Assessing infant neurocognitive development in resource-poor settings: the example of memory development in the UK and The Gambia.

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Thesis submitted to University College London for the degree of Doctor of Philosophy

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December 2018

I, Laura Kischkel, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Abstract

Infants and children in low and middle income countries (LMIC's) are at increased risk of compromised neurodevelopmental outcomes, due to exposure to a range of environmental risk factors. Neurocognitive research to date has focused almost exclusively on western, industrialised settings. For this reason, there is a lack of knowledge on what constitutes normative neurocognitive development in LMIC's. By understanding infant development more globally, an opportunity is created which ultimately will enable early intervention, targeting specific risk factors commonly encountered in LMIC settings.

This project assesses neurocognitive development in early infancy, with a particular focus on memory functioning. Infants were longitudinally examined in both the UK and in a rural village in The Gambia, West Africa. Assessments were conducted at 1, 5, 8 and 12 months of age using both neuroimaging measures (electroencephalography, functional near infrared spectroscopy) and behavioural methods.

Findings across these studies indicate differential developmental trajectories between the two cohorts. Electrophysiological measures indicate an attenuated developmental change in the Gambian cohort between 1 and the 5 months of age. Cortical haemodynamic responses differed between cohorts, in terms of their localisation and magnitude. Behaviourally, higher levels of retention of novel actions were observed in the UK compared to the Gambian cohort.

This thesis is part of one of the first projects taking a global perspective on early neurocognitive development, by exploring infants in a previously understudied population. The implementation of novel, objective neuroimaging methods has yielded results indicative of striking differences between the two cohorts. These data will provide a basis for future projects aimed at implementing interventions and thus alleviating some of the global burden of suboptimal neurocognitive development.

Impact Statement

This thesis is part of the first research project assessing infant neurocognitive development in rural Africa. As such, it is part of a newly emerging area of research concerned with understanding the impact of environmental adversity, as frequently experienced in low and middle income countries (LMIC's), on early cognitive development.

The presented research contributes to the field in two key ways. First, it provides evidence about the efficacy of neuroimaging methodology for the study of young infants in low-resource settings. This thesis in particular is the first to demonstrate the utility of electrophysiological markers of infant development in a low income country. This will help inform future research about both methodological considerations when setting up similar studies, as well as provide some context as to the observations made in other populations. Secondly, the findings presented in this thesis contribute to the definition of early markers of atypical cognitive development associated with environmental risk factors frequently encountered in LMIC's. This will be of importance for future research seeking to evaluate the efficacy of specific interventions, as well as in clinical practice seeking to identify infants most at risk of a compromised outcome.

Findings presented in this thesis have been disseminated within the wider scientific community through presentation at several national and international meetings. The rationale and importance of this line of research has further been communicated to participants in The Gambia and the UK during dedicated outreach events as well as to wider lay audiences, which has demonstrably increased awareness regarding the importance of healthy brain development and the need for global action.

Ultimately, this line of research will provide a foundation for the implementation and evaluation of interventions targeted at improving developmental outcomes for infants and children globally.

Acknowledgements

I would like to express my thanks to those who supported me during the completion of this thesis. First, I would like to thank my supervisors Prof Michelle de Haan, Dr Sarah Lloyd-Fox and Prof Clare Elwell. I consider myself lucky to have worked with three such inspirational scientists and role models, thank you for continuously providing encouragement, patient support and food for thought and in the process teaching me how to be a scientist. Further I would like to thank Charles Nelson and his group for their guidance during my visit to their lab.

Secondly, I would like to thank the entire BRIGHT project team. Nathan Hayes for helping me find my feet during my first year, Maria Rozkho, for having been such a fantastic testing partner week after week, Sam McCann and Christine Bartam-Torrance, for lovely Keneba afternoons, Anna Blasi and Luke Mason, for their unwavering patience, competence, and ingenuity in solving all the big and small problems. I further thank all other team members in Cambridge and in Keneba who did and continue to do such a wonderful job in tirelessly collecting the massive amounts of data that make the BRIGHT project so very special.

I have also greatly valued working alongside many smart and inspiring researchers at the Great Ormond Street Institute of Child Health, and I have to thank them for creating a supportive atmosphere and for their generous intellectual support to me as a student.

I am further indebted to my friends. To Denise and Birthe, for having been caring, supportive friends for two decades now- it has made a huge difference. To Jenny, whose sunny disposition and generously provided pep-talks are second to none. To Birgit, whose persistence and integrity have inspired me throughout our time working together. And to Barbara, for providing perspective on all the things that are important outside academia.

I would like to thank my family. My parents, Angela and Wolfgang for trusting that I could achieve whatever I set my mind to. My sister Katharina and her husband Mohammad as well as my brother Jan and his wife Elisabeth for their support, and for occasionally letting me practice with their babies… I would also like to thank my late granddad Paul, without whose support it is unlikely that I would have made the move to London. And of course, I wish to thank you Tobi, for joining me along the way. Your love and support is in every page of this thesis.

Last but not least I wish to extend my gratitude to all infants and families for their commitment to this project and for letting myself and the team be a part of their babies' first months and years of life.

It always seems impossible, until it’s done.

- Nelson Mandela

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Glossary

ASD	autism spectrum disorder
BF	bayes factor
BOLD	blood oxygen level dependent
BRIGHT project	Brain Imaging for Global Health project
CFC	cross-frequency coupling
DCT	dimensional card sorting task
dlPFC	dorsolateral prefrontal cortex
DST	Developmental Systems Theory
DTI	diffusion tensor imaging
EEG	electroencephalography
ERP	event related potential
FC	frontal cortex
FDR	false discovery rate
fMRI	functional magnetic resonance imaging
fNIRS	functional near infrared spectroscopy
HbO2	oxyhaemoglobin
HHb	deoxyhaemoglobin
HRF	haemodynamic response function
Hz	hertz
ICC	intraclass correlation coefficient
IFG	inferior frontal gyrus
IQ	intelligence quotient
IQR	inter quartile range
IS	Interactive Specialisation
ISI	inter stimulus interval
ITPC	inter trial phase coherence
LMIC's	low and middle income countries

MMN	mismatch negativity
MMR	mismatch response
MMR_{ERP}	mismatch response based on ERP
MMR_{TF}	mismatch response in time frequency domain
MRC	Medical Research Council
MRI	magnetic resonance imaging
MTL	medial temporal lobe
NTS	nearinfrared spectroscopy system
OP	object permanence
PAC	phase amplitude coupling
PET	positron emission tomography
PFC	prefrontal cortex
PSD	power spectral density
RCT	randomised controlled trial
ROI	region of interest
RT	reaction time
SES	socioeconomic status
SPECT	single photon emission tomography
TF	time frequency
TPJ	temporal-parietal junction
WASH	water sanitation and hygiene
WISC	Wechsler Intelligence Scale for Children
WM	working memory
WPPSI	Wechsler Preschool and Primary Scale of Intelligence

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Chapter 1. General Introduction

Children in low and middle income countries (LMIC's) are at increased risk of compromised development due to a range of adverse environmental factors. A recent study estimates that one third of young children in LMIC's fail to reach their neurodevelopmental milestones, the largest proportion of which living in sub- Saharan Africa (McCoy et al., 2016). Over 80 million children in LMIC's world-wide perform at a level below what would be expected for their chronological age in terms of neurocognitive development (McCoy et al., 2016), and may thus experience problems in processing information, paying attention, understanding and following directions, cooperating with peers and solving problems. One cognitive domain central to all these skills and therefore everyday functioning is memory. As memory processes are at the heart of virtually every higher order cognitive process it takes a crucial role in neurodevelopment of infants and children, with memory impairments holding potential for severe knock-on effects on functioning in other cognitive domains and everyday life.

Compromised neurocognitive development holds potential for detrimental effects on children's academic achievements, their socio-economic status as adults, social relationships and mental health. The reduction of poor neurodevelopmental outcomes in at-risk LMIC's has therefore been identified as a key priority for global health research in the United Nations Sustainable Development Goals (UN, 2015). At present, the reasons for the suboptimal developmental outcomes observed in early childhood are poorly understood, but are likely to lie in a multifaceted interaction of several risk factors frequently encountered in LMIC's. Infants and children in LMIC's are frequently at higher risk of being exposed to a range of social, nutritional and health challenges. Numerous associated risk factors have been associated with poor neurodevelopmental outcomes, including undernutrition (Grantham-McGregor et al., 2007) poor sanitation resulting

in increased rates of infectious disease (Joseph et al., 2014), and limited social interaction (Walker et al., 2007). While several studies have identified poor developmental outcomes in young children, very little is known about prior development during infancy, despite the fact that the impact of the aforementioned risk factors is already prevalent during this critical developmental period.

The first 1000 days of life, spanning conception to approximately 24 months of age, are a crucial period of infant development. During this time, foundations are lain for sensory-, social- and neurodevelopment, allowing individuals to thrive in later life. Development during this time is not only critical but also exceedingly vulnerable to adverse environmental factors (Anderson, 2003; Rice & Barone, 2000). A thorough understanding of infants' neurocognitive development during this early period therefore is important when seeking to understand the impact of environmental factors on later developmental outcomes. Only by prospectively studying infants' neurodevelopmental trajectories as well as different factors in their environment will it be possible to derive explanations for the mechanisms underlying developmental outcomes during childhood. A better mechanistic understanding of early brain and cognitive development in at-risk populations also provides the basis for targeted interventions, thus increasing their potential to be efficacious. In order to identify at-risk individuals in any given population, it is important to first gain an understanding of typical developmental trajectories, the definition of which helps to establish standards against which the efficacy of interventions can be evaluated.

This thesis seeks to define early developmental changes in infant's memory functioning, and apply known paradigms in a previously understudied cohort in rural Gambia. This chapter will provide background on both the cognitive concepts referred to throughout this thesis, as well as on the

current knowledge base regarding neurodevelopmental outcomes associated with early adversity and poverty. I will first provide background on models of memory functioning and memory development during infancy. I will then discuss directions of current neurodevelopmental research and evidence for associations of specific factors with cognitive development, with a specific focus on rural Gambia. I will I will then close the chapter with an outline of the studies contained in this thesis and the research aims and objectives.

1.1 Human Memory: Cognitive Models and Neural Bases

The memory system subserves every aspect of higher order cognition and is therefore one of the most fundamental cognitive systems to emerge in humans (Conway, 1997). In the following I will first discuss different conceptualisations of human memory in functional terms, in terms of different memory stores, as well as the neural bases associated with memory processes.

