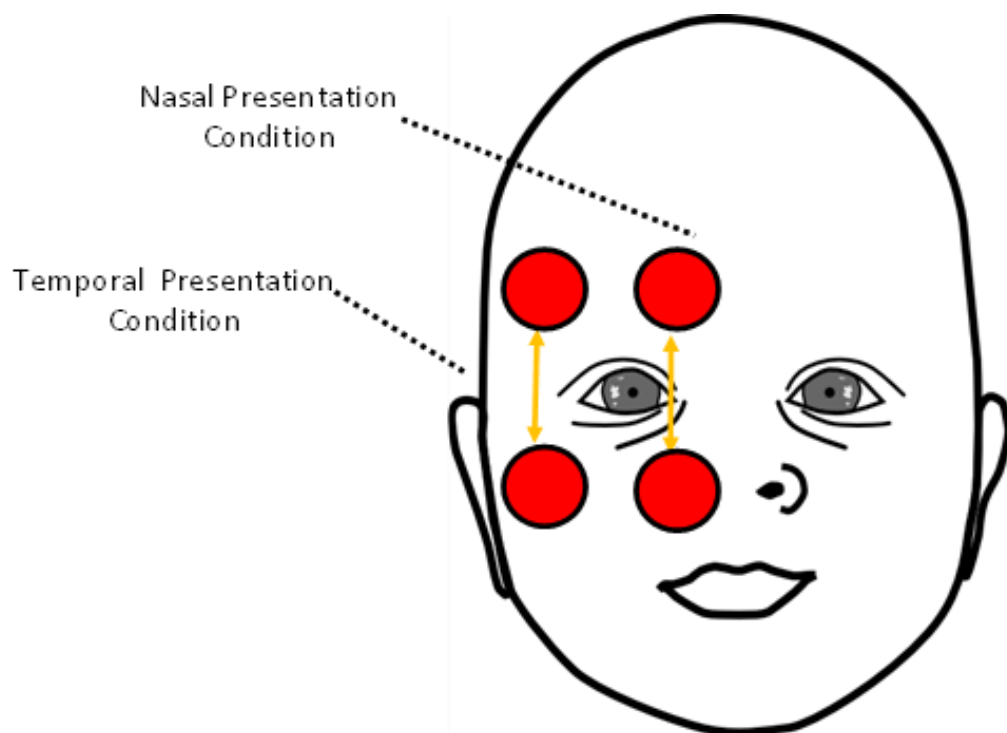


Figure 4.1 Presentation of the light stimuli to the fetus.

The light stimulus was presented in the coronal plane for temporal and nasal conditions. The direction of the arrows denotes the direction of movement of the stimulus along the fetal face.

4.3 Results and Discussion

On average, Figure 4.2 shows that more eye movements were made in response to the temporal stimuli ($M=6.55$, $SD= 6.21$) compared to the nasal stimuli ($M= 4.08$, $SD= 4.00$).

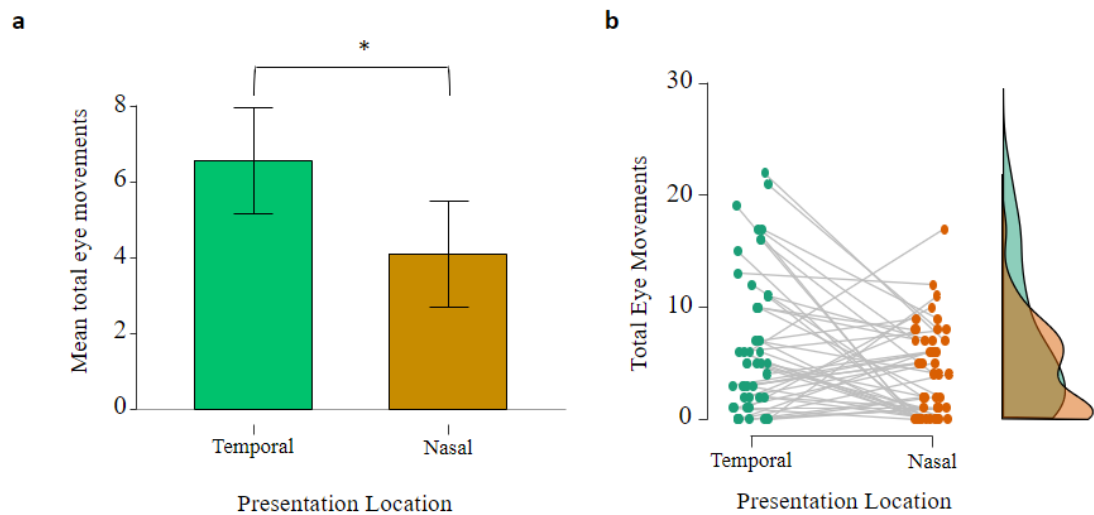


Figure 4.2 Eye movements as a function of stimulus presentation location.

a, Mean total eye movements as a function of stimulus presentation location. A Wilcoxon sign-ranked test indicated that there were more total eye movements to stimuli in the temporal presentation ($M=6.55$, $SD= 6.21$), compared to the nasal presentation ($M=4.08$, $SD = 4.00$), $Z= 2.336$, $W=879.500$, $p= 0.02$. Error bars are 95% CI. b, Total eye movements as a function of stimulus presentation location.

Fetuses were more responsive to light stimuli presented in their temporal visual field compared to their nasal visual field. These results provide initial insights into the parameters of typical fetal visual processing. Most notably, this work indicates variation in spatial processing of visual stimuli. The preferential orienting to temporal stimuli seen in the present study is consistent with both neonatal responding and the anatomical development of the fetal retina^{4,5,31,32}. These initial visual capacities of the fetus indicate a functional visuo-motor system, coupled with appropriate neural innervation to produce overt behavioural responses to environmental information.

This work thus provides the foundation for understanding visual developmental trajectories in humans.

Newborns exhibit preferential orienting to visual stimuli located in the temporal visual field⁵. Johnson³² proposed that this preferential orientation could be attributed to a subcortical pathway directly connecting the eye to the superior colliculus. This specific subcortical pathway is responsible for generating rapid and reflexive eye movements to stimuli and is driven by a majority of temporal field inputs³⁵. The current findings suggest that this subcortical pathway is operational prior to birth. Differential behavioural responses as a function of controlled visual stimuli offer valuable insights into the development of brain pathways. Future work imaging the fetal brain in response to visual stimuli during controlled behavioural experiments will build an integrated picture of visual system development.

The assessment of fetal vision allows for examination of the relationship between the external features of the visual system (i.e. the eye, retina and photoreceptors) and the brain. Immaturities in external components place limitations on the perceptual abilities of the fetus. However, to what extent does the maturation of the retina and other external features influence fetal visual perception? Clarifying the complex interplay between the anatomy of the eye and the evolving connections within the developing brain can reveal how the maturation of the physical anatomy of both the retina and cortical pathways influences both behavioural and neurological responses to visual stimuli.

The increased response to stimuli in the temporal visual space also underscores the significance of the peripheral visual field in both prenatal and postnatal visual development. Despite incorporating the majority of visual space, peripheral vision has been largely ignored in favour of foveal vision within the vision sciences³⁶. There is, however, growing acknowledgment and interest in infant peripheral processing^{37,38}.

The peripheral visual space is vital to visual search processes, including critical activities such as orienting attention to changes in the environment³⁹, detecting threats^{40,41} and proprioception^{42,43}. It is clear that spatial processing in peripheral space contributes to fetal vision and most likely underpins early fetal visual orienting. Fetal visual sensitivities also highlight the interplay between exogenous stimulation and internal neural processes. Cortical connections are sensitive to environmental stimuli that can induce and influence brain development³². Visual experiences in utero have the potential to shape cortical and sensory development. The exact role that the uterine environment, notably light, plays in the developing prenatal visual system is currently largely unknown. Determining fetal visual abilities is instrumental to understanding the intricacies of how this sensory system develops, including the requisite exogenous and endogenous inputs. Preterm infants have an increased risk of later developmental difficulties and delays⁴⁴. It is possible that this path is set due to an introduction to a more complex visual environment before the visual system is sufficiently prepared. Thus, understanding the interaction between the visual environment and visual perception is crucial for informing neonatal intensive care unit plans.

Fetal visual perception represents a key foundational stage for visual developmental trajectories. Examining the earliest manifestations of visual perception provides crucial insights into how visual stimuli are initially processed. This includes the underlying drivers of visual attention across development. Visual perception is one of the primary ways in which humans make sense of the complex and rich environments through which they move. At the most basic level, visual attention is a primary mechanism for gathering information⁴⁵. What captures attention during these formative stages and the mechanisms that drive this attention lays the groundwork for information processing, spatial awareness, and exploratory

fixations—all of which form the basis for complex cognitive processes such as learning and memory. Visuo-spatial processes are instrumental in perceiving and interacting with both physical objects and the dynamic social world of humans. Exploring visual behaviour has implications not only for comprehending fetal environmental information processing but for understanding core developmental processes more broadly.

These findings also aid in developing replicable and robust experimental methods for assessing fetal vision. The location that the stimuli is presented in visual space can elicit differential behavioural responses and requires specificity in fetal behavioural paradigms. Visual stimuli should be presented to the temporal visual field in a manner analogous to experiments with newborns. This is an important consideration for future work as the field of fetal experimental vision expands.

Presenting visual stimuli to a fetus comes with unique constraints. Infant and neonate studies of visual perception can present visual stimuli to precise locations within the visual field however; delivering light stimuli to fetuses requires a more generalised approach. Factors including projecting light stimuli through the maternal tissue, the fetal position in utero and fetal movement during testing all contribute to a decreased degree of specificity related to stimulus presentation. Our experimental study represents the first initial and general approach to quantify the parameters of fetal visuospatial processing. Subsequent experimental investigations with a greater range of presentation locations of visual stimuli, different stimuli designs such as shape, brightness and size, and utilising different paradigms including static or kinetic perimetry for the presentation of stimuli will provide a more comprehensive understanding of fetal visual capacities. Our results show it is possible to measure visual detection and orienting behaviors in the fetus, laying the initial groundwork for the field.

4.4 Conclusion

Visual perception plays a pivotal role in fundamental processes used to navigate both the physical and social world. This research is the first to explore fetal spatial sensitivities to visual stimuli in utero. It investigated differential sensitivities of fetal visual fields in the third trimester and demonstrated that fetuses preferentially orient to stimuli presented in their temporal visual field when contrasted with the nasal visual field, . This indicates that visual field preferences are present prior to birth. These insights into fetal vision substantially change our understanding of the development of the visual system and the brain. As the fetus has visual experiences in utero, this work highlights the emerging capacities underpinning prenatal visual search behaviours. These, in turn, determine the ontogenetic unfolding of the developing human visual system.

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Methods

4.4.1 Experimental Model and Subject Details

All pregnant participants received written information prior to study completion (compliant with Central Health and Disability Ethics Committee (HDEC) ethical requirements) and gave free and informed written consent before participation. All scans were conducted by one qualified and experienced sonographer at Waikato Hospital Ultrasound Clinic, Hamilton, New Zealand. All participants had singleton pregnancies with no known complications at the time of scanning. This study was approved by the Central Health and Disability Ethics Committee (HDEC), reference number: 2021 EXP 11521

The initial sample included 97 participants with 97 singleton fetuses of a gestational age between 33 and 36 weeks. Stimuli were presented by a researcher and fetal behavioural responses were recorded using 2D ultrasound. A research assistant was also present throughout the duration of the session to record experimental times.

Nine fetuses were excluded due to technical errors leading to corrupt source recordings. Ten fetuses were excluded because the maternal tissue thickness was greater than 50mm. Modelling suggested that tissue thickness greater than 50mm would result in significant scattering of the light stimulus and thus integrity was compromised¹. Two fetuses were excluded due to health conditions that could affect typical responses to stimuli and five fetuses were excluded due to unsuitable positioning (i.e. their eyes could not be visualised).

At the coding stage, a further sixteen fetuses were excluded. Twelve of these fetuses were excluded as they indicated zero eye movements throughout both experimental conditions and appeared to be in a deep sleep state, otherwise referred to as behavioral state 1F². A further four fetuses were excluded at the coding stage for data quality, specifically “uncodable” sections. This exclusion criteria was applied if, in 50% or more of either experimental condition, the fetal eye was not visible in the ultrasound image.

The final dataset for analysis, prior to the removal of outliers, consisted of 55 fetuses, gestational age between 231 and 252 days (M =239.75 days SD= 5.70). Maternal tissue thickness ranged from 13-48 mm (M = 3.03, SD = 0.85). Twenty-three fetuses were female, twenty-three were male and nine were sex unknown.

4.4.2 Ultrasound Acquisition

Ultrasound scans were performed using a Philips Epiq7 Elite Ultrasound System using a 5C1, or X6-1 transducer, keeping Ttb<0.3. Individual scans were recorded in their entirety as mp4 video files using OBS software³ and were saved to an external hard drive for offline analysis.

An experienced and qualified sonographer undertook the scanning. Images were captured in the parasagittal plane (a profile visualisation) of the fetal face for the experimental procedure. The parasagittal images provide optimal visualisation of the fetal lens and eye movements in response to a stimulus presented vertically in relation to the fetal face.

4.4.3 Stimuli

The light stimuli consisted of one custom-made red 4 mm LED emitting at 650nm. The light stimuli could be calibrated to one of three power levels for three different levels of maternal thickness. The three levels were 0.5mV for maternal tissue measurements below 15 mm, 1mV for maternal thickness between 15-30mm and 5mV for maternal thickness greater than 30mm. The light source design and rationale for the optical powers was based on the modeling and stimulus design in Reid⁴ and supported by Isaac¹. The light stimulus was presented within the predicted range of the fetal visual system at luminance that was safe for the fetal eyes.

4.4.4 Data Acquisition

4.4.4.1 Procedure

The participant was positioned in a supine position. Once comfortable the sonographer began the session by taking the fetal biometric measurements to ensure normal fetal growth and no fetal abnormalities. A set of fetal measurements (the fetal head position, placenta position, fetal biparietal diameter, head circumference, abdominal circumference, femur length, fetal weight and single deepest pocket) were calculated and recorded. Once it was confirmed that fetal biometry measurements demonstrated normal fetal growth and no fetal anomalies the experimental session would proceed.

The sonographer checked that one of the fetal eyes could be visualised and then calculated the maternal thickness measurements from the same maternal position where the stimulus was to be presented. Participants were requested not to talk during the study in order to control the sound and movement environment across the sample. All timings were controlled via a clock and noted by a research assistant.

The stimuli were delivered in two orientations ("nasal" and "temporal") to the maternal abdomen relative to the position of the fetus (breech presentation/head up, n = 1, cephalic presentation/head down, n = 54). The ultrasound image, interpreted by the ultrasound technician, informed the presentation parameters of the nasal and temporal conditions. In the nasal presentation condition, the start location of the light stimulus display was the middle of the fetal forehead in the vertical plane of the fetal nose. In the temporal presentation condition, the light stimulus start point was also the middle of the fetal forehead but moved in the vertical plane of the zygomatic bone of the eye being tested. Stimuli were presented to one of the fetal eyes (left, n = 26, right, n = 29).

The experimenter was not blind to the condition that was presented due to the requirement to present the stimulus in the correct orientation for each fetus. The administration of the stimulus was standardized across conditions by ensuring equivalent timing between conditions. In each condition the stimulus was then moved across the maternal abdomen in a vertical direction for six seconds at an average speed of one cm per second. This is consistent with speeds reported in newborn studies⁵ taking into account constraints specific to this population, i.e., the width of maternal tissue that was accessible in order to present stimuli and the space available for the fetus to move.

When the stimulus reached the lowest point on the presentation trajectory it was then moved upwards, retracing the path followed and returning to the initial start position. This process was then repeated ten times for a total of sixty seconds.

Movement and light onset was simultaneous and onset timing was controlled via a stopwatch in view of the experimenter who was delivering the stimuli. The light stimulus was programmed to turn off after 60 seconds, this ensured that the experimenter could attend to the movement of the stimulus and light exposure was standardised across conditions. Presentation of the first condition was followed by an interstimulus interval of 180 seconds where no light stimulus was presented, directly followed by the alternate stimulus condition. The total duration of the experiment, including the interstimulus interval was 300 seconds (5 minutes). The presentation order for temporal and nasal conditions of the stimuli were counterbalanced across the sample. Participants were blind to the presentation condition.

Light stimuli were presented in relation to the position of fetal head. In the event that the fetus exhibited a large amount of movement the experimental protocol would continue with the sonographer making the best attempt to obtain consistent imaging of the fetal lens and head. The experiment would not be paused and restarted to accommodate fetal movement. Large and frequent amounts of fetal movement meaning the lens and head could not be visualized would be reflected in the “uncodable” code (see data coding sub heading) and could lead to exclusion of the fetus from the final dataset

4.4.5 Data Coding

Behavioural coding of the fetal ultrasound was conducted on ObserverXT software⁶.

The number, duration and direction of eye movements was recorded for each condition. Coding was done for the single eye in which the stimulus was presented. A single-eye movement was unidirectional. An eye movement was defined as a lens movement from a stationary position through a position change to another stationary position. If the lens did not reach a stationary position but changed the direction of movement (i.e., from a superior to an inferior direction), this indicated a second eye movement. The start (onset) of the second eye movement was when the directional change began. The onset of an eye movement was when the lens movement began, and the offset was when the movement of the lens in that direction ended, either by coming to a stationary position or changing direction of movement.

In the parasagittal plane, the direction is noted as either an inferior or superior movement. A superior movement is when the lens moves from a stationary position towards the supraorbital foramen (towards the forehead, top of the eye socket, i.e. looking up). An inferior movement is when the lens moves from a stationary position towards the zygomatic bone (towards the nose, the bottom of the orbital socket, i.e. looking down). Full coding manual located in Appendix A.

Two coders completed the fetal eye movement coding. Coders were blind to the experimental condition. Cohen's weighted kappa was performed to determine interrater agreement on fetal eye movements. Cohen's Weighted Kappa was used to assess inter-rater agreement while accounting for the severity of disagreements between raters. Unlike the unweighted Kappa, which treats all disagreements equally, the weighted Kappa gives partial credit for disagreements that are closer in value and penalizes more heavily for disagreements that are further apart) and is appropriate to use with interval data. By incorporating weights, Cohen's Weighted Kappa provides a more nuanced measure of agreement, reflecting not only whether raters agree, but also how closely their ratings align⁷. The Kappa value was calculated on the total eye movements across experimental condition. There was a substantial agreement between two coders' judgments, $k = 0.947$ $SE = 0.037$. Behavioral measurement was thus deemed to be suitable for use in the hypothesis tests in the present study⁸.

4.4.6 Statistical Analysis

Statistical Analysis was completed using R statistical package⁹. Outliers of total eye movements were removed using the Tukey's method. Tukey's method identifies outliers by calculating the interquartile range (IQR) and defining any value outside 1.5 times the IQR from the first or third quartile as an outlier, providing a robust, non-parametric approach to detecting extreme values in datasets¹⁰. This gave a final sample of 49 singleton fetuses, gestational age between 231 and 252 days ($M = 239.27$ days $SD = 5.76$). Maternal tissue thickness ranged from 16 - 48mm ($M = 30.39$, $SD = 8.14$).

A Shapiro-Wilk test suggested a deviation from normality for both conditions, with Temporal ($W = 0.890$, $p < 0.001$), and Nasal ($W = 0.847$, $p < 0.001$). A comparison of the total number of eye movements across conditions was conducted using a Wilcoxon sign-ranked test.

Data Availability

The data reported in this paper will be available in the FigShare Digital Repository under the following DOI: <https://doi.org/10.6084/m9.figshare.25622310>

Competing Interests

The authors declare no competing interests.

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Prelude to Chapter 5

The preceding study empirically investigated visuospatial processing in the fetus, demonstrating that fetuses are more responsive to stimuli in their temporal visual field. This visual preference aligns with the anatomical and physiological development of the fetal retina and mirrors visual responses observed in postnatally in infants. Through exploring perceptual mechanisms, this study highlights the fetal capacity for visuo-spatial preferences. It also establishes eye movements as a viable measure of visual attention.

Eye movements can serve as behavioural indicators of both visual perception and cognitive functions during infancy (Aslin, 2007, 2012). The following study moves beyond perceptual mechanisms to explore attention within the framework of anticipatory gaze. Understanding what captures visual attention during this formative period, and the mechanisms underpinning such functionality, provides foundational insights into information processing, spatial awareness, and exploratory fixations. These are key foundational elements of complex cognitive processes such as learning and memory (Johnson et al., 2008).

Much remains unknown about prenatal vision and this thesis is the first foray across areas of the vision sciences. The study that follows reflects a deliberate attempt to integrate perceptual processes with broader cognitive concepts, underscoring the importance of building our understanding of fetal vision from the bottom up.

Chapter Five

Exploring anticipatory visual responding in the human fetus

Summary

Our knowledge of fetal visual perception is rudimentary, including how the fetus engages with the visual world. Understanding early visual perception is important as visual orienting practices during infancy are the building blocks for social processes including understanding facial expressions, eye contact, and action perception¹. Recent studies have begun to explore fetal abilities in utero². Here we show that fetuses can perform anticipatory visual gaze, specifically that fetuses can use an auditory cue to anticipate the appearance of a light stimulus. Using 2D ultrasound, we visualised the fetal lens and recorded fetal eye movements in response to visual stimuli. Employing a modified Visual Expectation Paradigm³, we found that fetuses demonstrated eye movements towards a specific location (in which no visual stimulus was present) when they heard an auditory cue previously paired with a visual stimulus presented in that same location. Our results illustrate that visual orientating and directed eye movements are present prior to birth. Understanding visual orienting in utero informs our comprehension of perceptual processes and the foundations of higher-level cognitive functions. These insights into fetal visual attention highlight emerging capacities that underpin prenatal visual experiences and have the potential to reshape our knowledge of developmental trajectories.

5.1 Main

Environmental information that captures and holds our visual attention provides insights into cognitive processes including learning, memory and attention^{4,5}. During infancy, analysing eye movements is an important tool for developmental psychologists seeking to understand early visual perception⁶. Visual orienting behaviours during infancy are key building blocks for social function, including understanding facial expressions, direct eye contact and action perception⁷.