1.1.1 Models of memory functioning.

Memory formation. The formation of any memory depends on initial processing of incoming information (encoding), subsequent consolidation of the information (storing) and a reactivation of the consolidated memory at a later time (retrieval). Regardless of which subsystem of memory is under investigation, they all rely on encoding, storage and retrieval in order to successfully carry over learned information to new situations. Failure in any one of these subsystems can lead to impairments in consolidating new information, or accessing what has previously been learned.

Short term- working- and long term memory. Since the early beginnings of memory research, a distinction has been made between memory that actively held in mind in a specific moment, and memory that is not currently

consciously available. In his now classical experiments of memory for nonsense syllables, Ebbinghaus (1885) was the first to describe that immediately after reading the sets of syllables he had an initial 'fleeting grasp of sets in moments of particular concentration" (Ebbinghaus, 1885, own translation), however this initial representation did not guarantee later retrieval. James (1890) defined two different types of memory that he referred to as primary and secondary memory. While only a small amount of information could be held in conscious present and therefore in primary memory, secondary memory was able to hold vast amounts of rich memories acquired over a lifetime. While long-term memory appears in virtually all models of memory functioning (Cowan, 2008), the capacity and specific properties of short term- and working memory (WM) have been, and continue to be, the subject of debate. One of the most influential conceptualisations stems from Atkinson & Shiffrin (1968), who regard short term memory as the capacity to temporarily hold a limited amount of information in consciousness, in a very accessible way. This definition bears substantial overlap with the concept of WM (Cowan, 2008), which has been further refined as that part of temporarily activated memory used to plan and carry out behaviour. In his model Cowan (2008) describes short term memory as an 'activated' subset of longterm memory, the currently attended portion of which is what is referred to as WM (Figure 1.1). WM itself has been conceptualised in a number of ways, the first and continually influential model was hereby drawn up by Baddely & Hitch (1986). As will be discussed in more depth in Chapter 5 in context of the WM task implemented as part of this thesis, the model encompasses sensory specific memory stores for visuo-spatial and verbal-phonological content, as well as attentional resources coordinating all processes.

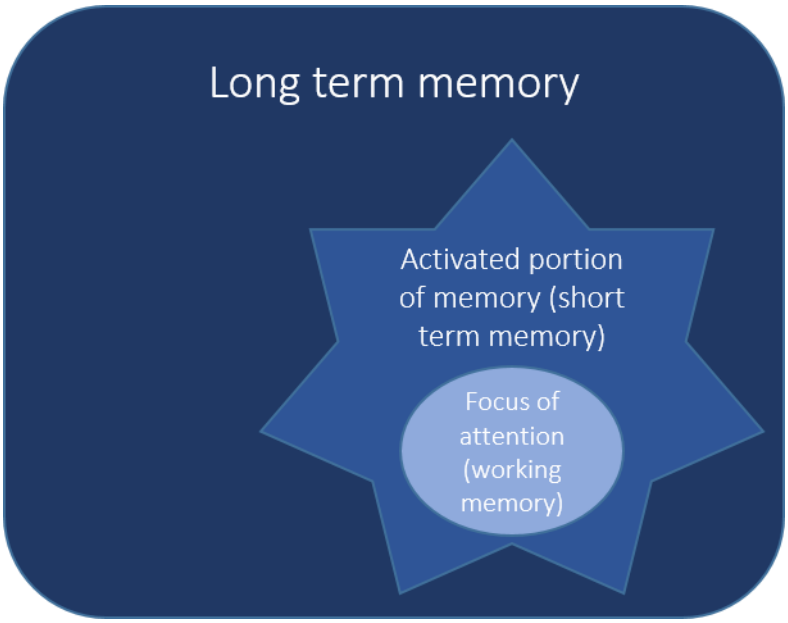


Figure 1.1. Adapted from Cowan (2008). Conceptualisation of the human memory system.

Explicit and implicit memory systems. Distinctions have further been made between different memory subsystems in relation to content. One of the first models was proposed by Tulving (1972) who described three different memory subsystems involved in consolidating different types of knowledge. He drew a distinction between procedural memory, or the knowledge on how to perform certain actions, semantic knowledge, or memory of facts, and episodic memory, or the memory for experiences and recollections.

The model was further refined by Cohen and Squire (1980) who proposed that both semantic and episodic memory belonged to that aspect of memory that one can be consciously aware of and that can be declared. They also proposed a distinct system to underlie procedural memories. Procedural memory content, such as knowing how to ride a bike or play an instrument are not easily brought to consciousness and are concerned with learned skills and habits. Procedural memories are therefore frequently labelled as 'implicit' whereas semantic and episodic memories are grouped to belong to 'explicit' memory. This categorisation is visualised in Figure 1.2.

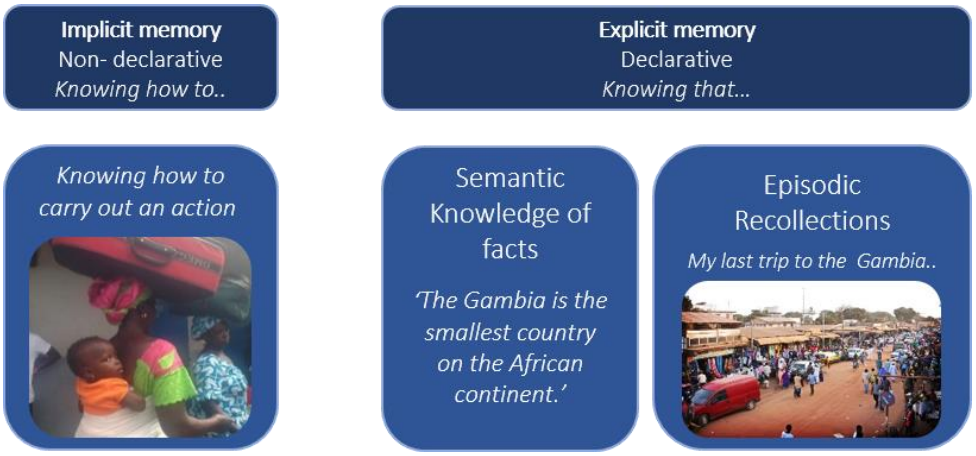


Figure 1.2. Breakdown of memory models proposed by Tulving (1972) and Cohen & Squire (1980).

Explicit memory refers to memories that can be brought to consciousness, such as the recall of events or places, or learned factual knowledge. Hereby, episodic memories are contextualised memories, for example of specific events that can vividly be remembered. Semantic memories on the other hand are of factual nature that are not necessarily embedded in spatio-temporal context. Both memory systems have been described to be associated with dissociable neural networks, as will be described in the following.

1.1.2 Neural bases of memory processing. Depending on context and specific task demands different neural networks have been shown to underlie specific memory processes. In Chapters 3, 5 and 6, I will discuss in detail the neural correlates associated with novelty detection, WM and imitation behaviour, respectively. Common to the majority of these networks however is the involvement of structures within the medial temporal lobes (MTL), which have been most strongly implicated in a variety of memory dependent processes. Figure 1.3 shows a schematic of the MTL network, as proposed by Mishkin et al. (1997), as well as an illustration of the approximate anatomical configuration of this network (Diana, Yonelinas &

Ranganath, 2007). Mishkin et al. (1997) propose for there to be a hierarchy with regard to neural structures underlying memory functioning, with both semantic and episodic memory being dependent on structures adjacent to the hippocampus, and episodic memory above that being reliant on additional processing of incoming stimuli by the hippocampus. Through incoming projections of the ventral stream, the perirhinal cortex is hereby proposed to provide input about the quality of a stimulus, whereas the parahippocampal cortex, which primarily receives projections from the dorsal stream, provides input regarding the location of a stimulus in space (Mishkin et al., 2007). Both the perhinal and the parahippocampal cortex input information to the entorhinal cortex, which in turn relays these inputs to the hippocampus itself.

The role of the hippocampus in integrating these inputs if further described in the 'binding- items- in- context model', proposed by Diana et al. (2007). Hereby it is proposed that the integrated retention of both stimuli themselves as well as the context that they were encountered in is at the very core of episodic memory, and that through its function of binding together these different inputs, the hippocampus is crucial for successful episodic memory formation.

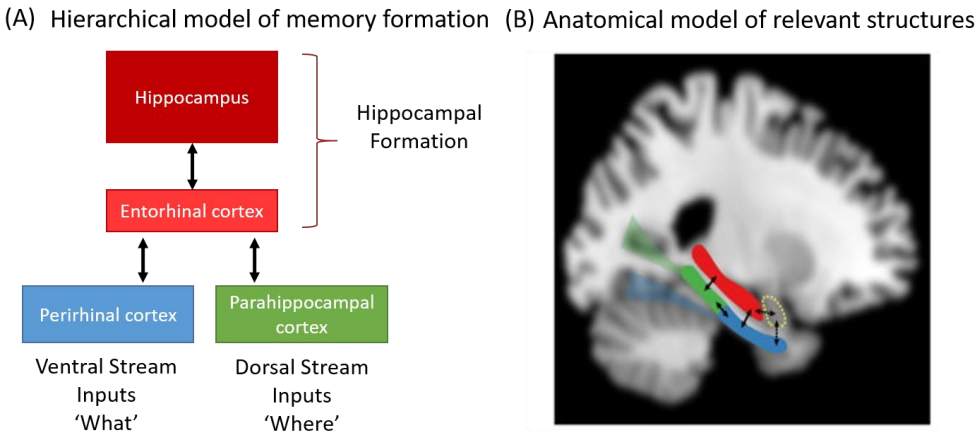


Figure 1.3. (A) Adapted from Mishkin et al. (1997). Hierarchical model of memory, with perirhinal and parahippocampal cortices providing input to hippocampal formation. (B) Reproduced from Diana et al. (2007). Anatomical localisation of structures proposed by Mishkin et al. (1997).

One of the first case studies to illustrate involvement of the MTL network with memory processes stems from pre- imaging studies on patients with lesions to the MTL area which reported cases of severe memory loss and anterograde amnesia. This is exemplified by the study of patient H.M. who suffered from severe epilepsy and therefore underwent neurosurgery resulting in the bilateral removal of his hippocampi as well as surrounding MTL structures (Squire, 2009). His resulting inability to consolidate any new explicit memories has been studied extensively and is taken as an exemplar case of what damage to the MTL network can result in.