Visual attention processes may have their foundations in the prenatal period, as modelling has suggested that the uterus is sufficiently illuminated in the third trimester to facilitate a visual experience^{8,9}. This work challenges long held assumptions that the visual system develops in the absence of exogenous stimulation^{10,11}. The prenatal period presents a distinct and rich environment that is central to understanding the development of early visual abilities.

Experimental research has demonstrated that fetuses respond to visual stimuli in utero. Prior neuroscience studies have presented flashes of light to the fetus with cortical activation demonstrated in response to visual stimuli¹²⁻¹⁵. Similarly, fetal magnetic resonance imaging has reported increased activity in the frontal cortex in response to a constant light source¹⁶. Further, Kiuchi et al.¹⁷, detailed increased eye movements and heart rate in response to a visual stimulus (a photographic flash) that was combined with vibroacoustic stimulation. The studies detailed above have reported responses to basic flashes of light, and more recently, the feasibility of presenting patterned stimuli has been demonstrated¹⁸. Reid et al¹⁸ simultaneously investigated face-processing capabilities in the fetus and explored a new method for presenting visual stimuli in utero. Using light arrays, based on the highly-replicated neonatal preference for face-like stimuli, the researchers presented a face-like stimulus and the same stimulus inverted. Fetal head movements towards the shapes

were recorded via 4D ultrasound imaging, with fetuses demonstrating significantly more head turns towards moving face-like stimuli. These results suggested that the preference for the face-like stimulus seen in neonates is present prenatally. Taken together, both behavioural and neuroscience studies demonstrate extant neonatal visual capabilities in utero.

Eye movements and looking times are fundamental for understanding visual perception in infancy and beyond⁶. Of importance for the current work, fetal eye movements can be visualised in utero. The lens of the fetal eye is easily distinguished as a white circle within the hypoechoic eyeball¹⁹. 2D ultrasound imaging can focus on the acoustic reflection of the eye lens and record how it moves within the orbital socket with eye movements detected in the fetus as early as 14 weeks¹⁷. To date, studies exploring fetal eye movements have been primarily observational, with a focus on indexing patterns of eye movements and their relationship to broad indicators of neurological development^{20,21}.

One of the first studies to investigate fetal eye movements in response to a targeted light stimulus found systematic movement patterns. Donovan²² analysed the number of fetal eye movements in response to a visual stimulus. Interestingly, they found a sustained number of eye movements post-presentation of a light stimulus. The authors posited two possible explanations for these results. First, the increased eye movement in the absence of light stimuli was the result of residual activation. The fetus had been stimulated by the light source, and the eye movements in the absence of stimuli were simply an indicator of latent arousal. A second hypothesis was that these eye movements in the absence of a visual stimulus were due to anticipatory visual search. The fetus had an expectation for a visual stimulus and was actively engaging with the uterine environment in anticipation of further light illumination. Anticipatory gaze has provided a unique window into cognitive

function during infancy. It is a proactive eye movement, where an individual saccades towards a location in space, before the visual stimulus is presented at that location²³. The act of anticipatory gaze is deceptively simple as complex cognitive processes are required. To perform an anticipatory gaze, the individual must hold an expectation that the stimulus will appear in that location and then initiate an eye movement towards the predicted location of the stimulus. An anticipation, visual or other, is evidence that an individual is not blindly responding to stimuli but forming representations and using memory to anticipate the future²⁴.

Anticipatory gaze was first assessed in infants using the Visual Expectation Paradigm (VExP)²³. Infants between the ages of three and seven months were presented with visual slides to alternating sides of their face. Infants learned where and when to expect a slide and would increasingly look to that location prior to the slide presentation^{23,25}. Anticipatory gaze in older infants has also been explored using auditory cues. Kovács and Mehler²⁶ repeatedly presented an auditory cue followed by presentation of a visual “reward” stimulus always in the same spatial-temporal location. Seven-month-old infants quickly learned that the auditory cue predicted the reward and looked in anticipation to the location of the visual stimulus. Pyykko et al.²⁷ demonstrated similar anticipatory gaze results in a large sample of seven and nine-month-old infants with the auditory-visual paradigm.

Infants as young as three months have been found to perform anticipatory eye movements²³ and no research to date has explored this phenomenon in a younger cohort. The results of Donovan et al²² may suggest that rudimentary anticipatory visual gaze could be present prenatally. Evidence of anticipatory visual search in utero would provide unique insights into the nature of cognitive processes in the fetus. To perform an anticipatory visual search, the fetus requires a memory of an earlier audio-cued light stimulus and needs to hold an expectation that the light

stimulus will be presented again following a repetition of this audio signal. Prior work has shown that the human fetus in the third trimester has the underpinning requisite ocularmotor, perceptual, and cognitive abilities to perform anticipatory gaze and has the capacity for multi-modal processing^{28,29}. Fetuses can both learn in utero³⁰ and perform anticipatory hand movements³¹ suggesting that the cognitive framework for performing basic anticipatory gaze may be present. The ability to perform anticipatory gaze would be further evidence that the fetus is an active participant in processing environmental information. The current study investigates the presence of anticipatory visual gaze in the fetus. Specifically, can fetuses use an auditory cue to anticipate the appearance of a light stimulus?

5.2 Experiment Summary

The final dataset includes 40 singleton fetuses between 33 and 36 weeks gestation (M=239.5 SD=4.75 days). 2D ultrasound was used to visualise the fetal lens and record fetal eye movements in the transverse plane. The transverse plane gives a visualisation through the head horizontally, viewing transverse images are similar to looking down on the top of the head in a birds eye view (Figure 5.1). A modified version of the Visual Expectation Paradigm used by Kovács et al.²¹ was employed to investigate anticipatory gaze (Figure 5.2). Fetuses first underwent six familiarisation trials where a 500Hz tone (anticipatory cue) was played for two seconds, followed by a light stimulus (dot diode emitting at 650nm) presented to the fetal temporal visual field for five seconds. Following the familiarisation trials, the fetus was presented with six test trials with two conditions: Light Absent (LA) and Light Present (LP). In LA trials, the two second auditory cue was played followed by no light stimulus presented. LP trials were an exact copy of the familiarisation trials. Test trials always began with a LA trial and alternated between LA and LP trials. Across all trials (both

familiarisation and test) the frequency of eye movements were recorded in the transverse plane with the view of measuring fetal visual engagement.

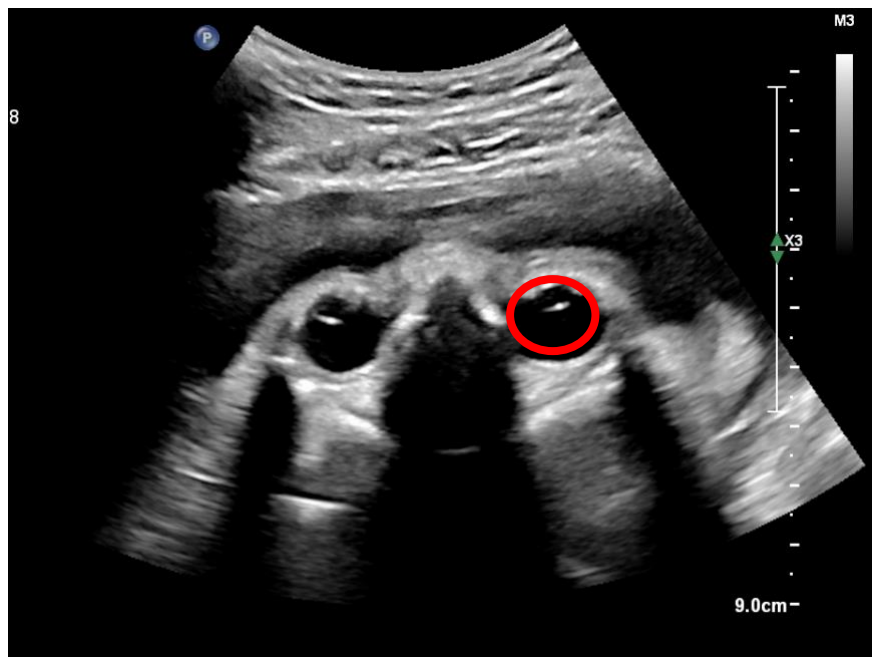


Figure 5.1 An image of a 2D ultrasound of a fetal head in the transverse plane.

The acoustic reflection from the lens of the right eye is highlighted in the red circle.

The stimuli were projected on the maternal abdomen and moved in a horizontal orientation to the face of the fetus. In both familiarisation and test trials the light stimulus was moved horizontally towards the periphery of the fetal face at a rate of 10 mm per second for five seconds. This was followed by a five second period featuring no sound or light stimuli to enable the fetus to return to the initial resting, or canonical, eye position. A trial was a total of twelve seconds in duration. The experiment consisted of six familiarisation trials and six test trials, making the total duration of the experimental presentations 144 seconds (2.4 minutes).

Stimuli were presented in an orientation to induce lateral eye movements. The number of eye movements in response to the stimuli was assessed using condition-blind coding of the 2D scans. Fetal eye movements were either coded as lateral or medial using Observer XT software³². Lateral movements were when the fetal lens moves towards the zygomatic bone (as if the fetus were looking to the side of their face, towards their periphery) and Medial movements were classified as a lens movement to the nasion (as if the fetus were looking in the direction of their nose). These two categories of eye movements were then collapsed to give the total eye movements for each trial type. The two categories were combined to give a comprehensive representation of fetal visual behaviour. Within the constraints of the current methods, it is not possible to ascertain a central fixation of the fetal eye or the precise location that stimulus is being presented in relation to the fetal eye. As such, a medial movement (lens movement towards the nose) could still be considered evidence of engagement with the stimulus in the periphery. The combined categories provide a pattern of eye movements, with the assumption that different levels of engagement with the stimuli would produce different patterns of movement.

We hypothesized that fetuses would demonstrate eye movements towards the specific location in which the light has previously been presented (where no visual stimulus is currently present) when they heard an auditory cue that had been previously paired with a visual stimulus presented in that same location.

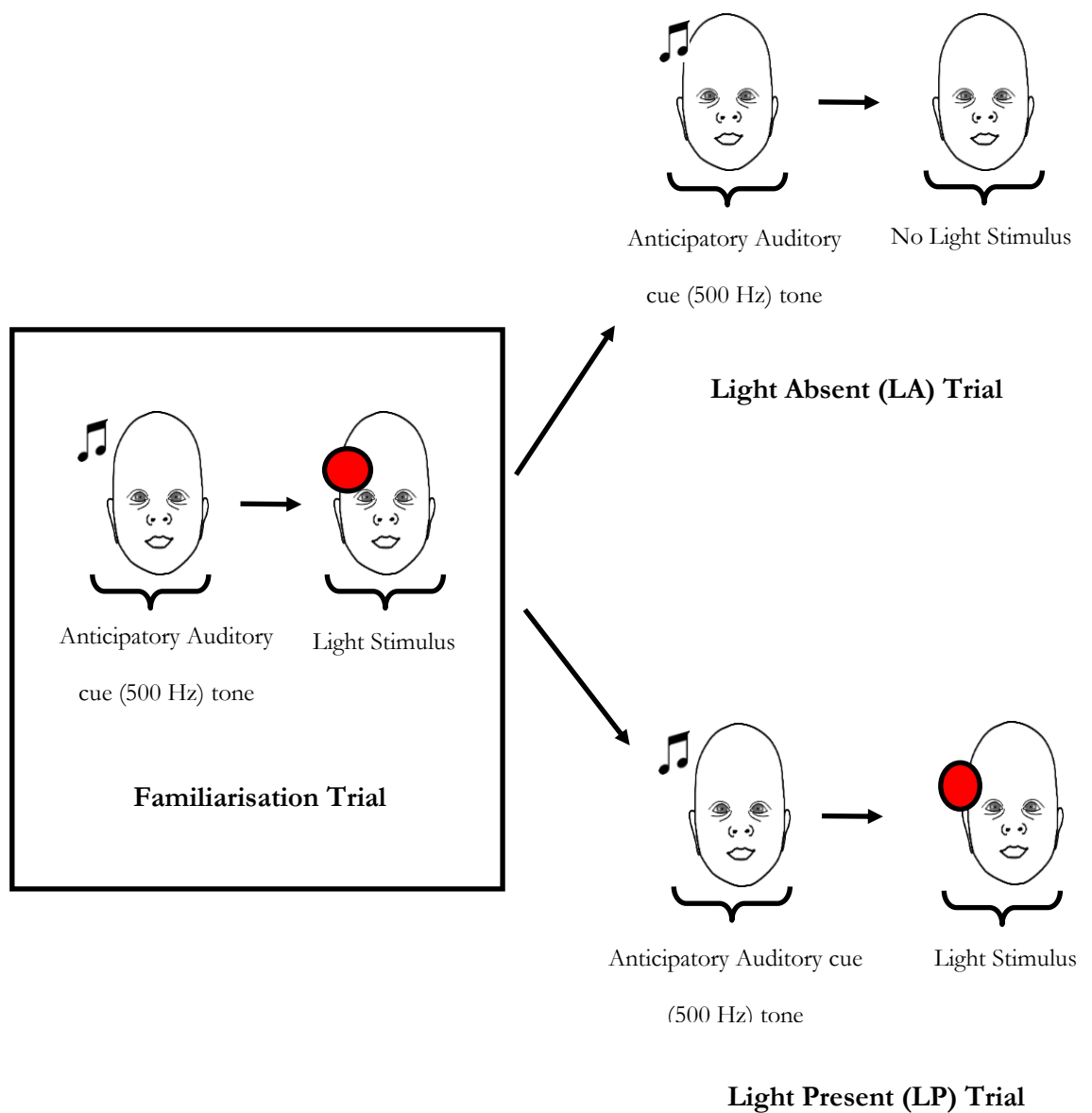


Figure 5.2 Visualisation of the anticipatory gaze experimental paradigm.

Fetuses first underwent six familiarisation trials. Following the familiarisation trials, the fetus was presented with six test trials with two conditions: Light Absent (LA) and Light Present (LP). Test trials always began with a LA trial and alternated between LA and LP trials.

5.3 Results and Discussion

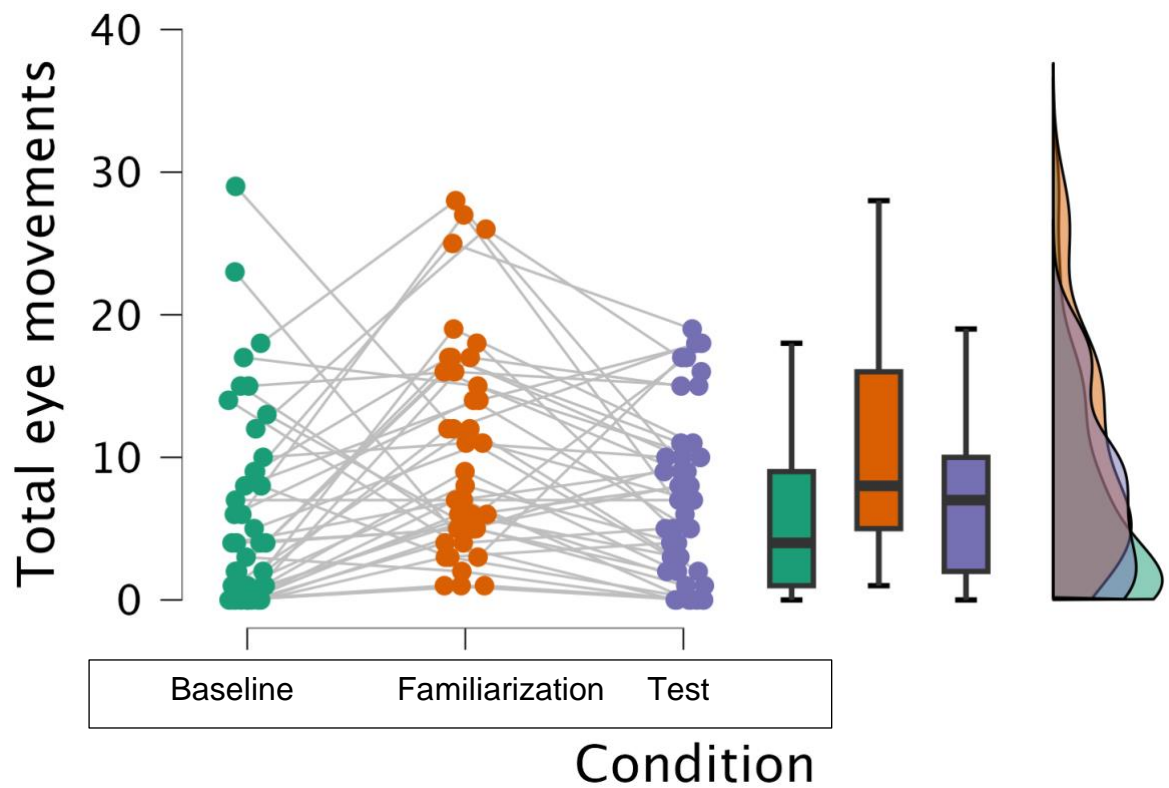


Figure 5.3. Total eye movements across experimental conditions

Comparison of total eye movements during baseline (Median = 4.0, SD = 6.121)

familiarization trials (Median = 7.5, SD = 7.59) and test trials (Median = 6.5, SD =

5.79). A Friedman test indicated significant differences among the three conditions,

$\chi^2(2) = 25.480$, $p < 0.001$. Post-hoc pairwise comparisons using Conover's test with

Holm correction showed that familiarization trials had significantly more eye

movements compared to baseline ($p < 0.001$) and test trials ($p = 0.008$). Test trials

also had significantly more eye movements compared to baseline ($p = 0.041$).

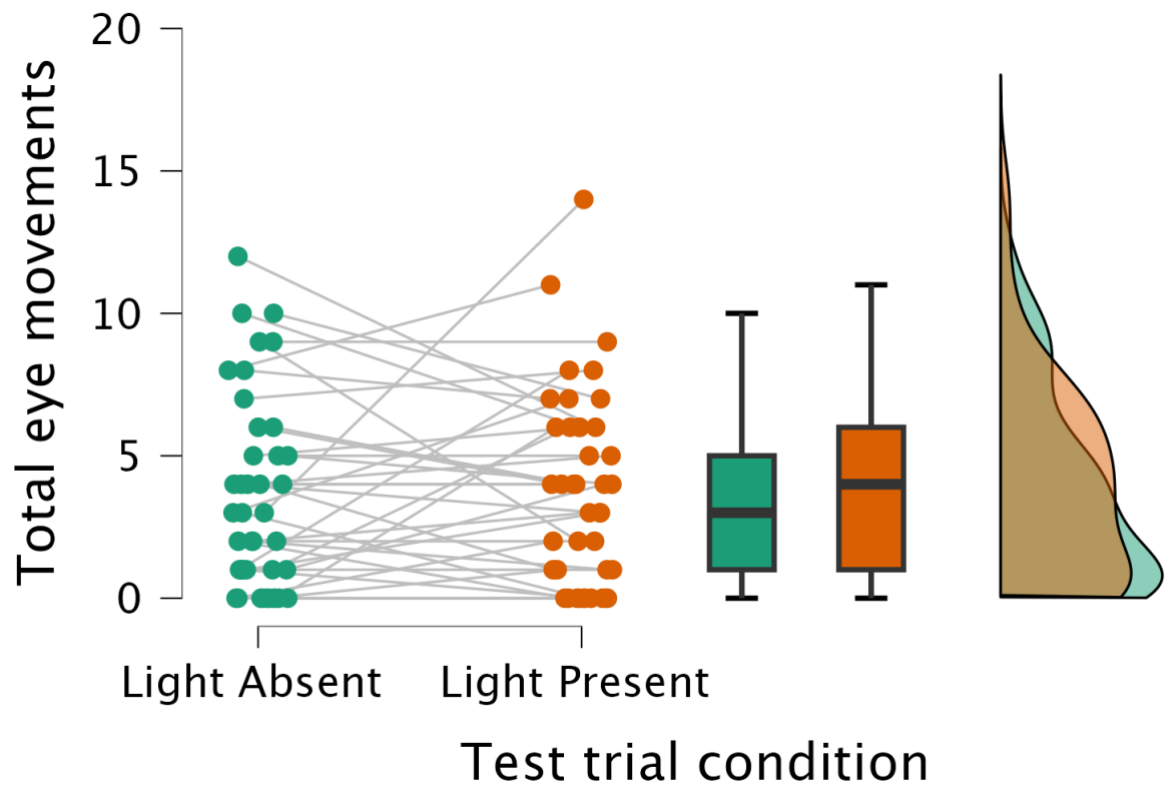


Figure 5.4. Eye movements as a function of test trial condition

A comparison of the total number of eye movements in test trials as a function of trial type (LA: light absent; LP: light present). A Wilcoxon sign-ranked test indicated that there were a similar number of total eye movements in the LA trials (Median= 2.5, SD=3.146) compared to the light present trials (LP) (Median= 3.5 SD= 3.428), $Z=-0.517$, $W=167.50$, $p= 0.611$.

There was a significant increase in the total eye movements in both the familiarisation and test trials compared to the baseline condition. This indicates that there were differential responses in the experimental conditions compared to baseline demonstrating that exogenous visual and auditory stimuli can provoke a behavioural response in the fetus.

A comparable number of eye movements were seen in the light absent trials (LA) (Median= 2.5, SD= 3.146) compared to the light present trials (LP) (Median= 3.5 SD= 3.428). These results are taken as evidence of anticipatory gaze in the fetus. In order for this to occur, during the familiarisation trials the fetus was required to associate the auditory stimulus as an anticipatory cue that preceded the presentation of a light stimulus in a specific location. In the test trials the fetus holds the expectation that the auditory stimulus will be followed by a visual stimulus in a specific spatial location. The presence of this expectation is evidenced by the fetus producing eye movements to the location where the light has previously been presented upon hearing the auditory cue, even in the absence of the light stimuli.

The pattern of eye movements in the test trials suggests that the fetus is actively engaging with the visual environment. The responses in the LA trials illustrate a move beyond a simple reactive response to an exogenous stimulus as anticipatory gaze requires shifts in visual attention that are generated endogenously and intentionally. The presence of anticipatory gaze supports the hypothesis posited by Donovan et al³³ that sustained fetal eye movements post the presentation of a visual stimulus could be taken as support for fetal awareness³⁴. Directed eye movements, as seen in both the familiarisation and test trials offer clear behavioural evidence of visual attention. The current study is among the first to investigate eye movements within an experimental framework. This approach provides a pathway for the further investigation of a wide range of fetal competencies, while also highlighting the crucial role of the prenatal environment in shaping the developing visual capacities of the fetus.

Visual anticipation reveals specific information about how individuals process and interpret complex situations. Anticipatory movements require the ability to use prior information to direct a future behaviour. The fetus anticipates that the visual

stimulus will follow the auditory stimulus, directing their gaze toward the location where it expects the stimulus to appear. If eye movements were merely reactive, this pattern of responding would not be present. Instead, we would expect to see an alternative pattern with increased eye movements in the LP trials compared to the LA trials. Within the visual expectation paradigm an “anticipation” is an action (i.e., eye movement) that occurs prior to an event (i.e., presentation of a visual stimulus)^{3,35}. Further manipulations of the paradigm measure behaviour when anticipatory expectations are violated by altering the presentation or pattern of presentation of the stimulus.

In the current study, modifications to infancy paradigms were required to align with the constraints of a fetal population. Notably, there is not a delay between the presentation of the anticipatory cue and visual stimulus, and there was only one stimulus presentation location. While undoubtedly a simplified version of the visual expectation paradigm^{3,35}, experimental methods must align with the population and the constraints of stimulus delivery. There is likely a decreased short-term memory trace and a requirement to keep the study within feasible time frames of fetal attention (for discussion on infant memory see Hayne³⁶). Further, it is rarely feasible to present visual stimuli to both sides of the fetus due to positioning in the womb. The current paradigm delivers a simple violation of expectation in the light absent (LA) trials when the light stimulus does not follow the auditory cue. Developing expectations of non-controllable external events permits visual behavior to be directional and indicates an element of partial internal control. The simple anticipatory actions seen in the fetus could be a fundamental cognitive construct on which more complex processes are built.

Understanding visual attention in utero via anticipatory gaze can inform our knowledge of perceptual processes and the foundations of higher-level cognitive

functions that may originate in the prenatal period. The performance of anticipatory eye movements requires memory, multisensory integration, and spatiotemporal processing, the implications of which are explored below.

To perform anticipatory gaze, the fetus must integrate both auditory and visual stimuli. The results are further support for the presence of multi-sensory processing in fetuses. Matuz et al²⁸ illustrated that fetuses have the ability to integrate visual and auditory stimuli in a habituation paradigm. Similarly, newborns are not naïve to the complexities of the audio-visual world, Orioli et al.³⁷ found that within hours of birth, newborns are sensitive to an audio-visual pairing incorporating visual size and auditory intensity. There is clear adaptive value to multisensory processing; the postnatal world is dynamic, filled with actions and events. It is advantageous to be able to make sense of auditory and visual cues together. Evidence of multisensory processing of audio and visual stimuli in utero suggests that the integration of separate sensory systems could have foundations in the prenatal period.

Anticipatory gaze has been referred to as “prospective” memory³⁸ as expectations imply future-orientated processing^{3,35,38}. To perform anticipatory gaze, the fetus must have a memory of the past and use that memory to anticipate the future. Within the current methodological paradigm, the performance of anticipatory eye movements requires a brief memory span. There is no delay from the presentation of the auditory stimulus to the presentation of the visual stimulus. Even though the temporal scale is small, memory and integration of the two stimuli are still required to perform the anticipatory eye movements. Fetal responses in the LA trials can be taken as a demonstration of simple, short-term memory. This type of response is within the remit of fetal abilities. Neural correlates of habituation, a type of short term memory, has been shown regarding both flashes of light and vibroacoustic presentations²⁸. Further investigation is needed to determine the length of a fetal memory trace and

whether it can extend far enough to be used for second-order regularities in which the fetus can apply a pattern or rule to a new, previously unseen sequence.

Anticipatory visual responses in the fetus also illustrate a rudimentary understanding of temporal and spatial order. The auditory stimulus precedes the visual stimulus, which is presented in a specific spatial location. Although these representations are simple, they are significant. To understand the ontogenesis of perception and higher cognitive processes, we must first comprehend the earliest iterations of these capacities.

Evidence for anticipatory behaviour contributes to shifting the debate towards an earlier emergence of consciousness than previously accepted. Consciousness in both content and structure is a highly contested concept^{34,39} and determining when and in what form consciousness first emerges is an important topic in the cognitive sciences³⁴. Given that attention, specifically visual attention, sensory integration, and intentional behaviours, have been identified as potential markers of the early emergence of consciousness³⁴, further consideration of visual orientating processes in the fetus could aid in understanding the foundations on which more complex cognitive processes, including consciousness, may arise.

Evidence of fetal awareness is contrary to a view in prenatal literature whereby the uterine environment renders the fetus in an unconscious sleep state⁴⁰⁻⁴³. In this framework, demonstrations of perceptual abilities are dismissed as the result of reflexive responses^{40,42}. Experimental research demonstrating fetal capacities^{2,28,33,44-47} are not easily reconciled with the notion of an unresponsive fetus held in a neuroendocrinological stasis. In a similar context, when assessing the fetal capacity to respond, fetal arousal and sleep state must be considered. It is clear that the fetus spends a large proportion of time in what appears to be REM sleep⁴⁸. Observational research integrating fetal movements, heart rate, and other physiological indicators

classified four fetal states (quiet sleep, active sleep, quiet awake, and active awake)⁴⁹ with research indicating that fetuses are predominantly in the "active sleep" state⁴⁹. In contrast to observational research, the current experiment presented exogenous stimuli to induce a behavioural response. It is unknown if the presentation of exogenous stimuli has the ability to arouse the fetus and shift the behavioural state. In the present study, the differential responses seen across the experimental conditions suggest the eye movements are not the result of different fetal states. In the ecological context, even if the "awake" periods are a small component of the fetal experience, they undoubtedly contribute to fetal development. Further investigation into fetal attention and the transitioning of behavioural states in the presence of external stimuli is required.

Presenting visual and auditory stimuli to a fetus comes with unique constraints. Infant and neonate studies of visual perception and anticipations can present visual stimuli to precise locations within the visual field and ensure that the infant is attending to the stimuli. Delivering light stimuli to the fetus requires a broader and more simplistic approach. A decreased degree of specificity related to stimulus presentation occurs due to factors including projecting light stimuli through the maternal tissue, the fetal position in utero and fetal movement during the experimental session. The current study represents a generalized approach to quantifying fetal visual capacities. Subsequent experimental investigations with greater precision at quantifying eye movements and stimuli presentation are required. Our results show that it is possible to measure visual detection and orienting behaviours in the fetus, laying the groundwork for the field of fetal visual perception.

5.4 Conclusion

This work investigated anticipatory gaze in the third trimester. Fetuses demonstrated eye movements towards a specific location (in which no visual stimulus was present) when they heard an auditory cue that has been previously paired with a visual stimulus presented in that same location. This indicates that visual orienting and directed eye movements are present prior to birth. This research is an initial attempt to explore directed eye movements and attention to visual stimuli in utero, with the results suggesting that visual attention prior to birth can be directed and that the fetus is an active participant in their own perceptual development. Visual perception plays a pivotal role in fundamental processes used to navigate both the physical and social world. These insights into fetal visual attention substantially change our understanding of the development of the visual system. As the fetus has visual experiences in utero, this work highlights the emerging capacities underpinning prenatal visual attention. Understanding fetal visual capabilities has the potential to reshape our knowledge of visual perception and developmental trajectories.

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5.5 Methods

5.5.1 Experimental Model and Subject Details

All pregnant participants received written information prior to study completion (compliant with HDEC ethical requirements) and gave free and informed written consent before participation (see Appendix B). This study was approved by the Central Health and Disability Ethics Committee (HDEC), reference number 2021 EXP 11521. All scans were conducted by one qualified and experienced sonographer at Waikato Hospital Ultrasound Clinic, New Zealand. All participants had singleton pregnancies with no known complications at the time of scanning.

The initial sample included 86 singleton fetuses of a gestational age between 33 and 36 weeks. Fetal behavioural responses to stimuli were assessed by a sonographer and a researcher using 2D ultrasound. A research assistant was also present throughout the duration of the session to record experimental times. Eleven fetuses were excluded due to technical errors leading to corrupt source recordings. Ten fetuses were excluded because the maternal tissue thickness was greater than 50mm. Modelling suggested that tissue thickness greater than 50mm would result in significant scattering of the light stimulus¹. Three fetuses were excluded due to health conditions and six fetuses were excluded due to positioning where the fetal lens could not be visualised.

At the coding stage twenty-two fetuses were excluded. Twelve of these fetuses were excluded as they indicated zero eye movements throughout both experimental conditions and appeared to be in a deep sleep state, otherwise referred to as behavioral state 1F². Four fetuses were excluded for exhibiting zero eye movements in the familiarization trials. Zero eye movements across the familiarisation trials indicated that the fetus had not attended to the stimulus pairing and therefore could not be considered for analysis in the test trials. A further six fetuses were excluded at the coding stage for image data quality, specifically “uncodable” sections. This exclusion criteria was applied if 50% or more of any one of the baseline, familiarisation trials or test trials conditions were coded as “uncodable.” An “uncodable” code was applied when the fetal eye was not visible in the ultrasound. This exclusion criteria was applied if, in 50% or more of the familiarisation, test, or baseline condition, the fetal eye was not visible in the ultrasound image.

The final dataset for analysis, prior to the removal of outliers, consisted of 45 fetuses (19 female, 22 male, 5 sex unknown), gestational age between 231 and 252 days ($M=239.69$ days, $SD=4.75$). Maternal tissue thickness ranged from 13 to 48 mm ($M=3.04$, $SD=0.81$).

5.5.2 Ultrasound Acquisition

Ultrasound scans were performed using a Philips Epiq7 Elite Ultrasound System using a 5C1, or X6-1 transducer, keeping $Tt_b < 0.3$. Individual scans were recorded in their entirety as mp4 video files using OBS software³ and were saved to an external hard drive for offline analysis.

An experienced and qualified sonographer undertook the scanning. Images were captured in the transverse plane of the fetal face for the experimental procedure. The transverse images provide optimal visualisation of the fetal lens and eye movements in response to a stimulus presented horizontally in relation to the fetal face.

5.5.3 Stimuli

The light stimuli consisted of one custom-made red 4 mm LED emitting at 650nm. The light stimuli could be calibrated to one of three power levels for three different levels of maternal thickness. The three levels were 0.5mV for maternal tissue measurements below 15 mm, 1mV for maternal thickness between 15-30mm and 5mV for maternal thickness greater than 30mm. The light source design and rationale for the optical powers was based on the modelling and stimulus design in Reid⁴ and supported by Isaac¹. The light stimulus was presented within the predicted range of the fetal visual system at a luminance, which was safe for the fetal eyes.

The sound stimuli was a 500Hz tone presented by a speaker positioned one meter away from the pregnant person. The tone was recorded at 90db at the maternal abdomen. Tone intensity and frequency are safe and within the range of the fetal auditory system⁵. The tone frequency and intensity is consistent with experimental studies examining auditory processing in utero (as reviewed in Dunn et al⁶).

5.5.4 Data Acquisition

5.5.4.1 Procedure

The participant was positioned in a supine position. Once comfortable the sonographer began the session by taking the fetal biometric measurements to ensure normal fetal growth and no fetal abnormalities. A set of fetal measurements including the fetal head position, placenta position, fetal biparietal diameter, head circumference, abdominal circumference, femur length, fetal weight and single deepest pocket were calculated and recorded. Once it was confirmed that fetal biometry measurements demonstrated normal fetal growth and no fetal anomalies the experimental session would proceed.

The sonographer checked that one of the fetal eyes could be visualised and then calculated the maternal thickness measurements from the same maternal position where the stimulus was to be presented. Participants were requested not to talk during the study. All timings were controlled via a clock and noted by a research assistant.

The light stimuli were delivered to the maternal abdomen relative to the position of the fetus (breech presentation/head up, $n = 1$, cephalic presentation/head down, $n = 44$). The ultrasound image, interpreted by the ultrasound technician, informed the light stimuli's presentation parameters. Stimuli were presented to one of the fetal eyes (left, $n = 20$, right, $n = 27$). Due to inherent requirements for variation in presentation position and angle of the light source onto the maternal abdomen, the experimenter was not blind to the condition that was presented during data collection. Participants were blind to the trial types being presented.

Light stimuli were presented in relation to the position of fetal head. In the event that the fetus exhibited a large amount of movement the experimental protocol would continue with the sonographer making the best attempt to obtain consistent imaging of the fetal lens and head. The experiment would not be paused and restarted to accommodate fetal movement. Large and frequent amounts of fetal movement meaning the lens and head could not be visualized would be reflected in the “uncodable” code (see coding sub heading) and could lead to exclusion of the fetus from the final dataset

5.5.4.2 Familiarization Trails

The experiment began with six familiarisation trials. Each familiarisation trial began with the auditory cue stimulus that was played for two seconds. Immediately following the auditory cue, the light stimulus was presented to the peripheral side of the fetal face moving at one cm per second for five seconds away from the fetal face. The light stimulus was presented in the same location across all trials. Each familiarisation trial was a total of seven seconds, followed by a five-second inter-trial-interval to allow the fetus to return to a canonical, resting position. The total time of the familiarisation trails was 72 seconds.

5.5.4.3 Test Trials

On completion of the familiarisation trials, the fetus was presented with a series of six test trials. There were two types of test trial, Light Absent (LA) and Light Present (LP). In LA trials, the auditory cue was played for two seconds followed by a period of ten seconds where no light stimulus was presented and a five second inter-trial-interval (Figure 5.2). LP trials were an exact copy of the familiarisation trials where the auditory cue was played for two seconds followed by the light stimulus for five seconds and a five second inter-trial-interval. Test trials were presented in an

alternating order, beginning with an LA trial. There were six test trials in total, three LA and three LP. The total time of the test trails was 72 seconds.

In each trial (both familiarisation and test) the light stimulus was then moved across the maternal abdomen in a horizontal direction (relative to the fetal head) for five seconds at an average speed of one cm per second. This is consistent with speeds reported in newborn studies⁷ taking into account constraints specific to this population, i.e., the width of maternal tissue that was accessible to present stimuli and the space available for the fetus to move.

The onset of sound and light stimuli were controlled by a custom build program. The experimenter pressed a button to start the trials. This ensured that the experimenter could attend to the movement of the light stimulus and that the light and sound presentation and timing was standardised across participants. Movement and light stimulus onset was simultaneous and was controlled by the experimenter who was delivering the stimuli.

The test trials were followed by a 180 second period in which no sound or light stimuli were presented referred to here as the baseline. The baseline period used for analysis was taken from the final 72 seconds of this period to match the familiarisation and test trial lengths. The baseline was measured from this time point as at the beginning of the session fetuses are commonly adjusting to the supine maternal orientation required for the ultrasound.

5.5.5 Data Coding

Behavioural coding of the fetal ultrasound was conducted on ObserverXT software⁸. The number, duration and direction of eye movements was recorded for each trial type and the baseline. Coding was done for the single eye to which the stimulus was presented.

Fetal eye movements were either coded as lateral or medial using Observer XT software⁸. Lateral movements were when the fetal lens moves towards the zygomatic bone (as if the fetus were looking to the side of their face, towards their periphery) and medial movements were classified as a lens movement to the nasion (as if the fetus were looking in the direction of their nose). These two categories of eye movements were then collapsed to give the total eye movements for each condition.

An eye movement was defined as a lens movement from a stationary position through a position change to another stationary position. A single-eye movement is uni-direction (lateral or medial). If the lens does not reach a stationary position but changes the direction of movement (i.e., from a lateral to a medial direction), this indicates a second eye movement. The start (onset) of the second eye movement is when the directional change begins. The onset of an eye movement is when the lens movement begins, and the offset is when the movement of the lens in that direction ends, either by coming to a stationary position or changing direction of movement.

Two coders completed the fetal eye movement coding. Coders were blind to the experimental condition. Cohen's kappa was performed to determine interrater agreement on fetal head movements. Cohen's Weighted Kappa was used to assess inter-rater agreement while accounting for the severity of disagreements between raters. Unlike the unweighted Kappa, which treats all disagreements equally, the weighted Kappa gives partial credit for disagreements that are closer in value and penalizes more heavily for disagreements that are further apart) and is appropriate to use with interval data. By incorporating weights, Cohen's Weighted Kappa provides a more nuanced measure of agreement, reflecting not only whether raters agree, but also how closely their ratings align⁹. The Kappa value was calculated on the total eye movements across experimental condition. There was a substantial agreement between two coders' judgments, $k = 0.977$ $SE = 0.009$. Behavioural measurement was thus deemed to be suitable for use in the hypothesis tests in the present study¹⁰.

5.5.6 Statistical Analysis

Statistical Analysis was completed using R statistical package¹¹. Outliers were removed using the Tukey's method. Tukey's method identifies outliers by calculating the interquartile range (IQR) and defining any value outside 1.5 times the IQR from the first or third quartile as an outlier, providing a robust, non-parametric approach to detecting extreme values in datasets¹². This gave a final sample of 40 singleton fetuses, gestational age between 231 and 252 days ($M = 239.5$ days, $SD = 4.75$). Maternal tissue thickness ranged from 4.8- 1.6mm ($M = 3.04$, $SD = 0.77$).

First, the total number of eye movements was calculated as a function of experimental conditions Baseline (Median = 4.00, $SD = 6.12$), Familiarisation (Median = 7.5, $SD = 7.59$), and Test (Median = 6.5, $SD = 5.79$). A Shapiro-Wilk test suggested a deviation from normality for all three conditions, with Baseline

($W=0.853$, $p<0.001$), Familiarisation ($W=0.908$, $p=0.003$), and Test ($W=0.919$, $p=0.007$).

A Friedman test shows that there were significant differences in the number of eye movements across the three conditions $\chi^2(2) = 25.480$, $p<0.001$. Conover's post-hoc pairwise comparisons with a Holm-Bonferroni correction indicated that there were significantly more total eye movements in the familiarisation trials compared to both the baseline ($Z=5.027$, $p<0.001$) and test trials ($Z=2.94$, $p=0.008$). There were also significantly more eye movements in the test conditions compared to baseline ($Z=2.08$, $p=0.041$).

The total number of eye movements was calculated as a function of trial type (LA: light absent; LP: light present). A Shapiro-Wilk test suggested a deviation from normality for both LA ($W=0.881$, $p<0.001$) and LP ($W=0.902$, $p=0.002$). A Wilcoxon sign-ranked test indicated that there were a similar number of total of eye movements in the LA trials (Median: 2.50, SD= 3.146) compared to LP trials (median= 3.50, SD= 3.428), $Z=-0.517$, $W=167.50$, $p=0.611$.

Data Availability

The data reported in this paper will be available in the FigShare Digital Repository under the following DOI: TBC upon publication

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Prelude to Chapter 6

The preceding set of experiments demonstrates that the fetus actively engages with exogenous light stimuli. **Chapter 4** had a perceptual focus (temporal vs nasal responding), whereas **Chapter 5** moved beyond perception to examine anticipatory capacities. The experimental study in **Chapter 6** builds on the previous study to examine a different aspect of fetal visual processing. This shift in emphasis is due to the significant lack of knowledge we have surrounding the psychological development of the fetus. Here we ask, do fetuses demonstrate preferences for specific types of motion?

The following study is notable for the visual stimuli used. The light stimuli presented in the preceding experiments of **Chapter 4** and **5** were the first to present targeted visual stimuli using a simple, single LED dot diode. In these experiments it is both the spatial and temporal presentation of the stimuli that provides insight into fetal visuo-perceptual processing. The following study presents the most ambitious and complex visual stimuli of the three experimental studies in this thesis. It marks the first attempt to present a visual scene to the fetus. The stimuli are pared down to the key essential elements, with two dots moving in different patterns of motion.

Investigating motion processing offers insights into a different facet of perceptual and cognitive processing while also extending our understanding of appropriate research paradigms with this population.

The shift in focus represents a deliberate expansion of inquiry. By addressing gaps in the knowledge of motion perception, the following chapter situates within the broader objective of this thesis; to build a foundational understanding of fetal visual perception. As with the two preceding experimental studies, **Chapter 6** provides

insight into another facet of fetal capabilities, collectively moving the field towards a more comprehensive understanding of fetal visual perception.

Chapter Six

Fetal responses to agentive motion cues

Summary

How an object moves through time and space provides invaluable visual cues via which to make sense of the world. Humans possess an remarkable ability to extract social meaning from the movement of even abstract shapes¹. Motion interpretation is a fundamental building block for social interaction². The ontogeny of the detection of animacy and social agents is unknown. New-borns can differentiate between types of motion events (causal and non-causal) and show a preference for events that demonstrate physical causality³. Might there be a prenatal predisposition to attend to motion cues that form the foundations of social cognition? The current study investigates fetal sensitivity to spatio-temporal cues that correlate with adult interpretations of agentive motion. 31 singleton fetuses between 33 and 36 weeks gestation were assessed. 2D ultrasound was used to visualise the fetal lens and record fetal eye movements. A modified version of Rochat et al.⁴ “chase” study was employed in which fetuses were presented with two separate point light displays of “agentive” and “control” sequences, consisting of two dots (dot diode emitting at 650nm) Dot A and Dot B in motion. 2D ultrasound was used to record eye movements to measure fetal visual engagement. There were no significant differences in the number of eye movements in the “agentive” compared to the “control” condition. This work further assists in mapping the parameters of fetal visual research and provides signposts for future work examining agentive motion processing in the fetus.

6.1 Main

The visual domain is rich and complex. How an object moves through time and space provides invaluable visual cues with which to help make sense of the world. Humans have a seemingly intuitive ability to extract social meaning from the movement of even abstract shapes^{1,2,5-7} This phenomenon is referred to as perceptual animacy, the view that an object can hold the properties of being alive due to movement characteristics¹. Graduations within perceptual animacy extend from an object being simply alive to having intentions, goals, and internal states^{8,9}. Motion interpretation moves beyond simple perception and is a fundamental building block for understanding social interaction².