The dependence on an intact MTL network has been demonstrated for declarative, explicit memories. Adult literature shows a strong link between the MTL and hippocampal activity during spatial- (Broadbent, Squire & Clark, 2004) episodic- (Moscovitch et al., 2016) and semantic- (Klooster & Duff, 2015) memory tasks. Different areas have been implicated for implicit memory processes, as these tap motor learning and conditioning, and depending on the task are subserved by different neural systems such as

the striatum, the supplementary motor cortex and frontal motor areas (serial reaction time tasks, visual expectation tasks), the cerebellum and basal ganglia (conditioning), as well as parietal, occipital, inferior temporal and auditory cortices, depending on which modality is targeted (Nelson & Webb, 2003). The notion of different neural systems being associated with implicit and explicit memory was also suggested in the context of patient H.M., who despite the fact of not being able to consolidate any new recollections or facts was shown to be able to learn novel skills (Squire, 2009).

Evidence from developmental studies. From a developmental perspective, early insults to structures of the MTL have been reported to lead to severe impairments in explicit memory functioning. One group shown to have particular difficulty with episodic memory, but relatively spared semantic memory, are patients suffering from 'developmental amnesia' (Vargha-Khadem, Gadian, & Mishkin, 2001). What these patients have in common is an early insult-, generally a hypoxic- ischemic event during birth or shortly after, resulting in severe episodic memory impairments with relative sparing of semantic memory. MRI images of these patients show the hippocampi to be severely atrophied, underlining the vulnerability of the hippocampus and surrounding tissue to these types of early life insults. From a developmental viewpoint, we see that MTL areas are activated in memory tasks in children (de Bie et al., 2015), and that any damage to them can lead to devastating outcomes for patients. It has also been shown, that changes in memory functioning coincide with maturation of MTL structures (Chai et al., 2010).

While the MTL are crucial to explicit functioning, a distinct and more wide spread neural network has been implicated in WM functioning. As will be reviewed in detail in Chapter 5, WM has been strongly associated with a network including the prefrontal and parietal cortices as well as the cingulum (Nelson, 2000). This distinction of the different types of memory

based on a neural level also translates to different developmental trajectories during infancy and childhood. In the following section, these adult models regarding cognitive conceptualisations of memory functioning as well as their neural underpinnings will be related to the infant period. Hereby, a conceptualisation of memory during infancy will be provided in reference to neurodevelopmental changes, before discussing paradigms frequently used to tap different types of memory. Lastly, developmental principles underlying memory development in general will be reviewed.

1.2 Memory in Infancy: Assessment and Developmental Principles

1.2.1 Models of infant memory development.

Infant memory has been conceptualised in reference to adult models of memory functioning. Nelson (1995) hereby proposed that implicit- as well as explicit- and WM processes can be dissociated early in development, and that certain developmental changes can be observed in relation to each of these subdomains.

As reviewed above, implicit memory comprises a diverse set of skills and therefore a range of different neural structures. Implicit memory processes in infants have been studied much less frequently than explicit and WM processes, resulting in limited evidence on its developmental progression. It has however been shown that starting during the first six months of life, there is evidence for the occurrence of basic, sensory implicit memory processes in infants, as will be reviewed below in context of implicit memory paradigms implemented in infant research.

In reference to explicit memory functioning it has been argued that developmental changes in the underlying neural circuitry first give rise to what has been termed pre-explicit memory, before a broader range of more complex functions gives rise to a more mature form of explicit memory (Nelson, 1995, Nelson & Webb, 2003). Nelson (1995) argues that pre-

explicit memory, relying primarily MTL structures, is expressed in infants' preferential response to novelty during the first six months of life. Over the second half of the first year, maturational changes within the MTL, as well as a recruitment of additional cortical areas gives rise to more complex explicit memory capacities which are qualitatively similar to adult explicit memory.

Lastly, Nelson (1995) reviews a distinct set of abilities, shown to be reliant on the pre-frontal cortex and conceptually more akin to adult WM. Significant improvements can be seen regarding WM abilities during the second half of the first year of life, with continuous improvements thereafter, which is in line with evidence regarding neural maturational processes.

Implicit, explicit and WM can be assessed during infancy using a range of paradigms and tasks, each tapping a slightly different aspects of memory development. The most commonly implemented of these paradigms will be reviewed in the following section, as will be their potential to highlight some of the developmental changes that have been proposed to occur during infancy.

1.2.2 Assessment of infant memory development.

Infant memory has been studied using a range of tools, which, depending on infant age, tax progressively higher level abilities. In line with the taxonomy proposed by Nelson (1995), the following section will review tasks suitable to assess implicit, pre-explicit, explicit and WM during infancy.

Priming. Priming paradigms are commonly used to assess implicit memory functioning. Priming hereby refers to a heightened sensitivity in to stimulus due to a recent encounter of a related stimulus (Tulving & Schacter, 1990). Perceptual priming is thought to occur through sensory similarities between

two stimuli, with no attribution of semantic meaning to either stimulus (Weldon, Roediger & Challis, 1989). Priming responses have provided evidence for the presence of implicit memory processes in infants as young as 6 months, as demonstrated by Webb and Nelson (2001). Infants were presented with a range of faces and compared event related potentials (ERP's) occurring in response to the first presentation of a given face, and its subsequent repeated presentation. Differential responses were observed to faces that had been presented previously and those that had not, which were not attributable to a mere novelty effect, as the difference did not manifest in ERP components known to be modulated by novelty effects (Webb & Nelson, 2001). It could further be shown that in correspondence to known developmental biases to attend different object features, infants between 4 and 5 months could be primed to attend to the shape of an objects, whereas 7-9 month olds could be primed to attend to colour features also. The precise developmental trajectory of priming processes is however not well documented (Nelson & Webb, 2001), and is likely to differ between sensory modalities due to close links to sensory processes. Another avenue in assessing implicit memory development that has been pursued in the beginning of infant memory research is the elicitation of conditioned responses, as will be reviewed in the following.

Classical conditioning. In conditioning paradigms a spontaneously occurring, unconditioned, response is paired with an unrelated, conditioned stimulus. After several repetition of this paired presentation, the conditioned stimulus suffices to elicit the unconditioned response, indicating a learned association.

While not widely used as a method to study infant memory development, some of the earliest studies in this field of research have utilised conditioning responses. In a study of 1 month old infants, Little et al. (1984) elicited an eye blink response through a small air puff in front of the infant's

eye, which was accompanied by a tone. Through several repetitions, the tone in isolation eventually sufficed to elicit the eye blink response, indicating a retention of the preceding pairing of conditioned and unconditioned stimuli. Conditioning paradigms have been implemented in even the youngest infants. In studies assessing the sucking rate on a modified dummy, it could be shown that infants not only modulate the rate at which they are sucking depending on whether they were exposed to, for example, their mother's or another woman's voice, but also learn what rate of sucking will lead to a tape of each voice to be played (Spence, 1996). This latter modification lead the infant to modify their behaviour to elicit a learned consequence, a process exploited in a range of operant conditioning tasks.

Operant conditioning. Operant conditioning tasks rely on the conditioned elicitation of a behavioural response. An example frequently used in infant research is the mobile conjugate reinforcement paradigm. In this task, a string is tied to one of the infant's ankles, which is attached to a mobile. Through increased leg kicking the infant is able to set in motion the mobile, which acts as a natural reinforcer through its appealing visual appearance. The rate of leg kicking can be compared before and after a delay, giving an indication of how well infants retained the cause- effect relationship. In a similar task, infants were presented with a train, which they could move through the press of a button. Response rates of the learning phase could thus be compared to those after a delay, giving an indication of infants' retention. Using both the mobile conjugate and the train task at the appropriate age points, different courses of retention and forgetting could be mapped out, demonstrating a linear increase in retention delay before decay over the course of the first 18 months of life (Hartshorn et al., 1995).

Both classical and operant conditioning procedures tap implicit memory, as retention occurs even in instances where subjects are not able to overtly

recall the encoded information. The following paragraphs will review paradigms, which group with early explicit, or pre-explicit memory, via habituation and novelty detection.

Habituation. Habituation paradigms are amongst the most frequently used in infant research and habituation processes have frequently been argued to form the foundation of more complex memory processes. In habituation paradigms, response decrements are quantified, which occur to a series of repeated stimuli. Paradigms assume that an initially response evoking stimulus becomes less and less salient when repeated, leading to a decreasing and ultimately diminished behavioural or neuronal response. Habituation can be assessed in a number of ways. In one frequently employed visual paradigm infants are exposed to an image for several consecutive trials. Their looking time on each trial is measured as is the total number of trials until habituation is maximal. Even newborns have been shown to habituate to repeated auditory or visual input in their sleep, illustrating that habituation can give an indication of early learning even in this young age group.

While purely habituation focused studies give an indication of how long the infant needs to encode the stimulus, most paradigms employ both a habituation as well as a dishabituation phase, yielding an additional measure of novelty detection.

Novelty detection. Novelty paradigms measure not just response decrements to the repeating but also response recovery- or dishabituation effects to a novel stimulus. These paradigms rely on the commonly evident novelty preference infants' exhibit which is also used to assess infants' encoding and retention of continuous stimulation. One of the most commonly implemented novelty paradigms is the visual paired comparison task. In this task, infants are first habituated to an image, before being

presented both with the habituated and a novel image. It has been found repeatedly that from an early age onwards, infants prefer looking at the novel image. Both novelty and habituation paradigms have been studied not only behaviourally, but also in neuroimaging paradigms. In infants, an abundance of work using ERP's has elucidated developmental trajectories of habituation and dishabituation processes, as will be laid out in detail in Chapter 3, in the context of the ERP study that is part of this project. More recently, habituation and novelty processes in infants have also been investigated using fNIRS (Nakano et al., 2009, Nakato et al., 2011). In recent study of infants in the UK and The Gambia between five and eight months Lloyd-Fox et al. (2019) described the haemodynamic response associated with habituation novelty processes, which was shown to develop in different trajectories between the cohorts.

Both habituation and novelty detection are grouped as belonging to what Nelson (1995) referred to as pre-explicit memory. As laid out above, pre-explicit memory can be thought of as the foundation of later explicit memory. Evidence for the distinction from priming or conditioning paradigms hereby stems from the neural structures known to underlie habituation novelty detection. As will be reviewed in detail in Chapter 3 in context of the novelty detection study implemented as part of this thesis, the MTL network has been shown to be of great importance for habituation and novelty detection processes. It is argued by Nelson (1995) that pre-explicit memory specifically supports novelty detection processes, which can occur even in presence of an immature MTL network. It is only through further maturational changes occurring in this network over the second half of the first year of life that infants can acquire more complex explicit memory processes.

One widely used task to measure of infant explicit memory is through measuring their ability to imitate novel actions, as will be reviewed in the following.

Imitation. The assessment of infants' ability to imitate novel gestures and actions represents a more advanced form of memory, as they require both to passively observe the novel action as well as to actively copy it. Imitation paradigms have primarily been used to study infants above 12 months of age, but have more recently also been found useful for the study of younger age groups (Jones & Herbert, 2006). Fundamentally, imitation studies rely on infants' retention of an unfamiliar action, which they are shown by the experimenter. Imitation can either happen immediately after demonstration (elicited imitation) or after a delay (deferred imitation). The latter is frequently used as a measure of explicit memory in pre-verbal infants, as rather than tapping motor learning by immediately copying an action, infants need to encode an abstract representation of the action to be able to successfully enact it at a later time. As will be reviewed in detail in Chapter 6 in context of the deferred imitation study implemented as part of this thesis, imitation has been shown to be reliant on structures within the MTL network (Adlam et al., 2005), which further corroborates its relation to explicit memory processes.

Object search. A final category of infant memory measures are aimed at understanding early WM development. While WM plays a role across sensory domains as will be laid out in Chapter 5, most tasks used to assess infants tap early visual spatial abilities. Paradigms are usually centred around infants' ability to mentally represent an object that is temporarily hidden from view. Infant's abstract concepts of an object are hereby most commonly assessed by examining their behavioural response to search for an occluded object. Infants begin to show a stable search response from around 9 months of age (Baird et al., 2002, Bell & Fox, 1997), and in parallel

also become much better at identifying the correct hiding location of objects that are subsequently hidden in one of two different places, indicating an increased flexibility of their representation (Cuevas et al., 2012, Bell & Fox, 1992, Diamond et al., 1997). Both tasks have been argued to tap early WM development (Nelson, 1995). As will be reviewed in detail in Chapter 5 in context of the WM study that is part of this thesis, extensive primate research has indicated that indeed a similar set of neural structures underpins both types of tasks (Goldman- Rakic, 1996, Ungerleider et al., 1998). With WM representing 'memory for action' it is highly predictive of performance on a range of tasks, rendering the study of its first emergence of great interest in context of neurodevelopmental research.

While each of the reviewed paradigms help elucidate different aspect of early memory development, there are some principles that generalise across tasks which underlie memory development during infancy, as is reviewed below.

1.2.3 Guiding principles of infant memory development.

Prolonged retention. The duration during which infants retain information has been reviewed and mapped out. Using the mobile conjugate task, Hartshorn et al. (1995) demonstrated that when infants between 2 and 18 months were trained to the same criterion to elicit the mobile movement and showed equivalent levels of retention after a 24h delay, more long-term retention increased linearly as a function of age. On average, two month old infants could still perform the mobile task after one day, whereas three month olds showed retention at three days but not at six. Six month olds performed above baseline at the task after two weeks, whereas nine month old infants performed well even after six weeks. Finally, 12 and 18 month olds were able to perform the task after 10 and 12 weeks, respectively. This evidence illustrates a gradual increase of tolerated retention delays with increased age. Even though this task taps a rather narrow band of memory functioning

reliant on conditioning, retention intervals at the different age points are similar to other studies, using imitation paradigms in similar age groups, suggesting that both tasks can inform more fundamental trajectories for memory development, rather than only elucidating a process specific to current task demands. (Meltzoff, 1995, Fivush & Hamond, 1989).

Generalisation across stimulus properties. Across several studies it has been found that in addition to an increased tolerance for delays between the demonstration and the imitation phase with increased age, infants also become better at generalising actions, regardless of properties specific to the used stimuli. For example, 12 month old infants have been shown to still be able to imitate an action even if features such as the objects colour changed, but not when its shape changed, whereas 18 month olds were still able to imitate the action even when a change occurred in both dimensions (Hayne et al., 1997).

Emerging context-independent retention. Infants also become better at retrieving a memory when fewer context specific cues that were present at time of encoding are present during retrieval. Evidence again stems from imitation studies, in which it could be shown that at 6 months infants could only copy novel actions when no contextual feature changes had occurred, such as a change from the home to a lab setting, but were able to generalise across these non-meaningful features at a later age point (Hayne, 2000). Both the context free retention and generalisation across object properties can be interpreted as increased efficiency of the memory system. By requiring fewer cues to be identical between encoding and retrieval and still being able to performed a cued action infants increasingly acquire the ability to learn about the world in more general terms rather than by repeated exposure to a range of highly similar situations.

Successful retention after minimal exposure. Habituation studies consistently find that fewer repetitions of the habituating stimulus are needed with increased infant age (Jeffrey & Cohen, 1971). Imitation studies have demonstrated that with increasing age infants are able to form a representation and retain it for later enactment after only a few demonstrations. Evidence from a range of deferred imitation studies indicates that while only a third of six month old infants are able to imitate an action after delay, even when they were demonstrated the action six times (Barr et al., 1996), these proportions rise continually over the first 18 months of life. At 9 months, approximately half of infants were found to be able to imitate after a 24h delay with only three demonstrations (Carver and Bauer, 1999), and at 14 months more than two thirds of infants could imitate after even just one demonstration of a novel action despite a long retention interval of a week (Bauer et al., 2000). The ability to more swiftly encode novel input also becomes increasingly more relevant in older infants as they begin to consistently copy their caregivers and peers in order to acquire relevant real-world behaviours. These behaviours frequently are part of more complex patterns or sequences, which infants have also been shown to become better at over time.

Increased memory capacity. Some evidence suggests that infants' memory capacity increases over the first two years of life. In a study of visual short term memory, Ross-Sheehy, Oakes and Luck (2003) presented infants with two sets of shapes, located on either side of the screen. One array of shapes stayed constant over repeated trials, whereas the other one changed from trial to trial. In infants who were able to encode the presented number of items, a preference for the changing display was expected, indicating a novelty preference. It was found that whereas four month old infants only reliably showed change preference for items sets of one, 13 month olds also showed this preference in sets up to four items. This indicates a memory

capacity for more items at this later age point. Similarly, it has been shown that infants become better able at remembering multiple action sequences in an imitation paradigm. In a study of German and Cameroonian infants, Graf et al. (2014) showed that in both groups infants imitated a greater number of possible target actions at 9 compared to 6 months, in the absence of higher baseline performance.

Cross-modal retention. One aspect of memory encoding that infants become much better at as development of their explicit memory progresses, is their ability to recognise an object in one sensory modality as familiar which was previously presented in another sensory modality. This type of memory arguable requires a level of abstraction of an object's properties during encoding. It has been shown that 6 month old infants allowed to explore an object by mouthing, but not seeing, it were are able to later visually recognise a of the explored objects (Rose, Gottfried & Bridger, 1981). In an ERP paradigm, Nelson et al. (2003) presented 6 month old infants with wooden objects in the tactile domain, pictures of which were subsequently presented on a screen. The Nc component of the ERP was shown to be significantly larger for the novel than the familiar objects, indicating that even at that young age are able to form abstract representations of objects. A developmental change was shown, indicative of a progressive developmental improvement of between 6 and 12 months of age, during which infants become better able to integrate sensory input from different sensory modalities, and established a unified cross-modal memory representation (Rose & Feldman, 2000).

The reviewed developmental principles underline how infants' abilities to encode, retain and retrieve input vastly improve over the course of infancy. Cognitively, infancy is a crucial time for memory development, and any environmental risk experienced in this period might have long-lasting, significant impacts. With the outlook of identifying key factors that impact

cognitive development in early infancy in order to devise targeted, and efficient interventions, this thesis will contribute to the knowledge base in three key ways. First, it will provide evidence from some of the first neuroimaging studies performed in infants in Africa and thus will help to further establish assessment tools and paradigms for broader use in LMIC's. Secondly, this thesis will chart out developmental trajectories on three separate studies on three different paradigms taxing infant memory in progressively complex ways. Thirdly, it will provide the foundation for further investigations into the specific mechanisms of how memory functioning might be affected by environmental influences. There are some strong indications for adverse neurodevelopmental outcomes associated with a diverse set of environmental risk factors that frequently are at play in context of growing up in LMIC's. The following section will review the evidence base on neurocognitive development in LMIC's, including risk factors and possible mechanistic pathways which will provide the rationale for the studies presented in this thesis.

1.3 Neurocognitive development in low and middle income countries

1.3.1 Directions in global neurodevelopmental research. Different approaches have been taken to begin studying child and infant development in LMIC's. As will be laid out in more detail in Chapter 2, conducting comparative research across cultures or in previously understudied populations frequently requires adaptation or development of novel methods. In order to obtain valid measures, groups frequently have developed their own scales suitable for the corresponding cultural context. In their work in a rural region of Kenya, Abubakar et al. (2008) modified previously available checklists of psychomotor development in Kenya and combined them with items from frequently used assessment batteries used in high income countries, such as the Bayley- (Bayley, 2006) and the Griffiths Scales (Griffiths, 1954). The resulting Kilifi Developmental Inventory

(Abubakar et al., 2008) was shown to yield good reliability in both an urban and a rural sample of 0-3 year olds, and could also demonstrate reduced scores in at risk groups such as children that were underweight or had a known history of neurocognitive impairment.

While the Kilifi Developmental Inventory is well-suited to study infants and children across Kenya, the degree of specialisation required for a particular community decreases generalisability to other groups. Projects taking a comparative approach have therefore frequently implemented a combination of those assessments tools best able to objectively measure children across cultures. In a large-scale, multi centre project including assessment sites in Brazil, India, Italy, Kenya and the UK, the International Fetal and Newborn Growth Consortium for the 21st Century Project (INTERGROWTH-21st, Fernandes et al., 2014) performed a review of widely used developmental scales and devised a selection based on criteria related to their objectivity, ease of administration, and duration of assessment. Ultimately, a combination of items from different scales was employed, tapping domains such as language development, fine and gross motor skills, and visuo-spatial skills. While the scale was reported to have good psychometric properties in terms of inter-rater reliability and consistency of their scale across sites, assessments were performed when infants were 24 months of age. Similar scales can be much harder to derive for younger age groups.