Animacy percepts are intrinsically linked to motion. Heider and Simmel's⁷ landmark work first explored social interpretations of movement. Adults viewed visual displays of simple geometric shapes (a circle and two triangles) moving in specific patterns of motion. In one animation a large triangle moves behind a smaller triangle as they circle around a stationary box. Individuals described the large triangle as "chasing" the smaller shapes. Participants uniformly ascribed functions typical of social interactions including "chasing" and "fighting" to these inanimate displays. Further, emotions (e.g. angry) and distinct personalities (e.g. shy) were attributed to the shapes. The objects did not touch each other (no physical causality was present), yet the motion of the individual shapes was observed to influence that of the other shapes. The inferences of social intention extended beyond the events displayed. The adult participants knew that these visual displays were lifeless, yet they still attributed social causality and agency to the shapes⁷.

With the knowledge that the motion of abstract objects provides enough cues to trigger perceptions of animacy, social intention and goal directed behaviour^{8,10}, the

investigation of the specific cues and parameters of motion required for perceptual animacy has been undertaken. Low-level perceptual cues including self-propulsion, speed changes (acceleration and deceleration), and spatio-temporal contingencies give rise to agency precepts in adults^{5,6,8,11-13}. The ability to ascribe agency and goal directed behaviour reflects higher cognitive functions; however, the motion cues used to inform this understanding are simple perceptual features. Within this framework, motion interpretation sits at the intersection of perceptual and cognitive abilities.

Humans are adept at interpreting simple spatio-temporal motion cues to infer social causality^{1,6,7}. But when in our development do we begin to understand animacy and social agents? Is there an innate predisposition to attend to motion cues that form the foundations of social cognition? Rochat et al.⁴ conducted seminal work into the precursor perceptual abilities of social causation. Infants between the ages of three and six months of age viewed two separate motion displays simultaneously in a preferential looking paradigm. Both displays contained two coloured discs moving across the screen. In one display, the discs moved independently of each other providing no motion cues of intention or agency. The second motion display contained a “chase” sequence. When one disc, the “chaser” got within a specific distance of the other disc, the “chasee”, would accelerate away from the “chaser” and would resume a standard speed of movement when it was at a specific and “safe” distance from the “chaser” disc. Three-month-old infants preferred to look at the “chase” display, a sequence with “social” interaction. Rochat et al.⁴ concluded that from the age of three months infants demonstrate a visual preference for motion that contains information of social causality.

Three month old infants also indicate the capacity to detect goal-directed behaviour from the motion of a self-propelled box¹⁴. In familiarisation trials, infants viewed a

box moving towards a target object. In test trials, infants attended more to events when the box moved towards a different object than the target object from familiarization trials. The authors interpreted this result in cognitive terms. Specifically, infants had the belief that the self-propelled box's motion was goal directed (to reach the target object) and were surprised when the box did not move in the direction to fulfil this goal. This suggests that, from a young age, infants are attuned to motion cues that underpin agency^{4,14}. To date no research has explored sensitivity to these cues of intention and social causality in infants under the age of three months; however, new-born research offers insight into motion perception at birth.

New-borns can differentiate between types of motion events (causal and non-causal) and show a preference for events that demonstrate physical causality¹⁵. Some experimental work also suggests there is a sensitivity to biological motion when contrasted with other forms of motion¹⁶. Two-day old babies prefer to look at a point light display of biological motion (a walking hen) compared to non-biological motion (rotating shape)¹⁶. Further to this, new-borns attend to cues of self-propulsion, a foundational element of animacy perception in adults¹⁷. Using a simple ball stimulus, Di Giorgio et al¹⁷ presented new-borns with two types of motion events. In the "self-propelled" event, a stationary ball started moving across the screen and out of view of the observer. In the "ambiguous" event, the ball appeared on the screen already in motion (providing no cues of self-propelled motion). Not only could new-borns discriminate between the two event types, they preferred to attend to the self-propelled event. Extending these findings, Di Giorgio et al.¹⁸ explored new-borns sensitivity to speed changes. Neonates preferred to look at visual displays with abrupt speed increase and decreases. Thus, there is consistent evidence that, from birth, humans demonstrate attentional biases to visual cues of motion.

Neonatal research demonstrates a sensitivity to motion cues that form the perceptual base of understanding animacy¹⁵⁻¹⁸. These findings have been interpreted as evidence that new-borns have innate attentional biases to specific motion cues^{19,20}. If this ability is in fact innate, it is plausible to expect that similar sensitivities would be present in fetal visual responding. How is motion perceived in utero? Do fetuses exhibit a preference for motion sequences that act as the building blocks for higher-level social cognition?

Prior experimental work has demonstrated that fetuses respond to visual stimuli in utero. Neuroscience studies have presented flashes of light to the fetus with cortical activation demonstrated in response to visual stimuli²¹⁻²⁴. Similarly, fetal magnetic resonance imaging has reported increased activity in the frontal cortex in response to a constant light source²⁵. Further, Kiuchi et al.²⁶, detailed increased eye movements and heart rate in response to a visual stimulus (a photographic flash) that was combined with vibroacoustic stimulation. The studies detailed above have reported responses to basic flashes of light, more recently, the feasibility of presenting patterned stimuli has been demonstrated²⁷. Reid et al.²⁷ simultaneously investigated face-processing capabilities in the fetus and explored a new method for presenting visual stimuli in utero. Based on the highly replicated neonatal preference for face-like stimuli, the researchers presented two separate light arrays to the fetus: a face-like stimulus and the same stimulus inverted. Fetal head movements towards the shapes were recorded via a 4D ultrasound, with fetuses demonstrating significantly more head turns towards moving face-like stimuli. These results suggested that the preference for the face-like stimulus seen in neonates is present prenatally. Taken together these behavioural and neuroscience studies demonstrate extant neonatal visual capabilities.

Fetal visual attention can be investigated by indexing eye movements. It has long been known that eye movements and looking times are fundamental for understanding visual perception in infancy and beyond²⁸. Of importance for the current work, fetal eye movements can be visualised in utero. The lens of the fetal eye is easily distinguished as a white circle within the hypoechoic eyeball²⁹. 2D ultrasound imaging can focus on the acoustic reflection of the eye lens and record how it moves within the orbital socket²⁶. Studies exploring fetal eye movements have been primarily observational, with a focus on indexing patterns of eye movements and their relationship to broad indicators of neurological development.^{30,31} Despite the observational nature of most research in this area, one of the first studies to investigate fetal eye movements in response to a targeted light stimulus found systematic eye movement patterns³².

The study outlined below aims to examine fetal sensitivity to spatio-temporal cues that correlate with adult interpretations of agentive motion. This study is not an attempt to imply that the fetus has the capacity to retain abstract concepts regarding agency and goal directed behaviour. Rather, it is an exploration into fetal visual attention that provides the earliest basis of developing complex social understanding. Simply, does fetal sensitivity to spatio-temporal cues correlate with adult interpretations of agentive motion?

6.2 Experiment Summary

The final dataset includes 31 singleton fetuses between 33 and 36 weeks gestation (M=239.5 SD=4.75 days). 2D ultrasound was used to visualise the fetal lens and record fetal eye movements in the transverse plane. The transverse plane gives a visualisation through the head horizontally, viewing transverse images are similar to looking down on the top of the head in a birds eye view (Figure 6.1)

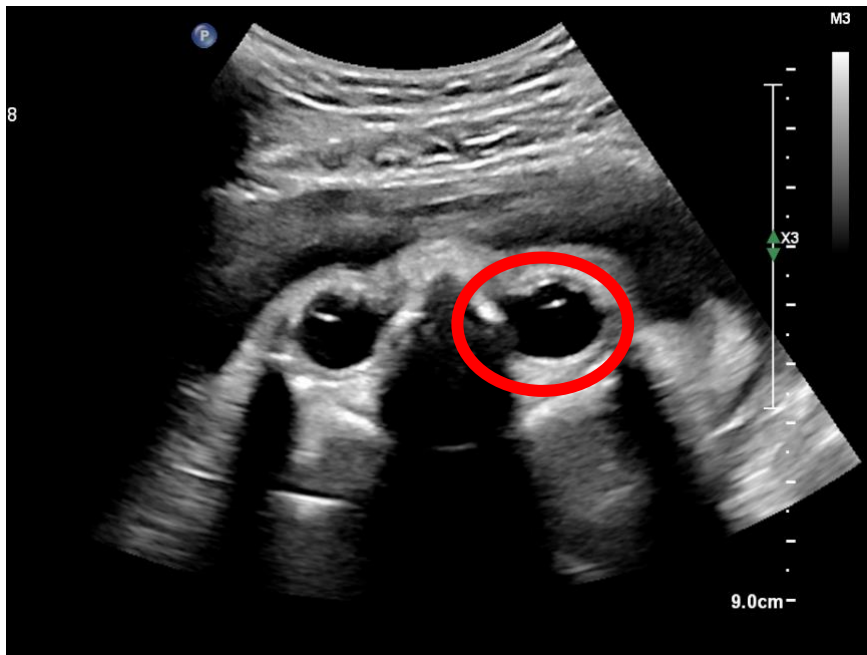


Figure 6.1 An image of a 2D ultrasound of a fetal head in the transverse plane. The acoustic reflection from the lens of the right eye is highlighted in the red circle.

Using an adaption of Rochat et al.⁴ “chase” study, fetuses were presented with two separate point light displays (“agentive” and “control” sequences) consisting of two dots in motion (see Figures 6.2 and 6.3). The relationship the two dots have to each other in time and space differentiate the cues of agency that the conditions confer.

In both conditions, the motion sequence the fetus viewed was twelve seconds in length. The fetus first viewed two stationary dots. Both dots initially flashed on and off for two seconds. In the “agentive” sequence (Figure 6.2), Dot A (located at the left of the sequence) began to move in a linear trajectory at a constant speed of 4.2 mm per second. When Dot A reached a specified point half way in the trajectory, Dot B (located at the right of the sequence) accelerated from a stationary position at a 45 degree angle towards Dot A at a speed of 8.4 mm per second. Both dots finished at the same time, closer together than at the start position, but not touching. The “agentive” sequence gives the appearance of Dot B accelerating towards Dot A.

Adult viewers rate this motion sequence as animate and infer that Dot B is trying to “catch” or “chase” Dot A (refer to the Methods section for adult sample pre-testing of stimuli) .

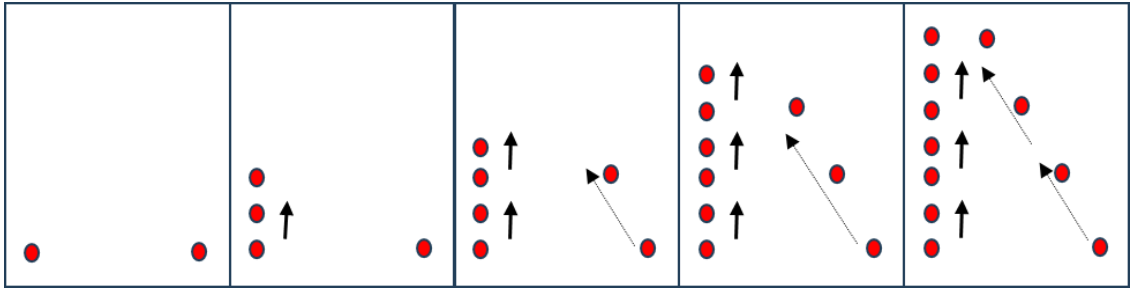


Figure 6.2 A pictorial depiction of the point light agentic motion sequence.

Dot A is located on the left of the panel and Dot B on the right. The sequence progression starts from the panel on the left and proceeds to the right. The arrows indicate the direction of movement of the dots.

In the “control”, motion sequence (Figure 6.3), Dot A and Dot B also start from stationary positions. As in the “agentic” condition, Dot A will begin to move in a linear trajectory at a constant speed of 4.2 mm per second. When Dot A reached a certain point in the trajectory, Dot B also began to move at a faster speed of 8.2 mm per second. This movement, however, is in the same direction as Dot A. It does not move at an angle towards Dot A, instead following a straight trajectory. The “control” motion sequence gives the appearance of Dot A and Dot B moving on parallel trajectories, independent of each other. The “control” motion sequence does not give the same cues of agentic movement that the “agentic” sequence holds to adult viewers.

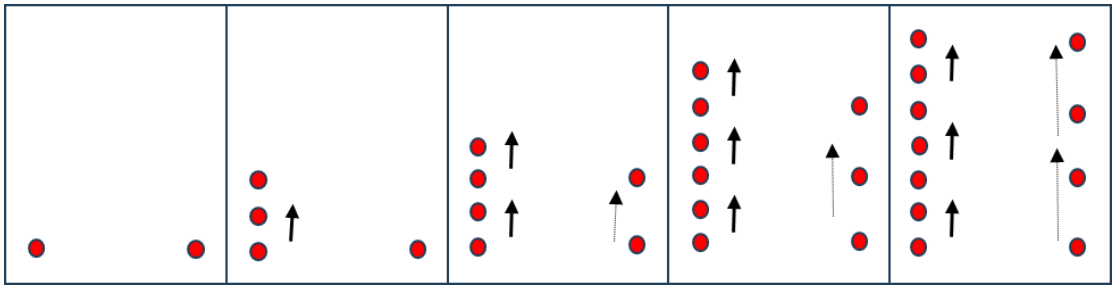


Figure 6.3. Pictorial depiction of the point light control motion sequence.

Dot A is located on the left of the panel and Dot B on the right. The sequence progression starts from the panel on the left and proceeds to the right. The arrows indicate the direction of movement of the dots.

The light stimuli for both conditions were constructed using a custom-built LED array with all 2mm dot diodes emitting at a wavelength of 650nm. All LED diodes in the array were programmed to turn on and off at specific times to give the illusory percept of motion. The LED arrays for each condition were a 100mm by 100mm square with the LED embedded in the specific organisation for the motion condition. The array was placed on the maternal abdomen by the experimenter and held in position. The onset of the light stimuli was controlled by the experimenter. The stimuli were projected on the maternal abdomen and the light stimuli moved in a horizontal orientation to the peripheral side of the fetal face.

At the end of both motion sequences, the two dots were removed from view and there were three seconds of no light present before the sequence of motion was repeated again. The motion sequence was repeated eight times for a total of 120 seconds (two minutes) stimulus exposure in each condition. Between stimulus condition presentations there was a 180 second (three minute) interstimulus interval to enable the fetus to return to the initial resting, or canonical, eye position. The total

duration of the experiment, including interstimulus interval, was 420 seconds (seven minutes). The condition presentation order was counterbalanced across the sample. Stimuli were presented at an orientation to induce lateral eye movements in the fetus. The number of eye movements in response to the stimuli was assessed using condition-blind coding of the 2D scans. Fetal eye movements were either coded as lateral or medial using Observer XT software³³. Lateral movements were recorded when the fetal lens moved towards the zygomatic bone (as if the fetus were looking to the side of their face, towards their periphery) and medial movements were classified as a lens movement to the nasion (as if the fetus were looking in the direction of their nose).

These two categories of eye movements were then collapsed to give the total eye movements for each trial type. The two categories were combined to give a comprehensive representation of fetal visual behaviour. Within the constraints of the current methods, it is not possible to ascertain a central fixation of the fetal eye or the precise location where the stimulus is being presented in relation to the fetal eye. As such, a medial movement (lens movement towards the nose) could still be considered evidence of engagement with the stimulus in the periphery. The combined categories provide a pattern of eye movements, with the assumption that different levels of engagement with the stimuli would produce different patterns of movement. We hypothesised that fetuses would demonstrate significantly more eye movements in the agentive motion sequence when compared to the control motion sequence.

The experimental conditions were followed by a 180 second (three minute) period in which no sound or light stimuli were presented, referred to here as the baseline. The baseline period used for analysis was taken from the final 120 seconds of this period to match the familiarisation and test trial lengths. The baseline was not measured

from the beginning of the session as fetuses often displayed movement in response to adjusting to the supine maternal orientation required for the ultrasound.

6.3 Results and Discussion

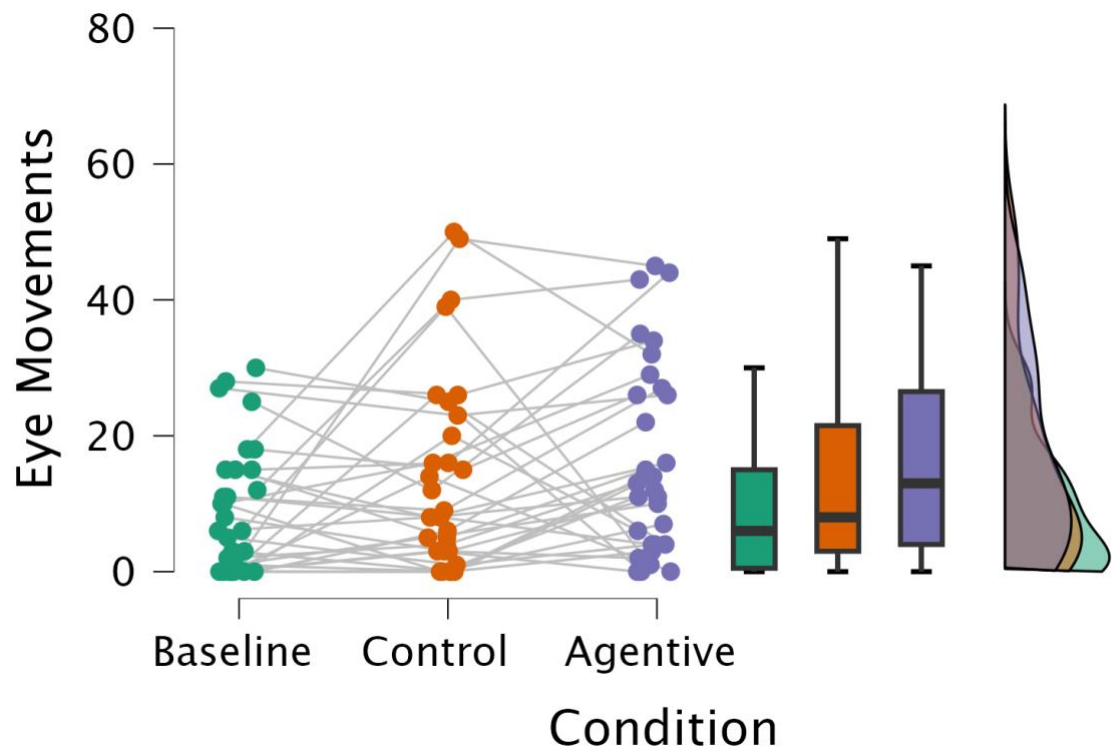


Figure 6.4. Total Eye Movements Across Experimental Conditions

Comparison of total eye movements was made during baseline (Median =6.00, SD = 9.423) agentive condition (Median = 13.00, SD = 14.031) and control condition (Median = 8.00, SD = 14.528). A Friedman test indicated significant differences among the three conditions, $\chi^2(2) = 7.277$, $p = 0.026$. Post-hoc pairwise comparisons using Conover's test with Holm correction showed that there were significantly fewer eye movements in the baseline condition compared to agentive ($p = 0.002$). There were no significant differences in total eye movements in the control condition compared to agentive ($p = 0.073$) or baseline ($p=0.439$).

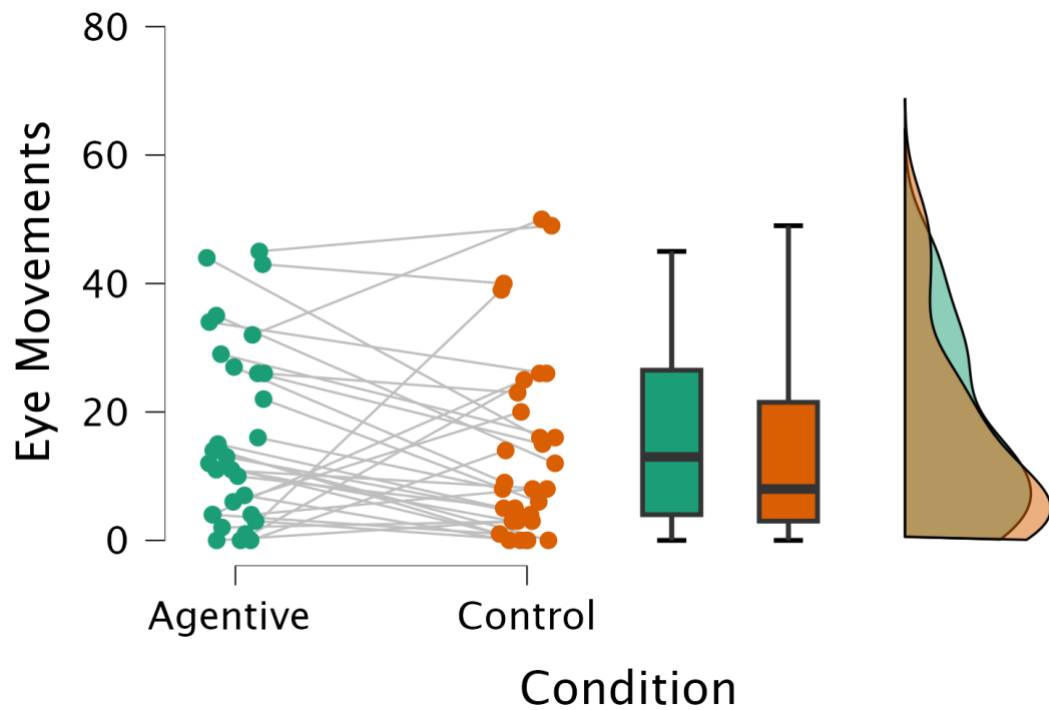


Figure 6.5. Eye movements as a function of trial type and experimental condition. A comparison of the total number of eye movements as a function of condition type (agentive and control). A Wilcoxon sign-ranked test indicated no significant differences in eye movements in the agentive condition (Median= 13.00, SD=14.031) compared to the control condition (Median=8.00, SD= 14.00), $Z=1.372$, $W=318.00$, $p= 0.173$.

Results demonstrated significantly more eye movements in the agentive condition compared to the baseline. While there was no significant difference between the number of eye movements in the control condition compared to the baseline, the results indicate a trend towards more eye movements in the agentive condition. These results can be taken as an indication of fetal visual attention towards the light stimuli in general, suggesting that fetuses perceive and respond to environmental changes in the visual environment.

There were no significant differences in eye movements between the control and agentive motion conditions. The results are symptomatic of the large standard deviations seen across the three conditions. Increasing the sample size may well mitigate this and reveal significant effects of condition type. Even though there is no significant difference between the agentive and control motion stimuli, there is a clear trend of increased eye movements in the agentive motion condition. While it must be acknowledged that this is a non-significant trend, it is nonetheless of interest to briefly speculate relative to the reasons for increased eye movements and, therefore, visual attention to the agentive motion sequence. A rich, high-level interpretation is that this trend is reflective of fetuses' sensitivity to motion cues that correlate with adult interpretations of agentive motion. Given that newborns are sensitive to motion cues that form the basis of animacy perception¹⁵⁻¹⁸ this interpretation is potentially plausible. Consideration must also be given to a lean, low-level perspective, where perceptual cues could contribute to this preference. In the agentive sequence, the two dots come together and are in closer proximity compared to the control sequence. This pattern and positioning of the dots are required to give the requisite agentive cues. Due to the constraints of presenting stimuli in utero (which are discussed in detail below), including a restricted visual presentation window and distortions of light stimuli passing through the uterine wall, it is possible that there is a more concentrated light stimulus near the end of the agentive sequence. It may not be the agentive cues from the movement of the dots that generate the preference but, rather, the general appearance of a greater light intensity as the dots move nearer to each other. However, these interpretations are currently conjecture as, within the current sample, the results demonstrate that fetuses did not exhibit preferential orienting to a motion sequence that contains spatial-temporal cues that correlate with agentive motion.

The null results should be interpreted with caution given that the lack of preferential orientating to agentive motion stimuli in this study does not necessarily indicate that fetuses are insensitive to cues of agentive motion. The field of fetal visual perception research is only a recent endeavour; as such, designing research paradigms requires consideration of a large number of unknown variables. While all effort is made to minimise and control for these variables, it is important to recognize that the complexity and novelty of this research area produces inherent limitations in the study design and, potentially, in outcomes. The challenges and considerations which could influence the results of the current experiment are discussed below.

The stimuli motion patterns used in the present study represent the first attempt to present a visual scene to the fetus. Studies assessing fetal visual perception have largely used generalized flashes of light (i.e. light/no light)^{21,23,26}. The patterned, face-like stimulus used in Reid et al.²⁷ was comparatively more intricate yet nonetheless was a static construct moved across the maternal abdomen. The face-like or inverted face-like pattern was moved across the periphery of the fetal visual field and the pattern itself was internally consistent and fixed across the presentations. This meant that for the face-like stimuli study²⁷, even if the fetus did not engage until a later part of a trial, the same visual information was present as to that of a fetus that had been attending at the start of the trial.

In contrast to previous fetal vision research, the light stimuli presented in the current study are relatively complex. In both the agentive and control conditions the fetus must attend to the entire sequence to detect any extant motion cues that differentiate the two sequences. Requiring visual attention for the entirety of each motion sequence presents multiple challenges. The parameters of fetal visual attention are still unknown. When designing the stimuli, consideration was given to striking a balance between making the sequence as brief in duration as possible (to account for

limited fetal attention) but still containing adequate agentive cues to differentiate between the two conditions. Even though the duration of the motion sequences was designed with reference to newborn studies with further allowance for a more immature fetal capacity related to movement and response times^{34,35}, it is still possible that the motion sequences extended beyond the ability of the fetus to attend towards each stimulus in its entirety. Given that processing the entire presentation was required for differentiation between conditions, factors related to fetal attention capacity may explain the results of the present study. Fetal fatigue was also a factor in the duration of the conditions. Each stimulus motion pattern was repeated eight times (over two minutes), in order to give the fetus an opportunity to engage with the stimuli. Increasing the number of presentations of each stimulus could provide the fetus with more opportunities to engage and differentiate between the conditions but at the potential cost of also inducing fatigue and preventing the fetus from responding. In this scenario, too many presentations may have produced a null result based on a lack of ability to engage with the stimuli over time. It should be acknowledged that at this point in time, the optimal duration of an experiment with visual stimuli is not known.

In contrast to infant studies, fetal attention and visual fixation to stimuli (using eye movements) cannot be measured in real time. In order to encourage the fetus to attend to the stimuli at the start of the motion sequence, an “attention grabber” (repeated flash of both dots at the start of every sequence) was included. However there is the possibility that the fetus was not attending to the light stimulus at the beginning of the motion pattern and only engaged at a later point, therefore not receiving all of the motion cues. Relatedly, prenatal studies do not have the ability to modify the initial position of the fetus. When this is taken into consideration with the generalized nature of the light stimulus presentation, there is the possibility that in

some presentations, the fetus did not see the stimuli, and thus, reduced eye movements were due to the fetus simply not engaging with the stimuli rather than indicating a specific attentional preference.

Presenting visual stimuli to the fetus through the uterine wall creates unique constraints for stimulus creation and delivery. Animacy experiments with infant samples can present an array of scenes with a range of visual cues, including multiple agents, shapes, and obstacles^{1,4,14,17,18}. In prenatal research, the stimuli are significantly constrained in size and complexity in order to be presented transabdominally. First, consideration must be given to what the stimulus will look like when projected through the uterine wall. Light is scattered when moving through the maternal tissues, with modelling suggesting stimuli becomes blurred and less defined³⁶. For this reason, point light displays depicting the behaviour of two single dots were selected in order to make the stimuli as uncomplicated as possible while still retaining agentive motion movement cues.

Stimulus size and pattern must also be accounted for within the restricted visual presentation field. Variations in maternal tissue and the fetal position in utero mean that stimuli will not always be delivered at a consistent distance from the eye. Further, the presentation location of stimuli and the fetal field of view is constrained. Due to the need to project stimuli through to the uterus, the presentation location of the visual stimuli does not have the same level of specificity that can be achieved in vision research conducted postnatally. The target location with respect to where the stimulus is presented in relation to the fetal head is informed by the sonographer. The consequence of this lack of specificity is that the stimulus presentation is to a more generalized area when compared to the precision accorded to postnatal vision science research. Stimuli for postnatal samples are often precisely determined across an array of visual parameters, such as degree of visual angle^{37,38}. Further, maternal

anatomy and the position of the fetus may influence how the stimuli might appear to the fetus, with the curvature of the maternal abdomen and fetal position relative to internal maternal anatomy (including bones and internal organs) potentially affecting the display of the stimulus. These variables all introduce potential variation in how the fetus views and, therefore, perceives the stimuli.

Consideration must also be given to the agentive cues in the motion sequences. Specifically, there was uncertainty over whether the content was overt enough to trigger animacy perception in the fetus. Both motion sequences were first presented to an adult sample to confirm that the displays conferred the percepts of agentive or independent motion (for a full description see the supplementary methods).

Descriptions by adults of the “agentive motion” sequence contained references to functional social relationships (i.e. Dot B was trying to “catch” to Dot A, “the dot on the right is trying to catch the dot on the left so they can be friends”). Descriptions of the “control motion” sequence did not contain references to intention or agency. A key question nonetheless remains in terms of whether the current stimuli contain enough agentive motion cues for a fetus. Examining neonatal attention preferences to postnatally adapted versions of the fetal stimuli would provide further insights into the efficacy of the current stimuli in terms of inducing agentive processing.

The design of prenatal vision experiments requires the construction of paradigms that control for an array of factors within unknown parameters. Despite the inherent challenges associated with investigating animacy and agentive motion perception in the fetus, this should not deter further exploration within this domain. The current study provides an initial starting point for a wider investigation of prenatal motion processing. It has long been known that newborns indicate a sensitivity to motion cues, which forms the foundation for perceiving animacy.¹⁵⁻¹⁸ Given that newborns have inherent attentional biases towards specific motion cues^{19,20}, it is plausible that

similar sensitivities would be present in fetuses during the third trimester. Extended investigation into fetal responses to agentive and broader motion cues has significant potential to advance our understanding of perceptual and cognitive development. Future studies will be informed by the methods and findings of the present study, with speed changes, causal motion, and cues of self-propelled motion all potential research avenues to pursue. Investigating different types of stimuli (i.e. point light displays featuring a single moving dot) and the modification of current infancy paradigms will enable significant insights into fetal perception of causality^{2,15}, animacy^{16-18,39}, and perceptual processing. Understanding the fundamental building blocks of motion also illuminates early visual attention mechanisms and how these processes change during in early development.

6.4 Conclusion

This work investigated sensitivity to agentive motion cues in the third trimester. A similar number of eye movements towards the agentive motion stimuli were seen when compared to those elicited during the control motion stimuli. These results do not provide evidence of a fetal sensitivity to agentive motion cues. The results do, however, indicate that significantly more eye movements were made in the agentive condition (when a light stimulus was present) when compared to a baseline condition (where no light stimulus was present). This demonstrates that visual orienting and directed eye movements towards a light stimulus are present before birth. This research is the first to explore sensitivity to agentive motion cues in utero. As animacy perception is a precursor to understanding how we perceive the social world^{1,2} it is important to understand its earliest iterations. This study provides a foundation for continued prenatal research into the perceptual cues used to detect animate movement. With each successive experiment knowledge is gained that further enables the refinement of prenatal research methodologies. Understanding

visual preferences in utero informs our knowledge of foundational perceptual processes that may well have their origins in the prenatal period.

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6.5 Methods

6.5.1 Experimental Model and Subject Details

All pregnant participants received written information prior to study completion (compliant with HDEC ethical requirements, appendix B) and gave free and informed written consent before participation. This study was approved by the Central Health and Disability Ethics Committee (HDEC), reference number 2021 EXP 11521. All scans were conducted by one qualified and experienced sonographer at Waikato Hospital Ultrasound Clinic, New Zealand. All participants had singleton pregnancies with no known complications at the time of scanning.

The initial sample included 66 participants with 66 singleton fetuses of a gestational age between 33 and 36 weeks. Stimuli were presented by a researcher and fetal behavioural responses were recorded using 2D ultrasound. A research assistant was also present throughout the duration of the session to record experimental times to aid later data analysis.

Five fetuses were excluded due to technical errors leading to corrupt source recordings. Six fetuses were excluded because the maternal tissue thickness was greater than 50mm. Modelling suggested that tissue thickness greater than 50mm would result in significant scattering of the light stimulus and thus integrity was compromised¹. Seven fetuses were excluded due to health conditions that could affect typical responses to stimuli and three fetuses were excluded due to unsuitable positioning (i.e. their eyes could not be visualised) as they were facing the maternal spine or hip area.

At the coding stage eight fetuses were excluded. Five of these fetuses were excluded as they indicated zero eye movements throughout both experimental conditions and

appeared to be in a deep sleep state, otherwise referred to as behavioral state 1F². A further three fetuses were excluded at the coding stage for data quality, specifically “uncodable” sections. This exclusion criteria was applied if 50% or more of either experimental condition was coded as “uncodable.” An “uncodable” code was applied when the fetal eye was not visible in the ultrasound image.

The final dataset for analysis, prior to the removal of outliers, consisted of 37 fetuses (20 fetuses were female, 14 were male and 3 were sex unknown), gestational age was between 231 and 256 days (M =232. 7 days SD= 7.06). Maternal tissue thickness ranged from 9-49 mm (M= 27.17, SD = 8.47).

6.5.2 Ultrasound Acquisition

Ultrasound scans were performed using a Philips Epiq7 Elite Ultrasound System using a 5C1, or X6-1, transducer. Both systems kept TIB<0.3. Individual scans were recorded in their entirety as mp4 video files using OBS software³ and were saved to an external hard drive for offline analysis.

Images were captured in the transverse plane of the fetal face for the experimental procedure. As we were interested in eye movements specific to engaging with a specific external visual stimulus, we acquired images in the transverse plane as they provide optimal visualisation of the fetal lens and lateral eye movements in response to a stimulus presented horizontally in relation to the fetal face.

6.5.3 Stimuli

6.5.3.1 *Stimuli Development - Agentive motion perception in an adult population*

The motion sequences were constructed to accommodate the constraints of presenting stimuli in utero. Stimuli must be simple enough to be presented in utero

but still confer percepts of agentive or independent motion. The development of the motion stimuli included an experimental study that presented the “agentive” and “control” motion patterns in the form of video animations to an adult sample. The aim of this study was to examine if simple point light displays contain spatio-temporal cues that are associated with adult interpretations of agentive and non agentive motion. This study was approved by the Human Research Ethics Committee of the Division of Arts, Psychology, and Social Sciences, University of Waikato (FS2021-73) and participants gave free and informed consent prior to participation.

6.5.3.2 Participants

The adult sample of 124 individuals between the ages of 18-65 (Mean= 23, SD= 6.23). The participants were recruited through the online platform MTurk and received monetary compensation for completing the questionnaire.

6.5.3.3 Motion Animations

Participants were presented with two animated videos: an agentive video and a control video. The videos were simplified adaptations of the motion sequences of Rochat et al.⁴ “chase” study, consisting of two red dots (Dot A and Dot B) in motion on a black background. The relationship the two dots have to each other in time and space differentiates the cues of agency that the conditions confer.

6.5.3.4 Agentive Motion Animation

The agentive video (Figure 6.6), conveys a “chase” sequence that demonstrates agentive motion. In the agentive video, Dot A and Dot B appear on the same horizontal axis at the bottom of the screen, separated by a distance of 60mm. At the start of the sequence, the two dots are visible for 250ms, then participants are

presented with a black screen for 250ms. This sequence is repeated 6 times for a total of 1500ms and gives the appearance of the lights flashing on and off. At time 1500ms, Dot A begins to move in a straight linear trajectory towards the top of the screen. Dot B remains stationary in the initial start position. When Dot A has been in motion for 1250ms, Dot B begins accelerating at double the speed of Dot A at an angle of 45 degrees in relation to Dot A. Both dots finish their path of motion together on the same horizontal plane, separated by 8mm. Dot A has been in motion for 2500ms. Dot B has been in motion for 1250ms. The “agentic” animation is designed to give the appearance of Dot B accelerating towards Dot A.

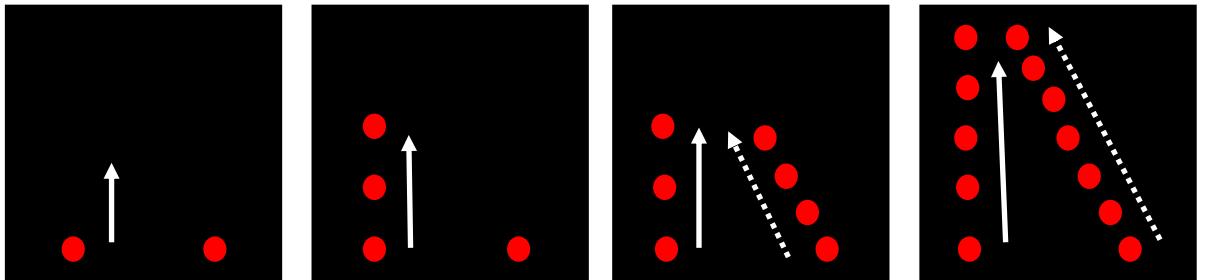


Figure 6.6 Pictorial depiction of the agentic animation used in adult sample.

Dot A is located on the left of the panel and Dot B on the right. The sequence progression starts from the panel on the left and proceeds to the right. The arrows indicate the direction of movement of the dots.

The control video (Figure 6.7) contains an “independent” motion sequence and is not intended to demonstrate agentic motion. In the control video, Dot A and Dot B appear on the same horizontal axis at the bottom of the screen, separated by a distance of 60mm. At the start of the sequence, the two dots are visible for 250ms; then participants are presented with a black screen for 250ms. This sequence is repeated 6 times for a total of 1500ms and gives the appearance of the lights flashing on and off. At time 1500ms, Dot A begins to move in a straight linear trajectory

towards the top of the screen. Dot B remains stationary in the initial start position. When Dot A has been in motion for 1250ms, Dot B begins accelerating at double the speed of Dot A in a straight linear trajectory towards the top of the screen. Both dots finish their path of motion together on the same horizontal plane, separated by 60mm. Dot A has been in motion for 2500ms. Dot B has been in motion for 1250ms.

The control video gives the appearance of Dot A and Dot B moving on parallel trajectories, independent of each other. The motion sequence in the control video is not intended to give the cues of agentic movement that the agentic video holds.

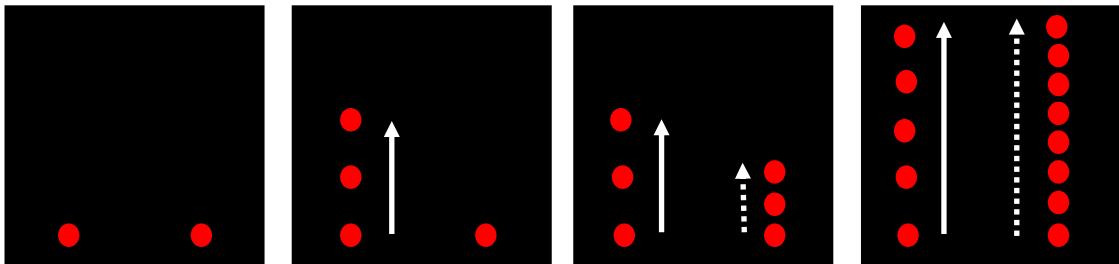


Figure 6.7 Pictorial depiction of the control animation used in control sample. Dot A is located on the left of the panel and Dot B on the right. The sequence progression starts from the panel on the left and proceeds to the right. The arrows indicate the direction of movement of the dots.

6.5.3.5 Procedure

Participants completed one online questionnaire (approximately 5-10 minutes) in which they were asked to view two short, animated videos; each video was four seconds long. Participants were able to replay the videos as many times as they liked. All videos contained two red dots (1.5mm radius) moving in different patterns across a black screen (presentation window 130mm by 130mm). After watching each animation, participants were asked to answer two questions:

Please briefly describe what you saw in the animation above (remember there are no right or wrong answers)

Please rate on a scale of one to seven how alive the motion of the dots appear to you. Where 1= definitely not alive and 7= definitely alive

The presentation order of the agentive and control videos was randomised across the sample.

We hypothesised that adult viewers would rate the agentive sequence as more animate (alive) than the control sequence and would be more likely to use agentive and chase descriptors to describe the animate sequence compared to the control sequence.

6.5.3.6 Data Coding

Participants free text responses to the question *“Please briefly describe what you saw in the animation above (remember there are no right or wrong answers)”* were coded under two separate categories: agentive motion description and a chase motion description. The coding criteria for each category are outlined below.

Agentive motion description category

A video description could receive a “1” or a “0” numerical code. A description received a code of “1” if the text referenced the dots as having agency, being animate or exhibiting goal-directed behaviour. Examples of descriptions that received a “1” code in the agentive category are:

Two dots hanging out apart, one moved away and the other wanted to join his friend so raced to the top to meet him.

The red lights are blinking on opposite sides of the bottom. The left one is chill and goes to the top slowly and the right one hustles diagonally to meet him.

The description received a “0” code if there was no reference to agentive or goal-directed movement. These forms of description were technical in nature and described the motion without ascribing internal states or goal-directed behavior to the dots. Examples of descriptions that would receive a “0” code are:

“red flashing lights moving up the screen”

“The dot starting at the left bottom corner moved steadily up the screen. The dot on the right was initially stationary and then moved diagonally towards a point horizontally adjacent to top left corner, arriving at the same time or just after the left dot.”

Chase motion description category

A video description received a code of “1” if the description referenced one of the dots as trying to chase, catch or touch the other dot. Examples of descriptions that received a “1” code are:

“right dot chases left one trying to catch it”

“Racing dots, one starts slow and the other is faster and catches him easily at the top”

A video description received a code of “0” if the description did not contain any reference to the dots trying to chase, catch or touch the other dot. Examples of descriptions that received a “0” code are:

- *“I saw the lights blink, then the left one moved upward and the other one crossed the screen diagonally”*

Two independent coders, blind to the experimental condition, assessed the qualitative data. The coders demonstrated a high level of agreement across items. In the two instances of disagreement, the coders discussed their assessments to reach a consensus, ensuring consistent and reliable coding across all data points.

6.5.3.7 Results

Agentive motion descriptions

The agentive video was described as agentive by 45.97% of participants, compared to 12.90% for the control animation. A chi-squared test revealed a significant association between video type and agentive ratings, $\chi^2(1) = 32.219$, $p < 0.001$.

Chase motion descriptions

The agentive sequence was described as a "chase" by 9.6% of participants, compared to 0.80% for the control sequence, indicating that the agentive motion video was more likely to be perceived as a chase. A chi-squared test confirmed a significant association between video type and chase ratings, $\chi^2(1) = 9.731$, $p = 0.002$.

Taken together, these results suggest that the agentive motion video was more likely to be described as having the spatio-temporal cues of both agentive motion and a chase.

Animacy Ratings

The agentive video received higher ratings of aliveness ($M = 4.5$, $SD = 1.915$) compared to the control video ($M = 4.02$, $SD = 1.867$). The Shapiro-Wilk test indicated a significant deviation from normality in the data, $W = 0.936$, $p < 0.001$. A Wilcoxon signed-rank test confirmed this difference, $z = 2.573$, $p = 0.009$, suggesting that participants rated the agentive video as significantly more alive than the control video.

Taken together, these results provide evidence that the two motion patterns were appropriately distinct and reliably perceived by participants, thereby validating their use for subsequent fetal studies. These findings suggest that the agentive sequence contains the spatio-temporal cues adults associate with agentive motion while the

control sequence does not. Therefore, using stimuli with these motion patterns would be well-suited for investigating agentive motion processing in the fetus.

6.5.4 Stimuli – Fetal Sample

Fetuses were presented with two separate point light displays (“agentive” and “control” sequences) consisting of two dots in motion (see figures 6.8 and 6.9). The relationship the two dots have to each other in time and space differentiate the cues of agency that the conditions confer.