To further objectify infant neurodevelopmental assessment and to make it non-reliant on infants' overt behavioural engagement, some groups have more recently begun to implement neuroimaging tools. In a study implementing single-probe functional near infrared spectroscopy (fNIRS) measurements in children between the ages of 1-3 and 5-7 years in Guinea-Bissau Roberts et al. (2017) observed that their simple resting state measurements of haemodynamic activity over frontal regions of the cortex

was correlated with performance on a behavioural WM task. While these measures yielded correlates between global cortical functioning and behavioural measures of cognitive development, other studies have shown that fNIRS can also been used to study young infants from birth, making inferences about localised cortical responses in the context of specific cognitive functions. The proof-of principle that this could be achieved was provided by Lloyd-Fox and colleagues who demonstrated feasibility to use fNIRS measures in rural Gambia to longitudinally study infant of multiple age ranges (Lloyd-Fox et al., 2014, Lloyd-Fox et al., 2017).

Other neuroimaging modalities have been used to derive objective markers of child development in rural Africa. In a series of ERP studies, Kihara and colleagues identified normative developmental trajectories (Kihara et al. 2010a), and subsequently showed divergence from these trajectories in children affected by different types of cerebral malaria (Kihara et al., 2010b). As will be discussed in more depth in Chapter 2, the implementation for neuroimaging holds great potential for use in young infants as it is not reliant on overt responses by the infant, and thus less affected by cultural expectations of infant behaviour. As described, the above studies have begun laying the foundation for understanding normative development in their respective populations of interest, which have been, or in the future will be, investigated relative to specific risk factors. From a range of other studies, we have gained an understanding of which factor(s) might be most implicated in the study of infant cognitive development in LMIC's as will be discussed in the following section.

1.4 Risk factors in LMIC's and their association with cognition

Associations have been made between a large range of risk factors prevalent in LMIC's and poor neurodevelopmental outcomes. Many of these factors have been shown to impact development from conception onwards,

thus affecting individuals pre-, peri- as well as postnatally. Factors tend to be interlinked and more often than not affect more than one domain, but can be broadly clustered into biological- (i.e. undernutrition, disease occurrence, exposure to toxins), healthcare and infrastructural- (i.e. access to sanitation and healthcare) and psychosocial factors (i.e. parental mental health, family structure, home environment). Each one of these factors has known associations with cognitive development, as is reviewed in Table 1.1. Papers examining cognitive outcomes were included based on the most commonly reported poverty related risk factors. Where possible, review papers were favoured over stand-alone empirical papers to better understand the generalised effects of a factor or a group of factors on cognitive outcome.

The reviewed papers encompass a number of large scale reviews which in sum, suggest links between said factors and childhood developmental outcomes, be it with regards to cognition, mental or physical health. The associations between the presented risk factors and adverse developmental outcomes are striking, warranting further research into mechanistic explanations of the observed outcomes. Also, it becomes apparent that while some studies have taken a broader view in taking multi-factorial approaches (Ruiz et al., 2016), as of now empirical evidence examining interlinking risk factors within the same cohort is still sparse.

1.4.1 Complex interrelation of risk factors.

It can easily be appreciated that the reviewed risk factors do not act in isolation, but rather are interacting with one another on multiple levels. A famine or temporary food shortage for example may affect infants' nutritional status via the mother's breastmilk, but may also lead to heightened levels of maternal anxiety. Another result might be that parents spend longer hours working to provide sufficient food, leading to a reduction in time spent interacting with the infant. In addition, temporary undernutrition might make the infant more

prone to infectious diseases, compounding and perpetuating the cycle of undernutrition for a prolonged period of time. It has been proposed in the context of nutritional deficiencies that duration, timing and severity greatly matter in terms of predicting later outcome (Georgieff, 2007). If this episode of food shortage endures for a long time or occurs repeatedly, long-term consequences are likely, such as elevated risk during pregnancy and child birth once the infant grows up to be an adult (Black et al., 2013), thus carrying over negative effects across generations.

In a recent paper, Jensen, Berens & Nelson (2017) conceptualised the compounding effects of co-occurring risk factors associated with poverty, by delineating mediating pathways between environmental risk and neurocognitive outcome (Figure 1.4). The authors conclude that more comprehensive studies are needed that collect data on multiple risk factors and outcomes, in order to more precisely understand these pathways in different at-risk populations. Further, they also highlight the importance to understand moderating variables, which could give an indication of possible protective factors, and as such form the first targets for randomised controlled interventions to alleviate some of the negative impact of certain risk factors on the developing brain.

Table 1.1. *Review of major publications relating to cognitive outcomes after exposure to one or multiple risk factors prevalent in LMIC's.*

Reference	Population	Risk factor examined	Task/Assessment	Domains affected
		Nutrition Related Factors		
DeRegnier et al., 2000	newborn infants	maternal diabetes	ERP's / Bayley Scales	A negative slow wave ERP component was found to be attenuated in the infants of diabetic mothers. Amplitude of said component at birth correlated with cognitive composite score on Bayley Scale at 12 months
Algarin et al., 2013	children with a history of iron deficiency anaemia during infancy tested at 10 years	iron deficiency	ERP (go/no go task) assessing inhibition	inhibition was negatively affected in formally iron deficient group, as evidenced by longer RT's and smaller ERP amplitudes
Tonoli et al., 2014	meta-analysis of studies assessing both children and adults with type 1 diabetes	Type 1 diabetes	meta-analysis, reviewed studies primarily focused on IQ	Small to moderate reductions in IQ, executive functions and motor speed in children, full-scale, verbal and performance IQ reductions in adults
Camargos et al., 2017	6-24 month old infants	Infant obesity	Bayley Scales	composite cognitive score found to be correlated with obesity biomarker based on blood lipid levels

Nutrition Related Factors (continued)				
Black et al., 2013	review of links between cognitive outcomes, maternal and child nutrition	maternal and child nutritional deficiencies	N/A – little detail provided	discusses links between a range of micronutrients (iron, vitamin A, zinc, iodine, folate, calcium, vitamin D), deficiencies of mothers or infants are negatively associated with cognitive outcomes and how associated factors such as stunting, underweight and wasting compound the problem (i.e. higher rates of infections)
Roberts et al., 2017	children 1-3 and 5-7 years in Guinea-Bissau	RCT of 11 week multi-nutrient supplementation	executive functions: WM and task switching, also brain functioning assessed through fNIRS measure (only post-intervention)	multi-nutrient supplement associated with better working memory performance in 1-3 year age group, magnitude of haemodynamic response was associated with task switching task.
Robles et al., 2017	children between 0 and 12 years	maternal diabetes	general cognitive functioning (Bayley Scales, WPPSI, WISC)	most studies either support poorer neurocognitive outcomes of children of diabetic mothers, or find mixed results in which good glycaemic control acts as a protective factor

Nutrition Related Factors (continued)				
Fugelstad et al., 2008	reviews associations of specific nutrients on brain development, children and adults	macro and micro nutrient deficiencies	structural brain scans, diverse neuropsychological batteries of general cognitive functioning	whole brain volume reductions, specific structures affected: hippocampus, PFC. Cognitively, impairments found in memory and language development, processing speed, WM, abstract reasoning motor development and attention
Water, Sanitation and Hygiene Related Factors				
Ngure et al., 2014	review of studies on infants and young children in LMIC's	poor access to sanitation as cause of enteropathy and undernutrition	Reviewed paper utilise a range of behavioural cognitive and motor tasks	reduced scores observed in context of exposure to cognitive, sensorimotor and socioemotional development, memory and processing speed
MacIntyre et al., 2014	review of studies on diarrhoeal diseases in infants and children	diarrhoeal disease	N/A – describes pathways not specific outcomes	proposes pathways in which diarrhoeal disease might be linked to sanitation and nutrition, leading to a persistent cycle of infections, all impacting cognitive development.

Water, Sanitation and Hygiene Related Factors (continued)				
Campbell et al., 2015	review proposing a framework on how WASH affects maternal reproductive- and in turn perinatal infant health	contaminati on of water, provision of sanitation and hygiene related behaviours	N/A – provides framework of compounding effects of WASH related factors	describes links between poor sanitation and hygiene infrastructure and poor cognitive outcomes
Parental Mental Health and Child Emotional Security				
Van den Bergh et al., 2005	review of studies on maternal stress in relation to child development in the fetal period, during infancy and childhood	maternal anxiety and stress	reviewed studies focus on neurobehavioural assessments, EKG recordings, mother infant interaction	maternal stress correlated with attention deficits in children, higher impulsivity, and increased memory problems

Parental Mental Health and Child Emotional Security (continued)				
Margolin et al., 2000	review of studies on children of a broad age range	exposure to community- or inter-parental violence, and child maltreat-ment	reviewed studies use various behavioural cognitive assessments	exposure to community or domestic violence found to relate to impairments in memory, verbal IQ, maths, academic achievement as well as structural brain abnormalities
Stein et al., 2014	review of studies on children between 3 months and 19 years	perinatal mental health	reviewed studies utilised primarily questionnaire based mental health measures, mother and teacher reports	maternal perinatal mental health was found to relate to mental health problems in children (depression, anxiety), fear regulation and problems with social engagement

Healthcare and Disease Related Factors				
Burger, 2010	review of programs supporting preschool-age children	no access to early education programs	reviewed studies used primarily teacher/parent reports, questionnaires, some domain specific neuropsychological assessments (IQ, reading, maths)	across the vast majority of programs positive associations were found between early education programs and tested cognitive domains
Stevens et al., 2006	infants, children adolescents (aged 0-19 years)	poor access to healthcare	measures of children's physical health	as would be expected child health status was found to be higher in children enrolled in health care programs
Mwaniki et al., 2012	review of studies assessing individual exposed in intrauterine and neonatal insults	intrauterine and neonatal insults	range of medical and cognitive assessments	most common outcomes were identified to be learning difficulties, developmental delays, or conditions such as cerebral palsy