In both conditions, the motion sequence the fetus viewed was twelve seconds in length. The fetus was initially presented with two stationary dots. At the start of the presentation, both dots flashed on and off for two seconds. In the “agentive” sequence (Figure 6.8), Dot A (located at the left of the sequence) will begin to move in a linear trajectory at a constant speed of 4.2 mm per second. When Dot A reaches a specified point half way in the trajectory, Dot B (located at the right of the sequence) will accelerate from a stationary position at a 45 degree angle towards Dot A at a speed of 8.4 mm per second. Both dots finish at the same time, closer together than at the start position, but not touching. The “agentive” sequence gives the appearance of Dot B accelerating towards Dot A. Adult viewers rate this motion sequence as animate and infer that Dot B is trying to “catch” or “chase” Dot A.

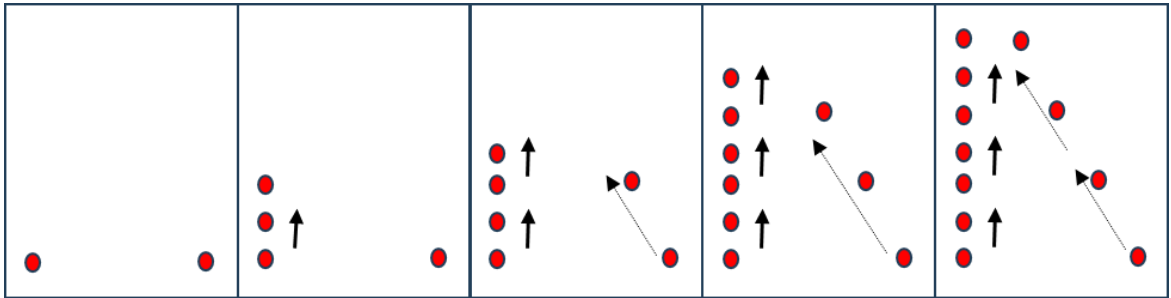


Figure 6.8 A pictorial depiction of the point light *agentive* motion sequence. Dot A is located on the left of the panel and Dot B on the right. The sequence progression starts from the panel on the left and proceeds to the right. The arrows indicate the direction of movement of the dots.

In the “control”, motion sequence (Figure 6.9), Dot A and Dot B also start from stationary positions. As in the “agentive” condition, Dot A will begin to move in a linear trajectory at a constant speed of 4.2 mm per second. When Dot A reaches a certain point in the trajectory, Dot B also begins to move at a faster speed of 8.2 mm per second. This movement, however, is in the same direction as Dot A. It does not move at an angle towards Dot A, instead following a straight trajectory. The “control” motion sequence gives the appearance of Dot A and Dot B moving on parallel trajectories, independent of each other. The “control” motion sequence does not give the same cues of agentive movement that the “agentive” sequence holds to adult viewers.

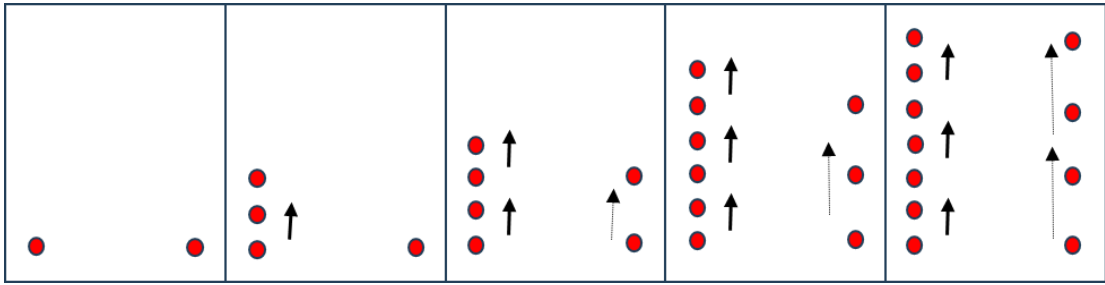


Figure 6.9 Pictorial depiction of the point light *control* motion sequence.

Dot A is located on the left of the panel and Dot B on the right. The sequence progression starts from the panel on the left and proceeds to the right. The arrows indicate the direction of movement of the dots.

At the end of both motion sequences, the two dots are removed from view. Three seconds of no light was present before the sequence of motion was repeated again.

The motion sequence was repeated eight times for a total of 120 seconds (two minutes) stimulus exposure in each condition. Between stimulus condition presentations there was a 180 second (three minute) interstimulus interval to enable the fetus to return to the initial resting, or canonical, eye position. The total duration of the experiment, including interstimulus interval, was 420 seconds (seven minutes). The condition presentation order was counterbalanced across the sample.

The stimuli for both conditions were delivered using a custom-built LED array with all 2mm dot diodes emitting at a wavelength of 650nm. All LED diodes in the array were programmed to turn on and off at specific times to give the illusion of motion between them. The agentive and control conditions each had separate LED arrays contained in a 100mm by 100mm square. The onset of the light stimuli was controlled by the experimenter. Once the experimenter started the motion sequence, the LED lights activated (on and off) at the predetermined times to give the

appearance of the specific pattern of motion for that condition. The LED array was placed on the maternal abdomen, held in place by the experimenter. The LED array was positioned so that the light stimuli moved in a horizontal orientation to the peripheral side of the fetal face.

The light stimuli could be calibrated to one of three power levels for three different levels of maternal thickness. The three levels were 0.5mV for maternal tissue measurements below 15 mm, 1mV for maternal thickness between 15-30mm and 5mV for maternal thickness greater than 30mm. The light source design and rationale for the optical powers was based on the modeling and stimulus design in Reid⁵ and supported by mathematical modelling of Isaac et al.¹. The light stimulus was presented within the predicted range of the fetal visual system at luminance that was safe for the fetal eyes.

6.5.5 Data Acquisition

6.5.5.1 Procedure

The participant was positioned in a supine position. Once comfortable the sonographer began the session by taking the fetal biometric measurements to ensure normal fetal growth and no fetal abnormalities. A set of fetal measurements including the fetal head position, placenta position, fetal biparietal diameter, head circumference, abdominal circumference, femur length, fetal weight and single deepest pocket of amniotic fluid were calculated and recorded. Once it was confirmed that fetal biometry measurements demonstrated normal fetal growth and no fetal anomalies, the experimental session would proceed.

The sonographer determined that one of the fetal eyes could be visualised and then calculated the maternal thickness measurements from the same maternal position where the stimulus was to be presented. Participants were requested not to talk

during the study in order to control the sound and movement environment across the sample. All timings were controlled via a clock and noted by a research assistant to enable later coding of offline data.

Light stimuli were presented in relation to the position of fetal head. In the event that the fetus exhibited a large amount of movement the experimental protocol would continue with the sonographer making the best attempt to obtain consistent imaging of the fetal lens and head. The experiment would not be paused and restarted to accommodate fetal movement. Large and frequent amounts of fetal movement meaning the lens and head could not be visualized would be reflected in the “uncodable” code (see coding sub heading) and could lead to exclusion of the fetus from the final dataset

The stimuli were delivered in two conditions (“agentive” and “control”) to the maternal abdomen relative to the position of the fetus (breech presentation/head up, n = 1, transverse, n=1, cephalic presentation/head down, n = 35). The ultrasound image, interpreted by the sonographer, informed the presentation parameters for the light stimuli. Stimuli were presented to the side of the visualised fetal eye (left, n = 17, right, n = 20).

The experimenter was not blind to the condition that was presented due to the requirement to present the stimulus in the correct orientation for each fetus. The administration of the stimulus was standardized across conditions by ensuring equivalent timing between conditions. This process was then repeated eight times for a total of 120 seconds.

Light stimulus onset timing was controlled via a stopwatch in view of the experimenter who was delivering the stimuli. To begin the motion pattern the experimenter would press a button on the stimulus and the LED array would

illuminate at the programmed time. This ensured that the experimenter could attend to the position of the stimulus and light exposure was standardised across conditions. The presentation of the first condition was followed by an interstimulus interval of 180 seconds, during which no light stimulus was presented, followed by the alternate stimulus condition. The first condition was then followed by the second condition. The total duration of the experiment, including the interstimulus interval, was 420 seconds (7 minutes). The presentation order for “agentive” and “control” conditions of the stimuli was counterbalanced across the sample. Participants were blind to the presentation condition.

The experimental conditions were followed by a 180 second (three minute) period in which no sound or light stimuli were presented, referred to here as the baseline. The baseline period used for analysis was taken from the final 120 seconds of this period to match the familiarisation and test trial lengths. The baseline was not measured from the beginning of the session as fetuses often displayed movement in response to adjusting to the supine maternal orientation required for the ultrasound.

6.5.6 Data Coding

Behavioural coding of the fetal ultrasound was conducted on ObserverXT software⁶. The number, duration and direction of eye movements was recorded for each trial type and the baseline. Coding was conducted for the single eye to which the stimulus was presented.

Fetal eye movements were either coded as *lateral* or *medial*. *Lateral* movements were when the fetal lens moves towards the zygomatic bone (as if the fetus were looking to the side of their face, towards their periphery) and *medial* movements were classified as a lens movement to the nasion (as if the fetus were looking in the direction of their nose). These two categories of eye movements were then collapsed to give the total eye movements for each condition.

An eye movement was defined as a lens movement from a stationary position through a position change to another stationary position. A single-eye movement is unidirectional (lateral or medial). If the lens does not reach a stationary position but changes the direction of movement (i.e., from a lateral to a medial direction), this indicates a second eye movement. The start (onset) of the second eye movement is when the directional change begins. The onset of an eye movement is when the lens movement begins, and the offset is when the movement of the lens in that direction ends, either by coming to a stationary position or changing direction of movement. Stimuli were presented at an orientation to induce lateral eye movements in the fetus. The number of eye movements in response to the stimuli was assessed using condition-blind coding of the 2D scans.

These two categories of eye movements were then collapsed to give the total eye movements for each trial type. The two categories were combined to give a comprehensive representation of fetal visual behaviour. Within the constraints of the current methods, it is not possible to ascertain a central fixation of the fetal eye or the precise location in space where the stimulus is being presented in relation to the fetal eye. As such, a medial movement (lens movement towards the nose) could still be considered evidence of engagement with the stimulus in the periphery. The combined categories provide a pattern of eye movements, with the assumption that different levels of engagement with the stimuli would produce different patterns of movement.

Two coders completed the fetal eye movement coding. Coders were blind to the experimental condition. Cohen's kappa was performed to determine interrater agreement on fetal eye movements. Cohen's Weighted Kappa was used to assess inter-rater agreement while accounting for the severity of disagreements between raters. Unlike the unweighted Kappa, which treats all disagreements equally, the

weighted Kappa gives partial credit for disagreements that are closer in value and penalizes more heavily for disagreements that are further apart) and is appropriate to use with interval data. By incorporating weights, Cohen's Weighted Kappa provides a more nuanced measure of agreement, reflecting not only whether raters agree, but also how closely their ratings align⁷. The Kappa value was calculated on the total eye movements across experimental condition. There was a substantial agreement between two coders' judgments, $k = 0.977$ $SE = 0.009$. Behavioural measurement was thus deemed to be suitable for use in the hypothesis tests in the present study.

6.5.7 Statistical Analysis

Statistical Analysis was completed using R statistical package⁸. Outliers were removed using the Tukey's method. Tukey's method identifies outliers by calculating the interquartile range (IQR) and defining any value outside 1.5 times the IQR from the first or third quartile as an outlier, providing a robust, non-parametric approach to detecting extreme values in datasets⁹. This gave a final sample of 31 singleton fetuses, gestational age between 232 and 256 days ($M = 238.33$ days $SD = 7.16$). Maternal tissue thickness ranged from 16-48mm ($M = 28.47$, $SD = 9.24$).

First, the total number of eye movements was calculated as a function of experimental conditions: baseline (Median = 6.00, $SD = 9.423$), agentive condition (Median = 13.00, $SD = 14.031$) and control condition (Median = 8.00, $SD = 14.528$). A Shapiro-Wilk test suggested a deviation from normality for all three conditions: baseline ($W = 0.865$, $p = 0.001$), agentive ($W = 0.821$, $p = 0.009$), and control ($W = 0.847$, $p < 0.001$) and therefore the non-parametric Friedman test was used to compare conditions.

A Friedman test indicated significant differences among the three conditions, $\chi^2(2) = 7.277$, $p = 0.026$. Post-hoc pairwise comparisons using Conover's test with Holm

correction showed that there were significantly fewer eye movements in the baseline condition compared to agentive ($p = 0.002$). There were no significant differences in total eye movements in the control condition compared to agentive ($p = 0.073$) or baseline ($p=0.439$).

When making a comparison of the total number of eye movements as a function of condition type (agentive and control), a Shapiro-Wilk test suggested a deviation from normality for both agentive ($W=0.905$, $p=0.009$) and control ($W=0.847$, $p < 0.001$). A Wilcoxon sign-ranked test indicated no significant differences in eye movements in the agentive condition (Median= 13.00, SD=14.031) compared to the control condition (Median:8.00, SD: 14.00), $Z=1.372$, $W=318.00$, $p= 0.173$.

Data Availability

The data reported in this paper is available upon request.

Competing Interests

The authors declare no competing interests.

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Chapter Seven

General Discussion

7.1 How does the fetus engage with the visual world?

The prenatal period marks the inception of all developmental processes. In order to understand the origins of human development, we first must understand the fetus. The aim of this thesis was to investigate fetal visual perception, guided by the central question: how does a fetus engage with the visual world, and what can that tell us about visual perception and cognition prior to birth? The fetal visual system has largely been ignored by psychological science. The last of the sensory systems to develop in utero (Graven & Browne, 2008), it was widely assumed that the fetus is shrouded in darkness, requiring no exogenous stimulation for prenatal visual development (Glass, 2002). This assumption, coupled with the inherent physical challenges of studying the fetus, has resulted in limited knowledge of fetal visual capabilities. In exploring fetal responding to visual stimuli, we aimed to address the significant gap in psychological literature, building the foundation for the emerging field of fetal visual perception.

7.2 Summary of findings

The first article in this thesis, *The role of light in prenatal visual development* (**Chapter 2**) presents a comprehensive literature review divided into four sections examining light as a critical environmental factor for both the visual system and beyond this domain to wider aspects of prenatal development. The first section has a strong biological focus and examines the components and capabilities of the fetal visual system

throughout gestation. This section establishes that by the third trimester, the fetus possesses the necessary abilities to engage with visual stimuli, while also highlighting distinct anatomical and physiological differences between the fetal and infant visual systems.

Part two of the article shifts to a mathematical perspective, detailing models of light in utero and presenting evidence that the uterine environment is sufficiently illuminated in the third trimester to allow for a visual experience. Part three of the review examines the potential role of light in shaping prenatal visual development. Animal models suggest that light during gestation is crucial for the formation of the visual system, with evidence indicating that prenatal visual experience can affect postnatal visual capacities in both animals and humans. Part four explores how light might influence sensory development and proposes that our understanding of postnatal abilities could be reappraised in the light of prenatal experiences and the uterine environment. The review concludes by asserting that the role of light is essential for understanding not only the prenatal visual system but also broader facets of early human development.

The literature summarized in **Chapter 2** also provides the foundational justification for the following experimental studies, notably in the third trimester: a.) The fetus has a functional visual system and responds to visual stimuli in utero; b.) the uterus is illuminated, enabling the fetus to have visual experiences.

Building on the review, central elements of fetal visual perception and cognition were explored in three separate experimental studies. Using a new research paradigm, informed by Reid et al., (2017), light stimuli were presented through the maternal abdomen to the fetus. 2D ultrasound visualized the fetal lens and recorded eye movements. This is a novel approach to assessing responses to visual stimuli in

utero.

The first of the experimental studies, *Visual Processing of Stimuli in the Human Fetus: A Peripheral Field Advantage?* (**Chapter 4**) investigated visuospatial processing in the fetus. Given the immaturity of the fetal central retina and the established neonatal preference for peripheral stimuli (Hendrickson, 2016; Yuodelis & Hendrickson, 1986), it was hypothesized that fetuses would exhibit a preference for stimuli presented in their temporal visual field. Eye movements in response to light stimuli presented in the temporal and nasal visual fields were recorded. The hypothesis was supported, with fetuses demonstrating more eye movements to the stimuli presented in the temporal visual field. This study represents the first attempt to quantify differential processing as a function of the fetal visual field. It highlights that the predisposition for visual field preferences is present prior to birth.

The second experimental study, *Exploring anticipatory visual responding in the human fetus* (**Chapter 5**) moves beyond simple perceptual abilities to investigate the emerging cognitive capabilities of the fetus. In this study, we show that fetuses can perform anticipatory visual gaze, specifically that fetuses can use an auditory cue to anticipate the appearance of a light stimulus. Employing a modified Visual Expectation Paradigm, we found that fetuses demonstrate eye movements towards a specific location (in which no visual stimulus was present) when they hear an auditory cue previously paired with a visual stimulus presented in that same location. This study illustrates that visual orienting and directed eye movements are present prior to birth. Further, this work provides evidence for integrative multisensory processing. Understanding visual orienting, specifically anticipatory gaze in utero, builds on our comprehension of perceptual processes while also providing insight into the foundations of higher-level cognitive functions.

The third experimental study, *Fetal responses to agentive motion cues* (**Chapter 6**) investigated fetal sensitivity to spatio-temporal cues that correlate with adult interpretations of agentive motion. Motion interpretation is a fundamental building block for social interaction (Scholl et al. 2000). The aim of this study was to investigate if there was a prenatal predisposition to attend to specific motion cues that underpin the detection of agency. A modified version of Rochat et al., (1997) “chase” study was employed in which fetuses were presented with two separate point light displays of “agentive” and “control” sequences, consisting of two dots in motion. There were no significant differences in the number of eye movements in the “agentive” compared to the “control” condition, though there were more movements in the agentive than the baseline condition. Despite the null result, this research further delineates the parameters required for fetal visual studies. It also offers guidance for future investigations into agentive motion processing in the fetus.

The three experimental studies yielded tangible insights into distinct aspects of fetal visual perception and attention. They also advance methods to directly investigate fetal visual perception via the measurement of fetal eye movements.

7.3 Theoretical implications

The research conducted in this thesis indicates that fetuses actively engage with the external visual environment. A further highlight of this thesis is the methodological advances developed throughout the studies. Positioned at the intersection of developmental psychology, medical imaging and vision science, this research provides new insights into visual processing. Fetal research to date has been primarily centred in observational studies, comparing fetal biometrics to later postnatal outcomes (DiPietro et al., 2015). The studies detailed in **Chapters 4, 5** and **6** mark a transition from observational to experimental research, thereby allowing for more

direct investigations of fetal visual processing.

7.3.1 Eye movements and experimental methods

The new methodologies explored in the experimental studies (**Chapters 4, 5, and 6**) advance research on fetal visual processing by indexing eye movements as a direct measure of fetal visual attention. Unlike previous research, which has relied on indirect measures such as head turns (Reid et al., 2017) and neural imaging (Eswaran et al., 2004b, 2005; Matuz et al., 2012; Sheridan et al., 2008), this approach provides a direct understanding of fetal engagement with visual stimuli. The ability to use eye movements as a measure in utero, broadens the methodological framework for future studies on visual perception in the fetus. This approach is particularly valuable, given the well-established importance of eye movements, gaze and looking time in infant research as critical indicators of perceptual and cognitive development (Aslin, 2007). Extending infancy research paradigms to fetuses provides new avenues for the study of early perceptual and cognitive development. The capacity to (a) present visual stimuli through maternal tissue via projected light and (b) precisely measure fetal visual behaviour via ultrasound, has the consequence that fetal research can employ modified visual methodologies and procedures similar to those seen in the infancy domain.

In the current experimental research outlined in **Chapter 4,5 and 6** the two categories of eye movements were then collapsed to give the total eye movements for each trial type. The two categories were combined to give a comprehensive representation of fetal visual behaviour. Future research could look at differentiating eye movements into a single direction on movement, increasing the precision of conclusions related to specific spatial locations.

7.3.2 Fetal Awareness - the fetus engages with the exogenous visual stimuli

The research conducted in this thesis indicates that fetal eye movements in the third trimester can be guided by exogenous visual stimuli. In the experimental studies, differential responses were observed across both experimental conditions (**Chapters 4, and 5**) and in comparison to baseline conditions (**Chapters 4, 5, and 6**). If eye movements were random and not affected by the external environment, differential patterns of eye movements to light stimuli would not be observed. A global conclusion from this work is that fetuses can respond to visual changes in the environment and that the fetus actively engages with external visual stimuli. This finding supports the earlier behavioral (Donovan et al., 2020; Kiuchi et al., 2000; Reid et al., 2017) and neurological research (Eswaran et al., 2002, 2004b; Matuz et al., 2012; Sheridan et al., 2008) that indicated prenatal populations exhibit visual processing abilities. This is significant because it not only provides evidence of fetal awareness but also suggests that if the fetus can perceive and respond to changes in the external uterine environment. It is plausible, therefore, that this environment can, in turn, exert an influence on fetal development.

The behavioural capacities of the fetal samples in the experimental chapters of this thesis contrast with a dominant position in prenatal literature which holds that the fetus is never “awake” or aware (Mellor et al., 2005; Padilla & Lagercrantz, 2020). and that the very nature of the uterine environment means that the fetus is held in an unconscious sleep state for the duration of pregnancy (Mellor et al., 2005). Under this assumption the fetus is unable to engage with, or perceive, the uterine environment (Lagercrantz, 2014; Mellor et al., 2005; Padilla & Lagercrantz, 2020). A downstream implication of this framework is that any visual system development is endogenously driven. This position is incongruent with the findings of the three experimental studies that demonstrate active visual engagement with exogenous light

and sound stimuli.

The results of current research must also be contextualized within the broader field of fetal awareness. While the current experiments are some of the first to examine direct measures of visual perception, they are not the first prenatal studies on fetal sensory perception (Hopkins & Johnson, 2005). There is a diverse range of evidence that the fetus is aware and engages with the prenatal environment across different sensory modalities (Hopkins & Johnson, 2005). The current research contributes to the growing body of evidence that emphasizes the necessity of considering the fetus as an active participant in the perceptual development of the self. Even though the precise mechanisms and parameters of fetal awareness remain unknown, it is evident from the current experimental work that the fetus in the final trimester at least (??not sure if worth including) cannot simply be dismissed as “unconscious” and “unaware”.