Healthcare and Disease Related Factors (continued)				
Kihara et al., 2010	children aged 4-12	former history of cerebral malaria	ERP novelty detection paradigm	children with a history of cerebral malaria (particularly with severe disease progression) showed a different ERP waveform profile in the ERP novelty paradigm, in line with a reduced ability to detect novel stimuli
Abubakar et al., 2007	children ages 7 months to 9 years	history of seizures or cerebral malaria	Kenyan Screening test with additional items from behavioural neurodevelopmental tests	no differences between children receiving prophylactic dose of anti-epileptic drug and placebo group
Exposure to Environmental Toxins				
Guxens & Sunyer, 2012	review of studies on a wide age range of children exposed to environmental toxins	air pollution and environmental toxins, inflammatory disease	reviewed studies used primarily behavioural cognitive assessments	deficits identified related to cognitive- (IQ, language, executive function), emotional- and motor development

Exposure to Environmental Toxins (continued)				
Bellinger, 2011	US children 0-5 years	environ-mental chemicals	behavioural IQ assessment	exposure to toxins related to reductions in full scale IQ
Multi-factor Approaches				
Ruiz et al., 2016	review examining a range of environmental factors on children of different ages	Comprehen sive review of over 100 factors	reviewed studies used a wide range of measures, primarily behavioural	Assessed demographics, inherent factors of child (sex, weight, height, birth complications), inherent factors of parents (parity, health status, mental health), diet (breastfeeding & duration), lifestyle factors (alcohol in pregnancy, smoking), social environment (family structure), natural environment (exposure to led and other toxins, house quality) associations found for inherent, behavioural social and physical environmental factors, negative cognitive outcomes were most consistently linked with demographic factors, maternal education, home and family environment and presence of environmental toxins

Note. ERP = event related potential, WM = working memory, RT = reaction time, IQ = intelligence quotient, RCT = randomised controlled trial, fNIRS = functional near infrared spectroscopy, LMIC's = low and middle income countries, WASH = water, sanitation and hygiene, WPPSI = Wechsler Preschool and Primary Scale of Intelligence, WISC = Wechsler Intelligence Scale for Children, PFC = prefrontal cortex.

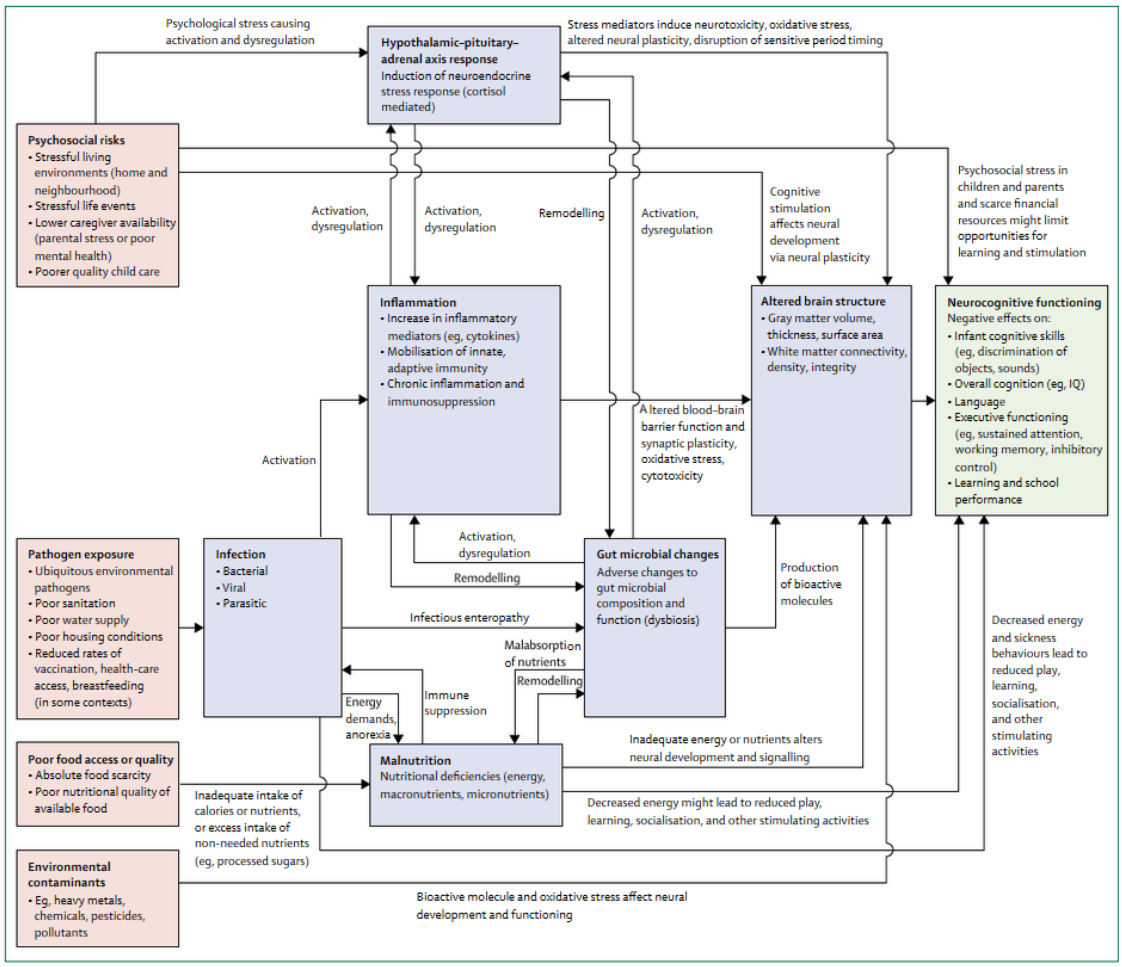


Figure 1.4. Reproduced from Jensen et al. (2017). Proposed biological pathways that mediate effects of selected poverty-associated risks to neurocognitive outcomes in children.

In terms of memory outcomes the model displayed in Figure 1.4 focuses specifically on WM, rather than long term memory. There are several possible reasons, for why WM might have received more focused attention over other forms of memory. First, as reviewed WM can be thought of as activated long term memory and as such provides an index of both attention and memory (Cowan et al., 2008). Alongside attention and inhibitory control, WM is further one crucial component of executive functions, which are of great interest due to their predictive power for later life outcomes (Alloway et al., 2004). While for this reason WM is of great

importance in determining present and future day to day functioning, a more diverse set of memory outcomes has previously been associated specific environmental factors as will be reviewed in the following.

1.4.2 Environmental adversity and memory development.

Similar to other cognitive domains assessed, memory development has been shown to be compromised in the context of a variety of factors both in utero as well as during the postnatal period.

From a nutritional view point, certain key nutrients have shown links with the neural architecture implicated in memory functioning and in consequence with memory performance. One of the most researched links hereby is the one between iron deficiencies and neurocognitive functioning. In rat models, foetal and neonatal iron deficiency have been shown to be associated with metabolic changes in the hippocampus and frontal cortex, leading oxygen to be metabolised less reliably (de Ungria et al., 2000). Further, structural changes within hippocampal dendrites have been shown to lead to less efficient synaptic transmission (Jorgenson, Wobken & Georgieff, 2003). Iron deficiencies have further been associated with altered profiles of fatty acids and myelin in the entire brain (Beard, Wiesinger & Connor, 2003) and whole brain changes further include reduced whole brain volumes in deficient individuals (Jorgenson et al., 2005). At the behavioural level, rodents have been shown to be impaired in hippocampally dependent processes such as their recognition- (McEchron et al., 2005), procedural- (Beard et al., 2006) and spatial memory (Felt & Lozoff, 1996).

While less is known about the links of iron deficiency and memory outcomes in human infants, it has been shown that newborns who are iron deficient due to severe maternal anaemia, intrauterine growth restriction, increased iron demands due to poorly controlled maternal diabetes or as a result of

premature birth show a suboptimal neurodevelopmental outcomes (Chockalingam et al., 1987, Georgieff et al., 1996, Petry et al., 1992). Studies examining neuropsychological development of these perinatal deficiencies later in life have reported that affected children showed lower school performance and impaired auditory recognition memory in infancy (Siddappa et al., 2004).

While iron deficiencies are the most well- documented in terms of their cognitive outcomes, some pathways have also been suggested for other nutrients. Zinc deficiencies have been associated with less efficient synaptic transmission with particular vulnerabilities of the orbitofrontal cortex (Frederickson & Danscher, 1990). Zinc deficient monkeys, were shown to have poor short-term memory (Golub et al., 1994), which has been interpreted as implying particular vulnerabilities in the MTL and frontal lobes (Georgieff, 2007). Further, some links have been proposed between protein deficiencies, which again was linked to a particular vulnerability of the memory system, having shown that both verbal and visual recognition memory seem to be impaired (Gottlieb, Biasini & Bray, 1988, Gorman & Pollitt, 2013). In summary, while some of these studies imply rather global deficits related to whole brain anomalies which could have an impact on memory (Beard et al., 2003, Jorgenson et al., 2005), but are not be specific to memory development, others (i.e. iron deficiencies and hippocampal abnormalities, Jorgenson et al., 2003) propose specific pathways for more selective deficits.

Another group of factors that has been associated with memory development are, birth complications and premature birth. Low birth weight and low weight for gestational age have also been linked to poorer memory outcomes (Isaacs et al., 2000), as has been prematurity (Vicari et al., 2004, Saigal & Doyle, 2008), even in otherwise healthy, low-risk babies (de Haan et al., 2000). Early insults, such as temporary oxygen deprivation in

the perinatal period can cause severe bilateral hippocampal atrophies, and in turn, memory impairments (Vargha-Khadem, Gadian & Mishkin, 2001). While studies showing reduced impairment in association with prematurity may affect individuals' academic attainment, these deficits associated with hippocampal atrophy have profound, life-long implications for affected individuals.

1.4.3 Implications for Interventions. From the reviewed literature it becomes apparent that rather than one factor having a large effect, it is more likely that many factors have small effects on the outcome which in their interaction have potential to amplify each other. This also suggests that when it comes to implementing interventions, targeting any single level will likely have a less pronounced effect than targeting a cluster of factors simultaneously. While this type of intervention has not yet been established in any LMIC with regard to neurocognitive outcome, a recent review of growth stunting in rural Gambia has posed the question of why reductions in proportions of stunted children have not been achieved thus far, despite continued efforts to intervene. The authors conclude that in addition to the nutrition specific (i.e. supplementation trials) and nutrition sensitive (i.e. sanitation and hygiene interventions) interventions themselves, other improvements in children's environments are needed to achieve the full effect (Nabwera et al., 2017). A similar conclusion was drawn by Nair et al. (2017) who implemented an intervention consisting of both women's groups and regular home visits to families with young infants from birth onwards. This intervention had previously been shown to have a positive effect on maternal mental health (Tripathy et al., 2010) and a review of similar interventions demonstrated significant reductions in neonatal mortality (Prost et al., 2013). Yet, examining the effects of participation in women's groups and regular home visits on infants' growth (length z scores) found no direct effects, even though neonatal mortality did decrease in the

experimental group and secondary effects were reported in terms of increased nutritional diversity and maternal hand washing prior to breastfeeding (Nair et al., 2017). The authors conclude that without a combination of measures, such as increased access to clean water and sanitation facilities accompanying behavioural change interventions effects were bound to be limited. They contrast the limited reductions in stunting in the Gambian context to other settings, in which a combination of public policy changes, income equality, education and access to water and sanitation has led to much more rapid reduction in growth stunting (Monteiro et al., 2010). The prevalence of growth stunting is among several issues prevalent in the Gambia, as will be reviewed in the following section.

1.4.4 Known risk factors in rural Gambia. For the past decade, universal education to the secondary level has been freely available across the country, however among the current parent generation, the parental and particularly maternal education levels are still low (Hennig, 2015). Further, there is some evidence for the prevalence of mental health issues in a proportion of new mothers. While still an understudied topic, some studies suggest that 7.5% of mothers experience elevated depressive symptoms at some point between the immediate antenatal period and the first six months postpartum (Bartram-Torrance, unpublished observations). There have been substantial improvements in this population over the past decades regarding access to clean water and sanitation, through the installation of latrines in each compound and standpipe wells at several points throughout the villages, greatly reducing infant and child mortality (Nabwera et al., 2017). Another factor is the universal, free healthcare that is offered to the population at several field stations throughout the country, and also provides antenatal care to pregnant women, and baby checks within 72 hours postpartum (Hennig et al., 2017).

As will be described in Chapter 2, many families support themselves through subsistence farming, meaning that during harvest time, many mothers are working in the fields. This leads to seasonality dependent differences in maternal engagement with the infant. Seasonality also plays a key role in the availability of nutrients throughout the year, as due to the alternating seasons the availability of crops varies throughout the year (Nabwera et al., 2017). Resulting nutritional deficiencies are reflected in anthropometric measures of infants' growth over the first months of life. From data gathered on infant growth over the past decades, it can be seen that while since the 1980's average values of head circumference measurements fall approximately within the mean range of WHO norms at the time of birth, infants do not consistently track the same trajectories thereafter (Figure 1.5). During the first two years of life, infants do not exhibit stable percentile ranks, but rather show a drop in z-score relative to WHO norms.

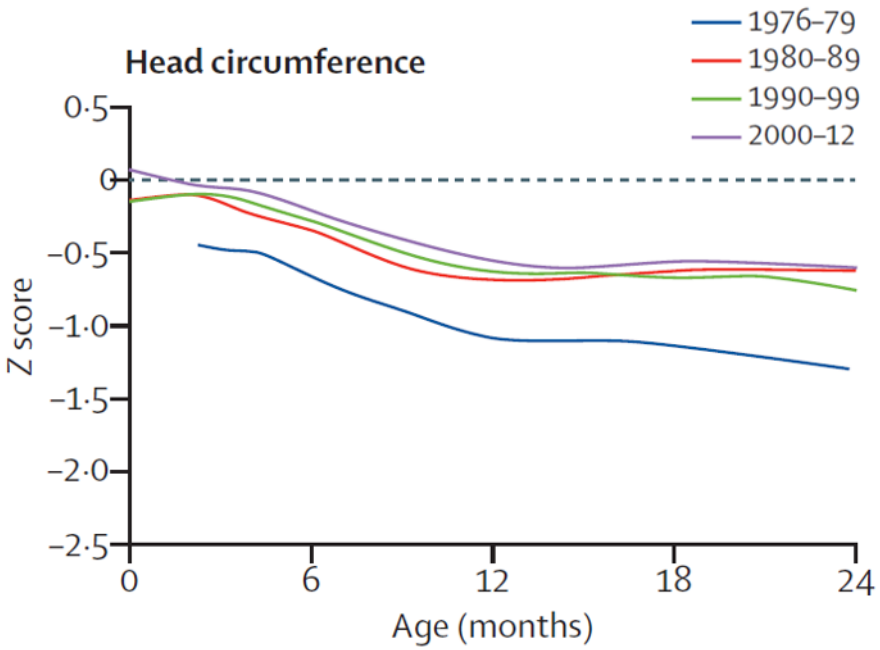


Figure 1.5. Reproduced from Nabwera et al. (2017). Infants' head circumference plotted against WHO z-scores. Trajectories are displayed per decade from 1976 -2012. All four lines show a decrease in z-score over the first 24 months of life. Even though since the 1980s head circumference measures at birth are closer to the WHO mean, across all four decades a decrease in z-score over the first 24 months of life is evident.

Head circumference measures are of interest when studying brain development. Even though brain function is dependent on more factors than just the mere size of tissue (Schoenemann et al., 2000), and there seems to be a relative sparing of the brain during periods of limited resources (Simmons et al., 1992), there still is reason to assume that this development is indicative of environmental deprivation that may affect cognitive functioning.

1.4.5 Brain Imaging for Global Health: The BRIGHT project. From the extensive data available on growth and nutritional outcomes in the rural Gambia, and the documented high prevalence of stunting and growth faltering over the first two years of life, the question has arisen regarding the extent to which brain and cognitive development are affected. To assess

this, a large scale, multi-centre project is currently underway that aims to establish longitudinal neurocognitive markers of infant development in Africa. The Brain Imaging for Global Health (BRIGHT, globalfnirs.org/the-bright-project) project is a collaboration of researchers in several UK institutions and the Medical Research Council at the London School of Hygiene and Tropical Medicine in The Gambia. The project longitudinally follows an infant cohort in the rural village of Keneba, The Gambia, and in Cambridge and surrounding villages in the UK. The project assesses a broad range of cognitive domains such as social cognition, novelty detection, early WM and language development. This is achieved through an extensive protocol of neuroimaging, eye tracking, behavioural and questionnaire-based measures, which is described in more detail in Chapter 2. In addition to these measures, data are collected on risk factors such as nutritional status, parental mental health, and family environment, ultimately allowing for an in-depth analysis of reasons behind the observed developmental outcome. The project is one of the first to collect neuroimaging measures in infants in resource poor settings and with the range of included studies will contribute to a holistic understanding of the factors implicated in early infant cognitive development and will therefore be yielding invaluable new insights on global infant development.

This thesis is based on a subset of data collected in context of the BRIGHT project. The project began at the same time as my PhD, and I have since the beginning worked closely with the team, being involved in piloting and setting up the specific studies that entered the protocol. My work has fed into many studies run as part of the BRIGHT protocol. My main role has been to implement and oversee the EEG studies of this project. While it had been determined that an EEG study would be included in the BRIGHT project before I started my PhD, these had not previously been piloted at the Gambian study site. Throughout the project, I led on the hardware set

up, experimental design, training of the team, day to day oversight of the data collection and analyses.

Over the first year, when refining my research questions, I reached an agreement with the project lead as to which other paradigms would be best to complement my thesis project, and I took on the oversight of one paradigm within the fNIRS protocol, as well as the design and implementation of the behavioural study included in this thesis. I have since been responsible for these paradigms, both in terms of training and addressing day to day issues, as well as analysing resulting data. The fNIRS working memory paradigm had previously been used in a pilot study in The Gambia, however I was able to modify the task to yield more meaningful data and to introduce it at a younger age point, to capture potential developmental changes. I saw through the design and implementation of the behavioural paradigm.

Due to the logistics of the project, I have primarily collected data at the UK site, for the full BRIGHT project protocol, most of which will be used by other researchers at both sites. In exchange, I was able to use all data from my studies from both sites.

1.5 Thesis outline and research aims

Over the following chapters of this thesis, I will first lay out the general methodology implemented in the empirical chapters (Chapter 2), before moving on to presenting the findings of the three studies conducted as part of this project (Chapters 3-6). Each empirical chapter contains a study targeted at capturing developmental changes in early memory functioning relevant to the studied age ranges. Hereby, different concepts related to memory will be tapped, starting at pre-explicit memory through an investigation of habituation and novelty detection, on to early explicit

memory by an investigation of infants' imitation behaviour and their emerging WM close to their first birthday. Chapter 3 will present a study examining the development of habituation and novelty detection between the ages of 1 and 5 months of age, using an auditory ERP paradigm. Chapter 4 will then follow up results from Chapter 3 using spectral EEG analysis approaches, which were explored as part of a small side project to this thesis. Chapter 5 will examine the development of early WM development between the ages of 8 and 12 months by measuring the haemodynamic fNIRS response in an object permanence and WM paradigm. Chapter 6 will present behavioural data, collected at 8 and 12 months on a deferred imitation paradigm. Each study allows for some specific predictions regarding previously reported developmental changes, which will allow for an investigation into the replicability of established findings in a new cohort, in which development might progress differently, due to a vastly different environmental experience and in context of known risk factors for early infant development. In the final chapter (Chapter 7) I will discuss all findings in relation to one another and draw some general conclusions regarding the implications for this line of research.

The overarching aims of the presented studies will be:

1. To establish normative longitudinal data on early memory development on a set of tasks in both the rural Gambia and the UK, in context of exposure of experienced environmental risk.
2. To examine the replicability of established paradigms assessing early memory capacity in the two cohorts.
3. To further develop paradigms to assess early memory development which can more easily be modified for use in a range of resource poor settings.

4. To identify possible differences and commonalities between the two cohorts regarding performance at each age point and subsequent developmental changes.

1.6 Implications for methodology

In order to address the aforementioned research question in the two populations investigated in this thesis, careful consideration had to be put into choice of adequate assessment methods and study paradigms. In Chapter 2, the methods suitable to assess young infants in partly remote, rural settings will be discussed. Different methods will be described with regard to the degree to which they enable more objective, culturally fair assessments, before describing in detail the methods and paradigms implemented in this thesis.