7.3.3 Origins of Human Psychological Development

Developmental biology has often attempted to understand the roles of experience and genetics in the development of sensory systems (Johnson & de Haan, 2015). Prior studies on visual preferences at birth have been attributed to genetic predispositions (DeCasper & Spence, 1986; Johnson & Morton, 1991) although a number of counter arguments have suggested that rapid postnatal learning, such as filial imprinting, could explain these visual preferences. The current research clearly shows that postnatal experience is not needed for the presence of differential visual perception.

This finding is of interest to broader debates in developmental psychology concerned

with the origins of human cognitive, social, and linguistic abilities. In these literatures, perspectives are primarily articulated along a range of nativist and constructionist views. Nativists argue for the existence of innate systems or mechanisms guiding development (Spelke, 2022), whereas constructionists emphasize the role of dynamic, multidirectional interactions between experiences and the individual at genetic, neural, and behavioral levels (Mareschal et al., 2007). Birth has often been considered the start of development, with neonatal abilities evidence of innate systems (Hopkins & Johnson, 2005). This argument could be extended to the fetus, with the preferential responding in the temporal field (**Chapter 4**) and anticipatory gaze (**Chapter 5**) offered as support of innate visual processes. As fetal visual experience is considered limited (especially in comparison to the postnatal environment) these fetal abilities demonstrated in the experimental could be interpreted as evidence of a system of knowledge present in the fetus that is functional on the first effective visuo-sensory encounter. Fetal abilities could be considered excellent evidence for nativist arguments. However, to definitively take this position, no exogenous visual experience must occur prior to birth and the uterine environment is not involved in shaping the fetal visual system. This conclusion must be reconsidered given the evidence present from **Chapter 2**, which demonstrates that the prenatal environment—including the illumination of the womb in the third trimester (Del Giudice, 2011)—can influence the development of the visual system through various pathways. To suggest that visual development in the prenatal period is purely the result of endogenous stimuli such as retinal waves (Spelke, 2024) is not easily reconciled with the body of literature discussed in **Chapter 2**. Studying fetal abilities enriches and extends the nativist/constructionist debate and offers new insights into the two theoretical stances. Determining the relationship between endogenous and exogenous visual stimuli in shaping the fetal sensory system will require further work. Irrespective of this need, it

is evident from **Chapter 2**, that the prenatal environment cannot be discounted. What is also clear from the experimental research in the current thesis (**Chapters 4, 5, and 6**) is that fetal experiences in utero must be considered. To understand the origins of development we must first begin with the fetus.

7.4 Practical Implications

The work presented in the current thesis offers exciting insights into early psychological development. There are also practical downstream implications for clinical applications. As previously noted, the study of the fetal visual system is an emerging field. Mapping fetal visual development can provide guidance into typical timelines and milestones during the third trimester. This knowledge can be used to better identify atypical developmental patterns and tailor prenatal care to the specific needs of the developing fetus (Hopkins & Johnson, 2005). Further, comprehensive knowledge of fetal development can inform public health policies aimed at improving maternal and child health, such as, prenatal care standards, and early intervention programmes. Educating expectant parents about fetal sensory development can promote healthier behaviours and environments that support optimal fetal visual development, leading to improved outcomes for both the fetus and the mother (Reissland & Kisilevsky, 2016).

An important application of fetal visual perception research lies in therapeutic interventions designed to support premature infants' sensory and neurological development. For premature infants, replicating the sensory environment of the womb in neonatal intensive care units (NICUs) could promote healthier development (Graven, 2000). It should be acknowledged that current guidelines are present, but further refinement informed by our increased knowledge of fetal visual development could lead to improvements and enhanced health related outcomes.

A frontier of neonatal care is partial ectogenesis. Partial ectogenesis is when a period of gestation occurs in an artificial environment outside of the human uterus (Kozlov, 2023; van der Hout-van der Jagt et al., 2022). Even though partial ectogenesis might appear to be in the realm of science fiction, the US Food and Drug Administration (FDA) is advising on the ethics of trialing artificial wombs for humans (Kozlov, 2023). This consideration is an acknowledgment that partial ectogenesis has the potential to dramatically change survival outcomes for premature babies (Kozlov, 2023). For partial ectogenesis to be successful, it will require the development of an artificial womb where the fetus can grow, helping to bridge the gap between premature birth and full-term development (Kozlov, 2023). Knowledge of fetal sensory systems and environmental requirements will be central to this process. In order to develop an artificial womb, scientists will need to know (amongst many variables) the environmental light requirements and sensory capabilities of the typically developing fetus (van der Hout-van der Jagt et al., 2022). Understanding how fetal visual development interfaces with the uterine environment will be an important consideration when developing these processes. This research demonstrates that light entering the womb has the potential to influence fetal development.

Understanding fetal sensory development is essential for advancing prenatal and neonatal care. This knowledge could inform predictive models of developmental trajectories, leading to personalized and effective healthcare strategies that support early intervention for potential delays or disorders. By integrating these experimental research insights into healthcare practices and policies, it may well be possible to enhance the overall well-being of both infants and parents (Graven & Browne, 2008).

7.5 Limitations

Fetuses are an inherently difficult population to study (Dunn et al., 2015). There are clear challenges and constraints when developing and implementing fetal research paradigms. Each experimental chapter (**4**, **5**, and **6**) contains limitations specific to that individual study. In this section, we will discuss the broader limitations that underpin our experimental approach.

The parameters of fetal attention and fatigue are not currently established. In the experimental research (**Chapters 4, 5, and 6**), protocols were designed with a conservative approach. The emphasis was on paradigms being brief and simple while still allowing the fetus enough time to process and respond to the stimuli.

Implementing a paradigm where the population cannot process stimuli due to fatigue is unproductive and would produce a false negative outcome. It is for this reason that all current experiments used a maximum of two experimental conditions and were designed for brief session times. We saw differential responses to stimuli, which indicates that the current paradigms are within the bounds of what is appropriate for the fetal population. However, a more nuanced understanding of fetal visual attention will help optimize experimental paradigms, enabling studies featuring multiple stimuli or more complex methods. Until such time as the capacities of the fetus are known, experiments that are simple and rapid are more likely to yield significant results.

Discussions of fetal attention often involve the quantification of fetal behavioral states and sleep patterns. From 32 weeks gestation, fetal behavioral states have been categorized into four distinct patterns—quiet sleep (1F), active sleep (2F), quiet awake (3F), and active awake (4F)—based on heart rate, body movements, and eye activity, reflecting varying levels of arousal and sleep-wake cycles (Nijhuis et al.,

1982). Fetuses, like newborns, spend a significant amount of time in a sleep state (Mirmiran et al., 2003). It is known, however, that the periods of 'quiet awake' and 'active sleep' periods remain significant (Brändle et al., 2015). It is reasonable to expect an increased likelihood of responding during the 3F and 4F states. Consequently, testing fetuses during sleep states (1F and 2F) is likely to be less conducive to active engagement and response.

In acknowledgment of fetal behavioral states, one of the exclusion criteria in the experiments in this thesis required the removal of those fetuses who showed zero eye movements across baseline and experimental conditions. This criteria was based on a 1F classification, the fetal state analogous to deep sleep where the fetus is largely unresponsive and therefore would not be expected to attend to visual stimuli (Kiuchi et al., 2000; Nijhuis et al., 1982). It should be recognized that zero eye movements across the entire experimental protocol are a low exclusion threshold. Is a fetus who exhibits one eye movement across all conditions in a different arousal state from a fetus that exhibits zero eye movements? For the current research, exclusion criteria needed to be set, but the current operational definition introduces possible floor effects that could be better mitigated with clear measures of fetal behavioural states.

Further, across the three experiments there were a large number of null responses in experimental conditions. There are several ways these responses could be interpreted: a.) the fetus is in an unconscious, deep sleep state (Nijhuis et al., 1982), b.) the fetus is simply not perceiving the stimuli because of the constraints on the visual system c.) the visual stimuli are not being presented within the fetal field of view. Improved methods of determining fetal behavioural states will allow for a more precise interpretation of the visual responses.

Fetal behavioural states have been determined by observational research, in the

absence of external stimulation (Nijhuis et al., 1982). In contrast to observational studies, the experiments described in this thesis measure eye movements in response to the onset and offset of light (and in the case of the anticipatory gaze study (**chapter 5**), light and sound stimuli). A question pertinent to this research is whether external visual stimuli can shift behavioural states? Specifically, can a light source arouse the fetus or shift a behavioural state to one of increased wakefulness? Previous research by Kiuchi et al., (2000) suggests that a visual stimulus (photic flash) can shift fetal states to a more “wakeful” 3F and 4F states. The shifting of behavioural states is more likely to occur between 3F to 4F states than 1F or 2F. Refining the quantification of fetal behavioral states and the boundaries between sleep and wakefulness states will enable us to improve fetal research models.

In infancy research, attention grabbers, often visual and auditory pairings such as a dancing elephant (Pyykkö et al., 2019), are frequently employed to ensure that a participant is focused on the experimental stimuli. In **Chapter 6**, flashing lights were introduced at the start of the experimental protocol to capture fetal attention. However, alternative methods may be more effective for the fetal population. Vibroacoustic stimulation, as shown in Kiuchi et al., (2000), can shift behavioral states, increasing arousal regardless of the initial fetal state. Including an initial vibroacoustic stimulation in the protocol could be an appropriate method to ensure that the fetus is in an optimal state of awareness for experimental participation.

A better understanding of fetal attention parameters will facilitate more complex experimental designs, enabling the exploration of multiple conditions and intricate stimuli. Quantifying behavioral states will also facilitate a more accurate interpretation of the behavioral responses. The simplicity of current studies reflects the developing nature of research in fetal visual perception. Future experimental research will enhance our ability to define the limits of fetal visual attention and

enable the development of more sophisticated experimental designs.

Administering visual stimuli to the fetus entails distinct challenges not typically encountered in postnatal visual research. Visual stimuli cannot be presented directly to the fetus. Instead, as we see in the current experiments, light stimuli are presented to the mother's abdomen and must pass through the maternal tissue to reach the fetus. A detailed review of these challenges is outlined in the discussion section of **Chapter 6** and is summarised below.

The stimuli used in this research are constrained in size and complexity due to the need for transabdominal presentation. The primary consideration is how the stimulus will appear when projected through the uterine wall, as light scattering through maternal tissues causes the stimuli to blur and lose definition. To address this, simple point-light displays of a maximum of two dots were chosen.

Additionally, the stimulus size and pattern must be tailored to the limited visual presentation field, which is affected by variations in maternal tissue and fetal positioning. The distance from the fetus's eye to the stimuli is also inconsistent, and the presentation location is less precise than in postnatal research. The stimuli are delivered to a generalized area based on sonographer guidance. This is in significant contrast to the specific visual parameters used in postnatal studies. Moreover, maternal anatomy and fetal positioning may influence how the stimuli are perceived, as factors such as the curvature of the maternal abdomen and internal anatomical structures may well alter the display. These variables introduce potential variations in any fetal visual experience or perception of the delivered stimuli.

The challenges and limitations of the current studies provide opportunities to further refine and advance the field of fetal visual perception. Fetal visual perception is an emerging field and with each successive experiment we learn more. The three

experimental studies and their research paradigms should be viewed as initial starting points for further research.

7.6 Future Directions

The possibilities for future research are wide ranging. The fetal responses observed in the experimental studies indicate that infancy paradigms can be successfully adapted and applied to a fetal sample. This approach opens up a multitude of opportunities to explore fetal development through well-established rigorous methodologies present in infant research.

Future investigations into fetal visual perception can significantly enhance our understanding of early sensory development. Initial studies, such as the exploration of visuospatial processing, can be further extended to accurately quantify the parameters of the fetal visual field. Investigations into fetal preferences for different coloured stimuli, such as orange vs red, and their relationship to retinal development, will provide deeper insights into the maturation and functionality of the visual system. Basic work related to visual capacity is required, such as the processing of luminance. The anticipatory gaze study (**Chapter 5**) demonstrated evidence of multisensory processing in the fetus, highlighting the integration of auditory and visual stimuli required to perform anticipatory gaze. Adapting infancy experimental paradigms integrating auditory, visual, gustatory, and tactile stimuli, such as those used by Orioli et al.(2018), Bremner, (2017) and Steri et al. (2012), will serve to extend our understanding of cross-modal processing in utero.

Perceptual research is crucial for clarifying the capabilities of the developing visual system and informing the design of optimal visual stimuli. By mapping out the fundamental parameters of the visual system, researchers can better explore how

perceptual abilities guide the development of more complex cognitive functions, offering a comprehensive view of fetal sensory development.

Eye movements provide a valuable index for investigating higher cognitive functions (Aslin, 2007). The results from the anticipatory gaze study (**Chapter 5**) indicate that fetal visual research can extend beyond the assessment of perceptual abilities to explore emergent cognitive capacities. Future research could explore fetal habituation, memory, paired preferences, and engagement with both motion (both agentive and causal). For example, there is evidence of numerical understanding in fetuses, previously demonstrated through auditory tones (Schleger et al., 2014), suggesting the potential for similar investigations using visual stimuli, such as presenting one, two, or three lights and tracking eye movements to determine differentiation between conditions. These extensions would enhance our understanding of fetal cognition and contribute to a broader knowledge of foundational psychological abilities.

Future experimental studies could consider the inclusion of sham stimuli conditions. In Chapter four, the inclusion of a condition that included physical touch (moving the light stimulus probe along the maternal abdomen with no light present). Chapter five experiment could have a separate condition where the sound/light pairings in an unpredictable sequence (i.e. not simply light present or light absent). Inclusion of these conditions could provide further support for results being in response to the experimental conditions and not general arousal.

Tracking fetal eye movements provides a direct measure of fetal visual attention. Incorporating additional behavioral measures (i.e. fetal heart rate monitoring) and neural imaging techniques (i.e. fetal magnetoencephalography and fetal functional magnetic resonance imaging) can build a comprehensive understanding of fetal visual

perception. Advances in neural imaging technology facilitate a deeper exploration of how fetal visual perception interfaces with the establishment and development of neural structures, ultimately enhancing our understanding of cognitive development precursors. By applying paradigms from current experimental studies, projecting patterned light stimuli through maternal tissue, and recording the resulting neural responses as evoked visual potentials, it would be possible to uncover the origins of occipital lobe activity and its connections with the frontal cortex. The detection of rudimentary neural signatures in the fetus, which, despite being delayed in latency compared to neonates, has the potential to demonstrate the foundations of early cognitive development.

A comprehensive understanding of developmental trajectories requires longitudinal research. Investigating nascent visual perception across gestation and bridging the prenatal and postnatal periods (trans-natal research) will provide critical insights into early developmental processes and, in turn, help disentangle the relationship between prenatal experiences and subsequent developmental outcomes. By tracking individuals from the prenatal stage through various postnatal stages, researchers can identify how early biological and environmental factors influence both the visual system and psychological processes across the lifespan. This approach not only clarifies the continuity and change in development but also informs targeted interventions that can promote optimal developmental trajectories from the earliest stages of life. It must be acknowledged that longitudinal research is expensive; thus, a cross-sectional research is a logical approach for an initial investigation of developmental capacities.

7.7 Conclusion

The research conducted in this thesis suggests that even before birth, fetuses have

emerging visual perception abilities that could form the foundation for later social interactions and cognitive functions. By mapping experimentally induced visual responses in utero, this work enhances our understanding of how the developing visual system interacts with the uterine environment. The three experimental studies provide some of the first insights into the parameters of the prenatal visual system and relationships with cognitive function. They also advance our understanding of appropriate methodologies for fetal visual research paradigms. In utilizing eye movements as a behavioural index, this research has not only contributed to understanding the parameters of fetal visual perception but also established critical groundwork for future investigations into visual processing in the fetus. The experimental framework for exploring social-cognitive development is possible in the fetal period. This thesis challenges us to reconsider how we understand postnatal visual attention and preferences, suggesting that they must be viewed in the context of prenatal experiences and fetal abilities. There is compelling evidence to move from simply asking *do* prenatal experiences shape neonatal visual preferences, to *how* do prenatal experiences shape visual perception. The results of this thesis suggest that prior to birth the fetus is already an active participant in their perceptual development. The foundations of perceptual, social, and cognitive development are established in the prenatal period. To truly grasp the origins of these functions, we must now ask: what does the fetus know?

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Appendices

Appendix A NPK Coding Manual - Fetal Sonography Scans

NPK Coding Manual - Fetal Sonography Scans

Sonography Scan Orientation

Start: Orientate to the scan you are looking at

- 1.) Identify the orientation of the scan (transverse or parasagittal)
- 2.) Identify the orbital socket and which eye you are viewing (left or right).
- 3.) Identify the lens (white dot in the orbital socket). The point/corner of the lens is the most consistently visible and best to use as the point to code from. View the lens descriptions for further information on visualising the lens.
- 4.) When viewing in the *transverse* section
 - a.) Identify the **nasion** (also known as the bridge of the nose), the midline bony depression between the eyes where the frontal and two nasal bones meet, just below the glabella.
 - b.) Identify the **zygomatic bone** (the bone that forms the prominent part of the cheek and the outer side of the eye socket)
- 5.) Viewing in the *parasagittal* section
 - a.) Identify the **nasal bone**
 - b.) Identify the **zygomatic bone** (the bone that forms the prominent part of the cheek and the outer side of the eye socket)

c.) Identify the **supraorbital foramen**, a bony elongated opening above the orbit (eye socket) and under the forehead. It is part of the frontal bone of the skull.

6.) Familiarise the other anatomical markers, including:

- a.) Maternal tissue
- b.) Amniotic fluid
- c.) Placenta (if visible)
- d.) Ear (if visible in transverse section)
- e.) Mandible (in parasagittal section)
- f.) Spine (in parasagittal section)
- g.)

Fetal Lens

The fetal lens movement is used to code an eye movement. The lens appearance is inconsistent and can change depending on the angle of incidence in which the eye is being scanned.

Figure 1: Anatomical detail of the fetal eye that can be obtained on ultrasound

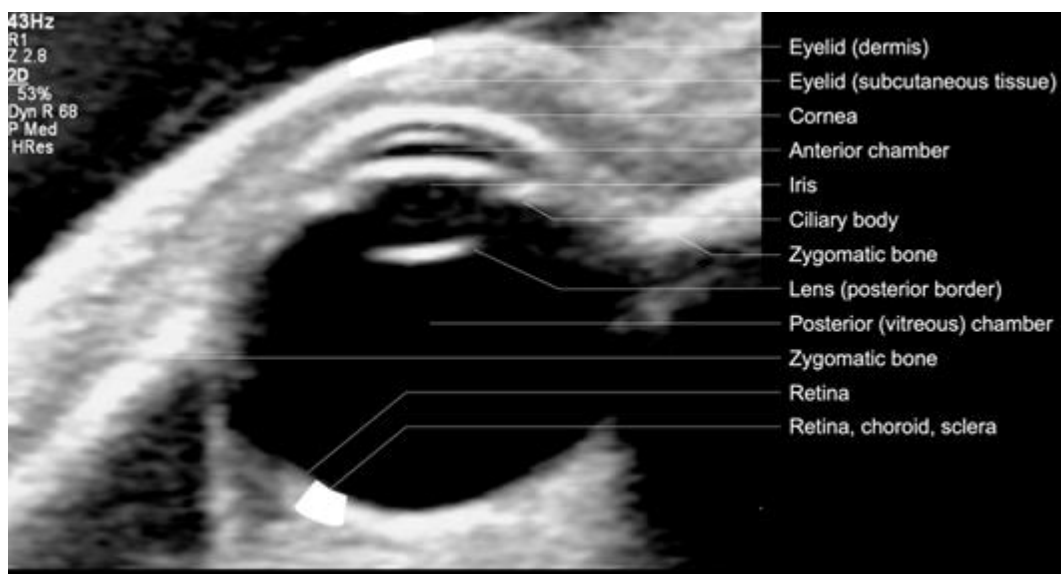
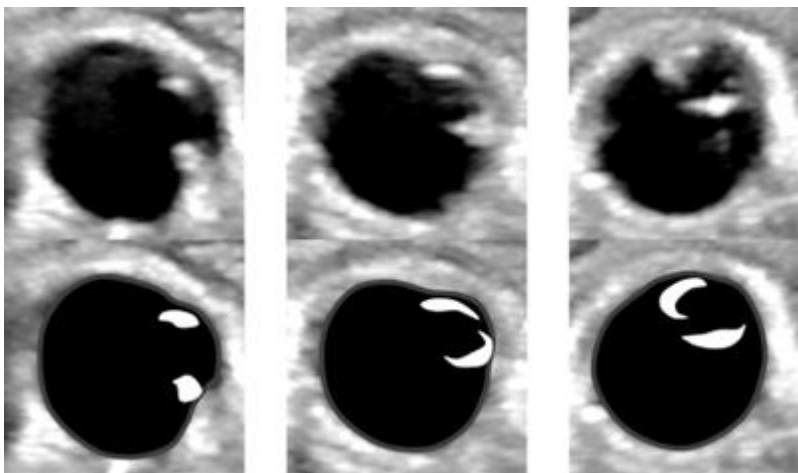


Figure 2: The effect of the angle of incidence on the visualisation of the lens during fetal eye movements. Ultrasound images (top) with corresponding diagrammatic representation (bottom).



Coding Eye Movements

General Definition of an Eye Movement (EM)

In its simplest form, an EM is defined as a lens movement from a stationary position through a position change to another stationary position. A single-eye movement is in one direction.

In some instances, the lens does not reach a stationary position but changes the direction of movement (i.e. from a lateral to a medial direction). A directional change indicates a second eye movement. The start (onset) of the second EM is when the directional change begins.

The onset of an EM is when the lens movement begins, and the offset is when the movement of the lens in that direction ends.

When coding an eye movement, consideration must also be given to the following:

- the direction of the lens movement
- the movement of the fetal head
- the movement of the sonography probe

Direction of EM

Coding an eye movement needs to include the direction of the lens movement within the orbital socket.

- 1.) The initial stationary lens is the reference point. This reference point informs what directional EM codes are applied to the movement.
 - a.) There are two eye movement direction codes (*lateral* and *medial* for the transverse section and *up* and *down* for the parasagittal).

Coding EM in the Transverse Plane

In the transverse plane, the direction of lens movement is coded as either lateral or medial.

Lateral movement is when the eye moves from the stationary position towards the zygomatic bone (side of the face).

Medial movement is when the lens moves from the stationary position towards the nasion point (towards the nose).

Coding eye movements in the parasagittal plane

In the parasagittal plane, the direction is noted as either an inferior or superior movement.

Superior movement is when the lens moves from a stationary position towards the supraorbital foramen (towards the forehead, top of the eye socket, i.e. looking up).

Inferior movement is when the lens moves from a stationary position towards the zygomatic bone (towards the nose, the bottom of the orbital socket, i.e. looking down).

Time of lens movement

- There are no time qualifiers for eye movement. It is simply the movement of the lens and can vary across participants and conditions.

Distance of lens movement

- No distance qualifiers exist for eye movement. The movement of the lens should be easily distinguished by the human eye. Generally, eye movements are easily distinguishable. If you need clarification, code the EM as best possible and make a note in the comments section for review.

Eye movement and Fetal Head Movement

When coding an EMs, consider fetal head movement.

When coding an eye movement, if there is:

No head movement (the nasion point is stationary): An EM is when the lens moves from one stationary position to another.

The head is moving in the same direction as the lens (nasion point is moving): To qualify as an EM, the lens must move faster than the movement of the nasion point

Head is moving in the opposite direction to the lens: Possible fixations - lens stationary in one place, but the fetal head is moving (note this in the comments

section as possible fixation), no EM is coded in this instance, but a head movement will be.

Further eye movement coding considerations

Eye movement out of plane - Due to the angle of the ultrasound the lens can move out of the plane of view, meaning a second stationary point (a qualifier for an EM) is not visible. The lens can then come back into view, sometimes in the same position it was last visible but sometimes in a different position to where it was last seen.

This is still classified as an eye movement. The eye movement ends (offset time) when the last point of the lens is visible.

If this occurs, please also note it in the “comments” column for review.

Fetal Head Movements

Coding head movements in the transverse plane

In the transverse plane, the direction of head movement is coded as either left or right. The direction of movement is in relation to the fetal body (i.e. head moves to the fetus's left). This is why it is important to orientate the scan.

- Left movement is when the head moves from the stationary position towards the left of the fetal body
- right movement is when the head moves from the stationary position towards the right of the body.

Coding head movements in the Parasagittal Plane

In the parasagittal plane, the direction of head movement is noted as either flexion or extension.

- *Flexion* is when the head moves from a stationary position towards the chest (i.e. looking down)
- *Extension* is when the head moves from a stationary position away from the chest (i.e. looking up).

Other Fetal Movements

Tonic movement

Tonic movement is a repetitive and consistent movement where the whole fetal body is moving as one. This can consist of a gentle rocking motion, often due to maternal breathing. This is something to be aware of when coding head and eye movements. Other repetitive motions, such as hiccups and thumb sucking, can also be seen in some fetal scans and should be considered when coding head and eye movements.

NB: If you are unsure if a movement is EM/HM or simply tonic movement, note this in the comments section for review

Un-codable data

There are two un-codable codes. They are un-codable lens and un-codable head. It is important to qualify the amount of un-codable data for each participant.

Uncodable lens: this code is noted if the lens is not visible in the orbital socket.

There are multiple reasons the lens might not be visible:

- Fetus has moved their head and or body, meaning that the lens or orbital socket is no longer visible
- The sonography probe has moved (this can occur simultaneously with a fetal head/body movement).

- The fetus has moved a limb in front of the eye (hand, arm, foot), blocking the view of the orbital socket.
- Discretion can be applied to the un-codable lens code. Tonic movement causes the lens to consistently moves in and out of view. If the lens always remains in the same place, you do not need to code this as un-codable. Please note, however, in the comments that there is tonic movement.
- If the lens is not visible for under **one second** but is in the same position, you do not need to code that as un-codable lens

Uncodable head: this code is used if you can not code a head movement (and therefore can not code an eye movement). Uncodable head is used when the fetus is moving around, rapidly changing position, or has moved to a position where their head is not visible or cannot be clearly distinguished.

- Note the whole head does not need to be in view. Anatomical markers including the fetal ear, zygomatic arch and frontal orbit can be used to determine head movement direction.

General Coding Notes:

Coding General

- € Use the “comments” column. If you see anything unusual or are unsure which code to use, note it in the comments. You can also use the comments column to make general notes about the scan (i.e. tonic rocking, fetus moved arm above face etc.).

Videos:

- € Remember to listen to the videos on **mute**. This is so coding remains blind. The presentation conditions are spoken about in the video as the researcher and sonographer orientate the probe and stimulus.
- € Speed of the video can be adjusted (refer to the Coding in Observer XT Manual)

- € Videos are stored on the NPK external hard drive in a file labelled “AVI - SONO” ensure that you use these files as they are in the correct format (codec and container) for use in ObserverXT.

Coding in ObserverXT

Help Manual Link:

Reference Manual is located on the desktop labeled: Reference Manual - The Observer XT 16 Help

If you encounter issues with Observer XT when coding, remember to add them the ObserverXT log sheet (NPK drive > “Observer XT Log”) and let Jess know.

Appendix B

Participant Information and Consent Forms



Participant Information Sheet

Fetal Visual Responding

Lead investigator: Professor Vincent Reid

Contact phone number: +64 7 838 4080 extn 9222

Ethics committee ref.: 2021 EXP 11521

Kia ora and thank you for taking the time to read through our information sheet.

You are invited to take part in a study on fetal visual perception. Whether or not you take part is your choice. If you don't want to take part, you don't have to give a reason, and it won't affect the care you receive. If you do want to take part now, but change your mind later, you can pull out of the study at any time up to and during the study appointment and up to fourteen days after you have taken part in the study.

This Participant Information Sheet will help you decide if you'd like to take part. It sets out why we are doing the study, what your participation would involve, what the benefits and risks to you might be, and what would happen after the study ends. We will go through this information with you and answer any questions you may have. You do not have to decide today whether or not you will participate in this study. Before you decide you may want to talk about the study with other people, such as family, whānau, friends, or healthcare providers. Feel free to do this.

If you agree to take part in this study, you will be asked to sign the Consent Form on the last page of this document. You will be given a copy of both the Participant Information Sheet and the Consent Form to keep.

This document is 9 pages long, including the Consent Form. Please make sure you have read and understood all the pages.

VOLUNTARY PARTICIPATION AND WITHDRAWAL FROM THIS STUDY

Your participation in this study is voluntary, and that you may withdraw from the study at any point before, during and up to two weeks after participation, should you wish to do so. You do not need to explain why you have made this decision, and there will be no adverse consequences for you.

You will be asked before ultrasound scanning begins whether you are happy to take part and you will be asked to sign a consent form. Should you wish to withdraw from this study, any data collected will be destroyed and not used in the data analysis.

WHAT IS THE PURPOSE OF THE STUDY?

The aim of the investigation is to determine whether we can use low levels of light to show a number of shapes to fetuses and record how their eyes move in response to the movement of these shapes.

Babies in the womb are used to low levels of light, though will have never before have seen shapes from outside the womb like we will be presenting to them.

When a baby is first born, they prefer to look at certain types of movement, will look to where they think an object might appear, and will respond more to shapes presented to the sides of their focus. We want to understand if babies have these preferences and abilities before they are born. This will help us to understand whether having experience of seeing objects after birth is required for these preferences. It is also possible that we will see these preferences before babies are born.

HOW IS THE STUDY DESIGNED?

This study is taking place in the Waikato region. We aim to recruit 120 participants including 120 fetuses of a gestational age between 33 and 36 weeks. This study will not be investigating twins or triplets.

WHO CAN TAKE PART IN THE STUDY?

Pregnant people who are pregnant with one fetus aged between 33-36 weeks gestation. To be eligible to participate, mothers will have experienced a routine pregnancy with no known complications and, at the start of the pregnancy, have a BMI of 30 or less. Maternal BMI needs to have been below 30 to allow for the light stimulus to travel through the maternal tissue to the fetus.

WHAT WILL MY PARTICIPATION IN THE STUDY INVOLVE?

Participation in this study will involve attendance, on an appointment basis, at one 2D ultrasound scan at approximately 34 weeks gestation. While the scanning is taking place we will present light and sound to your baby and observe their eye movements. We will also take some measurements of your baby so we can see if different sized babies respond differently at this stage of development.

Study participation will last around 60 minutes in total.

Information regarding the scan

The scan that we will use is a 2D scan. With 2D ultrasound we are able to see your baby's eyes and how the eyes move. There is no anticipated harm as a consequence of this study, where routine prenatal care will remain unchanged with the addition of 2D imaging procedures. Ultrasound exposure is not to be known harmful to the unborn child (Abramowicz, 2007). In case your baby is inactive, you may be asked to walk around for 15

minutes and then will return to be scanned. When your baby is born, we will access your patient notes for information regarding your delivery method and details of you and your baby's health at birth

Information regarding the light

We will shine a red light on your abdomen. We will do this after making calculations to determine how bright the light should be given the distance between the light and your baby. This will be an individually tailored level of light, as every person is different. The amount of light that will reach your baby will be the same as if you were sunbathing outside on a clear day, without clothes. This level is deliberately set so that it is bright enough that your baby can see it, but not so bright that it will be unpleasant for your baby to look at.

Information regarding the sound

We will play a simple tone through speakers close to, but not touching your abdomen. There is no anticipated harm to your baby, as guidelines for the sound levels appropriate for babies in the womb will be followed. The American Academy of Paediatrics Committee on Environmental Health has recommended safe sound levels (Committee on Environmental Health, 1997). These 1997 recommendations have been updated by an expert team of practitioners and broadened to include fetuses (Graven, 2000). The recommendation for maximum decibel levels and method of delivery of auditory sounds will be carefully followed.

WHAT ARE THE POSSIBLE RISKS OF THIS STUDY?

There is no risk to you or your baby from taking part in this study.

Should the sonographer find unexpected results during the scan, normal procedures of the clinic will then take place, overriding the procedures of this study. You may be informed of the abnormality, your lead maternity carer, or GP, may be contacted and you may be referred, if necessary to the appropriate clinical team. The study will finish immediately to allow these procedures to take place.

WILL ANY COSTS BE REIMBURSED?

You will receive a \$20 petrol voucher acknowledging the transportation costs of getting to and from the study location. You will also receive a \$10 Warehouse voucher as a thank you for taking part in the study.

WHAT IF SOMETHING GOES WRONG?

If you were injured in this study, you would be eligible **to apply** for compensation from ACC just as you would be if you were injured in an accident at work or at home. This does not mean that your claim will automatically be accepted. You will have to lodge a claim with ACC, which may take some time to assess. If your claim is accepted, you will receive funding to assist in your recovery.

If you have private health or life insurance, you may wish to check with your insurer that taking part in this study won't affect your cover.

WHAT WILL HAPPEN TO MY INFORMATION?

During this study the researchers and sonographer will record information about you and your study participation. This includes the ultrasound scan and the demographics questionnaire.

Identifiable Information

Identifiable information is any data that could identify you (e.g. your name, date of birth, or address). Only members of the research team will have access to your identifiable information.

De-identified (Coded) Information

To make sure your personal information is kept confidential, information that identifies you will not be included in any report generated by the researchers. Instead, you will be identified by a code. The researcher will keep a list linking your code with your name, so that you can be identified by your coded data if needed.

The results of the study may be published or presented, but not in a form that would reasonably be expected to identify you.

The following groups may have access to your coded information

- Approved auditors appointed by the New Zealand Health and Disability Ethics Committees, or any relevant regulatory authority or their approved representative reviewing my relevant medical records for the sole purpose of checking the accuracy of the information recorded for the study

The results of the study may be published or presented, but not in a form that would reasonably be expected to identify you.

Security and Storage of Your Information.

Your identifiable information is held at the University of Waikato during the study. Recorded sonography scans will be saved to an external hard drive. A second copy of the data will be made and transferred to a backup external hard drive in the event that data is compromised on the first hard drive.

The external hard drives and paper copies of participant consent forms and participant demographics questionnaires will be stored in a locked filing cabinet in a locked research office within Te Kura Whatu Oho Mauri, School of Psychology on the University of Waikato, Hamilton campus. The only individuals with access to the filing cabinet and office will be members of the research team.

All data collected will be stored securely as outlined above for ten years in accordance with New Zealand law for health data (National Ethics Advisory Committee 12.13). After this time all raw data will be destroyed.

Rights to Access Your Information.

You have the right to request access to your information held by the research team. You also have the right to request that any information you disagree with is corrected.

If you have any questions about the collection and use of information about you, please feel free to ask Professor Vincent Reid (contact details provided on page 4).

Rights to Withdraw Your Information.

You may withdraw your consent for the collection and use of your information at any time, by informing a member of the research team.

If you withdraw your consent, your study participation will end, and the study team will stop collecting information from you.

If you agree, information collected up until your withdrawal from the study will continue to be used and included in the study. You may ask for it to be deleted when you withdraw unless you withdraw after the study analyses have been undertaken.

UNIVERSITY OF WAIKATO- INFANT RESEARCH GROUP DATABASE

You are invited to sign up to the University of Waikato - Infant Research Group database. If you chose to join the database you may be contacted by researchers from the University of Waikato with opportunities to take part in further infant and child research. Your contact details will be stored on a password-protected server and you will be able to withdraw from the database at any time.

CAN I FIND OUT THE RESULTS OF THE STUDY?

You can request a summary of the results on the consent form. At the completion of the study (which is expected to be March 2023) a summary of the results will be sent to you via your chosen method, either electronically or through the postal service.

WHO IS FUNDING THE STUDY?

This study is funded by a Marsden Grant (20-UOW-022) from the Royal Society Te Apārangi.

CULTURAL CONSIDERATIONS

We understand that Māori may have cultural considerations when participating in any study. We, the researchers, respect the tikanga and understand that participation in this type of study requires careful consideration. We support the process whereby some participants will want to discuss the study with whānau or other trusted members in the community/iwi.

We acknowledge the experience and knowledge shared by participants is a taonga; data collected is treated as invaluable and will be treated with respect. Further, the Waikato DHB respects the importance of tikanga, so please inform us if you wish to have whānau support present.

WHO HAS APPROVED THE STUDY?

This study has been approved by an independent group of people called a Health and Disability Ethics Committee (HDEC), who check that studies meet established ethical standards. The Central Health and Disability Ethics Committee has approved this study.

WHO DO I CONTACT FOR MORE INFORMATION OR IF I HAVE CONCERNS?

If you have any questions, concerns or complaints about the study at any stage, you can contact:

Professor Vincent Reid
Head of School,
School of Psychology, University of Waikato
Phone: +64 7 838 4080 extn 9222
Email: vreid@waikato.ac.nz

If you want to talk to someone who isn't involved with the study, you can contact an independent health and disability advocate on:

Phone: 0800 555 050
Fax: 0800 2 SUPPORT (0800 2787 7678)
Email: advocacy@advocacy.org.nz
Website: <https://www.advocacy.org.nz/>

If you require Māori cultural support, talk to your whanau in the first instance. Te Puna Oranga are available for cultural support throughout the duration of the trial. A member of Te Puna Oranga can be contacted at Waikato Hospital.

Phone: 07 834 3644 extension 97844

You can also contact the health and disability ethics committee (HDEC) that approved this study on:

Phone: 0800 4 ETHIC
Email: hdecs@health.govt.nz

References

- Abramowicz, J. S. (2007). Prenatal exposure to ultrasound waves: is there a risk? *Ultrasound in Obstetrics and Gynecology*, 29(4), 363–367. <https://doi.org/10.1002/uog.3983>
- Committee on Environmental Health. (1997). Noise: A Hazard for the Fetus and Newborn. *Pediatrics*, 100(4). <https://doi.org/10.1542/peds.100.4.724>
- Graven, S. N. (2000). Sound and the Developing Infant in the NICU: Conclusions and Recommendations for Care. *Journal of Perinatology*, 20(S1). <https://doi.org/10.1038/sj.jp.7200444>

Consent Form

Fetal Visual Perception

Please tick to indicate you consent to the following

I have read, or have had read to me in my first language, and I understand the Participant Information Sheet.	Yes <input type="checkbox"/>	
I have been given sufficient time to consider whether or not to participate in this study.	Yes <input type="checkbox"/>	
I have had the opportunity to use a legal representative, whanau/ family support or a friend to help me ask questions and understand the study.	Yes <input type="checkbox"/>	
I am satisfied with the answers I have been given regarding the study and I have a copy of this consent form and information sheet.	Yes <input type="checkbox"/>	
I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time without this affecting my medical care.	Yes <input type="checkbox"/>	
I consent to the research staff collecting and processing my information, including information about my health.	Yes <input type="checkbox"/>	
If I decide to withdraw from the study, I agree that the information collected about me up to the point when I withdraw may continue to be processed.	Yes <input type="checkbox"/>	No <input type="checkbox"/>
I consent to my GP or current provider being informed about my participation in the study and of any significant abnormal results obtained during the study.	Yes <input type="checkbox"/>	
I agree to an approved auditor appointed by the New Zealand Health and Disability Ethics Committees, or any relevant regulatory authority or their approved representative reviewing my relevant medical records for the sole purpose of checking the accuracy of the information recorded for the study.	Yes <input type="checkbox"/>	
I understand the compensation provisions in case of injury during the study.	Yes <input type="checkbox"/>	
I understand that my participation in this study is confidential and that no material, which could identify me personally, will be used in any reports on this study.	Yes <input type="checkbox"/>	
I know who to contact if I have any questions about the study in general.	Yes <input type="checkbox"/>	

I understand my responsibilities as a study participant. Yes

I wish to receive a summary of the results from the study. Yes No

If you selected 'Yes' please provide an email or post address that we can send the results summary to.

Contact Address (email and/or postal):

I wish to sign up to the University of Waikato - Infant Research Group database. By providing my contact details I may be contacted by researchers from the University of Waikato with opportunities to take part in further infant and child research Yes No

If you selected 'Yes' please provide your contact details below:

Contact email address:

Contact phone number:

Declaration by participant:

I hereby consent to take part in this study.

Participant's name: _____

Signature: _____ Date: _____

Declaration by member of research team:

I have given a verbal explanation of the research project to the participant, and have answered the participant's questions about it.

I believe that the participant understands the study and has given informed consent to participate.

Researcher's name: _____

Signature: _____ Date: _____

Appendix C

Co-Authorship Forms



Co-Authorship Form

School of Graduate Research
 The University of Waikato
 Private Bag 3105
 Hamilton 3240, New Zealand
 Phone +64 7 838 5096
 Email: SGR@waikato.ac.nz
 Website: <http://www.waikato.ac.nz/students/research-degree>

This form is to accompany the submission of any PhD that contains research reported in published or unpublished co-authored work. **Please include one copy of this form for each co-authored work.** Completed forms should be included in your appendices for all the copies of your thesis submitted for examination and library deposit (including digital deposit).

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work.

Chapter 2: Leov, J., Dunn, K., & Reid, V.M (2024). Seeing the Light: The Role of Light in Prenatal Visual Development Manuscript in preparation for submission to Developmental Review

Nature of contribution by PhD candidate	Conceptualisation, methods, formal analysis, writing -original draft, review and editing
Extent of contribution by PhD candidate (%)	80

CO-AUTHORS

Name	Nature of Contribution
Prof. Vincent Reid	Supervision, conceptualisation, methods, writing -review and editing, funding acquisition
Dr Kirsty Dunn	Supervision, conceptualisation, methods, writing -review and editing, funding acquisition

Certification by Co-Authors

The undersigned hereby certify that:

- ❖ the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- ❖ that the candidate wrote all or the majority of the text.

Name	Signature	Date
Professor Vincent. M. Reid		20.09.2024
Dr Kirsty Dunn		25/09/2024

July 2015



Co-Authorship Form

School of Graduate Research
 The University of Waikato
 Private Bag 3106
 Hamilton 3240, New Zealand
 Phone +64 7 838 5096
 Email: SGR@waikato.ac.nz
 Website: <http://www.waikato.ac.nz/students/research-degree>

This form is to accompany the submission of any PhD that contains research reported in published or unpublished co-authored work. **Please include one copy of this form for each co-authored work.** Completed forms should be included in your appendices for all the copies of your thesis submitted for examination and library deposit (including digital deposit).

Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work. **Chapter 4**

Leov, J., Dunn, K., & Reid, V.M (2024). Visual Processing of Stimuli in the Human Fetus: A Peripheral Field Advantage? Manuscript in preparation for submission to: Developmental Psychology

Nature of contribution by PhD candidate

Conceptualisation, methods, data collection, data analysis, writing- original draft, writing -editing and review

Extent of contribution by PhD candidate (%)

80

CO-AUTHORS

Name	Nature of Contribution
Prof. Vincent Reid	Supervision, conceptualisation, methods, writing-editing and review and funding acquisition
Dr Kirsty Dunn	Supervision, conceptualisation, methods, writing-editing and review and funding acquisition

Certification by Co-Authors

The undersigned hereby certify that:

- ❖ the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- ❖ that the candidate wrote all or the majority of the text.

Name	Signature	Date
Prof. Vincent Reid		20/09/2024
Dr Kirsty Dunn		25/09/2024

July 2015



Co-Authorship Form

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Please indicate the chapter/section/pages of this thesis that are extracted from a co-authored work and give the title and publication details or details of submission of the co-authored work. Chapter 5:

Chapter 4:Leov, J., Dunn, K., & Reid, V.M (2024). Anticipatory Visual Responses in the Human Fetus Manuscript in preparation for submission to: Nature

Nature of contribution by PhD candidate

Conceptualisation, methods, data collection, data analysis, writing- original draft, writing-editing and review

Extent of contribution by PhD candidate (%)

80

CO-AUTHORS

Name	Nature of Contribution
Prof. Vincent Reid	Supervision, conceptualisation, methods, writing-editing and review and funding acquisition
Dr Kirsty Dunn	Supervision, conceptualisation, methods, writing-editing and review and funding acquisition

Certification by Co-Authors

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- ❖ that the candidate wrote all or the majority of the text.

Name	Signature	Date
Prof. Vincent Reid		20.09.2024
Dr Kirsty Dunn		25/09/2024

July 2015