Chapter 2. General Methodology

One limiting factor in studying neurodevelopmental trajectories in LMIC's is the limited availability of assessment tools that can offer objective, mechanistic insight into brain development, with proven validity across cultures. Technological advances, especially in the field of neuroimaging have yielded several tools which lend themselves to the study of young infants from birth onwards. The high levels of objectivity that these tools offer makes them well- suited for use in cross cultural research. Their portability enables the assessment of a broad range of cognitive functions in even remote and rural settings. While the potential of these novel methods so far has not been fully realised, they present a promising avenue within the study of global infant development, as will be laid out in the following.

This chapter describes the context and the general methodology underlying the three studies relevant to this thesis. First, I will describe the two study cohorts and the participant populations in Cambridge, UK and in Keneba, The Gambia. I will then discuss the methodological implications of conducting neurodevelopmental research in rural, remote settings before laying out the design and procedure for the electroencephalography (EEG), functional near infrared spectroscopy (fNIRS) and behavioural imitation study which form the basis of this thesis.

2.1 Study sites

2.1.1 Keneba, The Gambia. Studies in The Gambia are conducted at the Keneba field station of the MRC Unit The Gambia at the London School of Hygiene & Tropical Medicine (MRC Keneba), which is one of several MRC field stations situated in different regions throughout the country. The Gambia is the smallest country on the African continent located in the far west and surrounded entirely by the neighbouring state of Senegal (Kebbeh, 2014). Its capital Banjul is located at the Atlantic coastline from where the river Gambia runs upcountry and divides the land into the North- and the

Southbank regions. The majority (60%) of the roughly 2 million inhabitants live in the coastal regions surrounding the capital, with the remainder of the population living rurally, often supporting themselves through subsistence farming (Hennig et al., 2015). The Gambia is one of the lowest ranking countries with regard to gross national income, years of schooling and life expectancy (UN, 2011), with over half of adults in The Gambia never having received formal education (Hennig et al., 2015). For the last decade however, education levels have risen as there is now free universal education which 97% of children attend to primary level (UNESCO, 2014).

Marriages are commonly polygamous with over half of wives living with one or two co-wives (Hennig et al., 2015). Over the past decades infant and child mortality has decreased, birth spacing has increased, and overall family size has gone down (Nabwera et al., 2017).

While there are multiple ethnic groups in the country, mostly associated with their own language and cultural customs, the largest part of the population belongs to the Mandinka ethnic group (Jukes & Grigorenko, 2010). This is particularly true for the region surrounding MRC Keneba. For this reason, it was decided to only enrol families of the Mandinka group into our study, to avoid confounds in our sample due to translation of stimuli and questionnaires into multiple languages.

The MRC Keneba provides healthcare to the population in the West Kiang region and has a long-standing tradition of research into population health and nutrition. This is partly due to the contrasting weather patterns which alternate between six months of heavy rains and six months of extreme dryness, which directly affects the availability of key nutrients (Moore et al., 1997). Due to the long-standing collaboration with the MRC and the local community, interest by the population to participate in studies at the MRC

is very high and the majority of families are enrolled in ongoing research projects.

2.1.2 Cambridge, United Kingdom.

Data collection for the UK branch of this project primarily took place at the Evelyn Perinatal Imaging Centre (EPIC) at Rosie Hospital, Addenbrookes Hospital Cambridge, and to a lesser extent at the Centre for Brain and Cognitive Development in Cambridge. Families were all recruited from the Cambridgeshire area. Demographically, the population in Cambridgeshire is representative of that across the UK with regard to ethnicity, employment rates and family structure (Cambridge City Annual Demographic and Socio-Economic Report, 2011). The area however differs from the rest of the UK with regard to levels of education within the population, with twice as many inhabitants holding a higher education degree (Cambridge City Annual Demographic and Socio-Economic Report, 2011).

2.2 Recruitment

2.2.1 Keneba.

In the West Kiang region in which MRC Keneba is situated, pregnant women across all neighbouring villages are routinely reported to the Demographic Surveillance System which captures information about the local population. After first identification of a pregnancy, women undergo clinical assessments at MRC Keneba field station to determine gestational age and to aid early identification of potential complications related to the pregnancy. For recruitment into the BRIGHT project, women were approached by a field worker of the Gambian BRIGHT project team during this antenatal clinic visit.

Women were explained the aims of the BRIGHT project, the structure of the longitudinal visits and the assessments used at each visit and were shown pictures of infants performing each assessment. Women were given an

information sheet (Appendix 2.1) and a consent form written in English (Appendix 2.2), which was explained thoroughly in Mandinka by the recruiting field worker and were then asked to take the information home and share it with their husbands. Potential participants were then approached again the next day by local village assistants and those that decided to participate were formally enrolled in the study. Interested women were enrolled unless testing capacity of 10 infants per month was already reached for the period within which it was anticipated for the infant to be born. Participants were excluded from the study if (a) the infant was born prior to 37 weeks of gestation (b) the baby was found to have any severe medical or neurological deficits at birth (c) if a multiple pregnancy was identified (d) if testing capacity for the anticipated birth month had already been reached. A summary of the numbers approached, consented as well as reasons for withdrawals can be found in Figure 2.1. At the beginning of each study visit, mothers were explained all procedures again and reminded that each part of the visit was voluntary and that they could withdraw at any time.

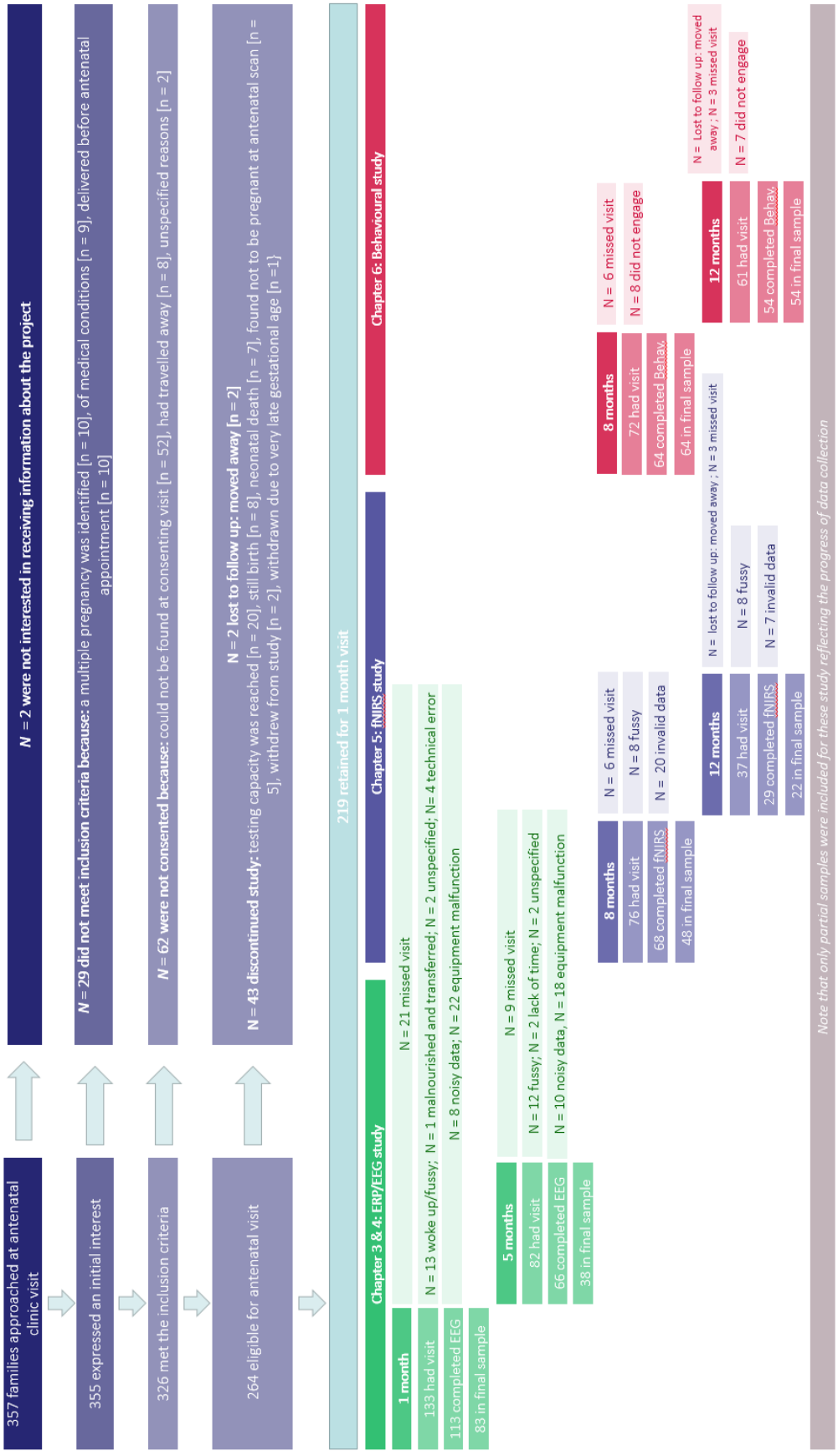


Figure 2.1. Numbers of participants approached, consented and retained at Keneba site for each study in this thesis.

2.2.2 Cambridge. Pregnant women and their partners were approached during their visit to the birth centre at 36 weeks of gestation at the Rosie Hospital at Addenbrookes Hospital in Cambridge. One researcher who was generally accompanied by a research midwife explained the aims of the project and the structure of the longitudinal study visits. Potential participants were shown images of the different assessments and given some context specific to the brain imaging measures employed. Those who indicated an interest were then given some additional information on a parent information sheet to take home (Appendix 2.3). Verbal consent was obtained from interested couples to be contacted at a later date. A member of the research team would contact those who had expressed an interest a few days after the initial contact and schedule the antenatal home visit with families that decided to participate. Written consent was obtained from both parents at the home visit (Appendix 2.4). Participants had to be excluded if (a) the infants were born prior to 37 weeks of gestation, (b) if a multiple pregnancy was identified, (c) the infant was diagnosed with any major medical or neurological deficits at birth. Due to the higher precision of gestational age checks in this cohort, no participants had to be withdrawn due to testing capacity limits. Rather, recruitment was put on hold for periods during which capacity was reached.

At the beginning of each study visit, parents were thoroughly explained all measures that were going to be performed on the day and asked to sign a sessional register confirming that they consented for their infant to participate in the assessments or, if applicable, that they objected parts of the assessment should they wish to. A summary of the numbers approached, consented as well as reasons for withdrawals can be found in Figure 2.2.

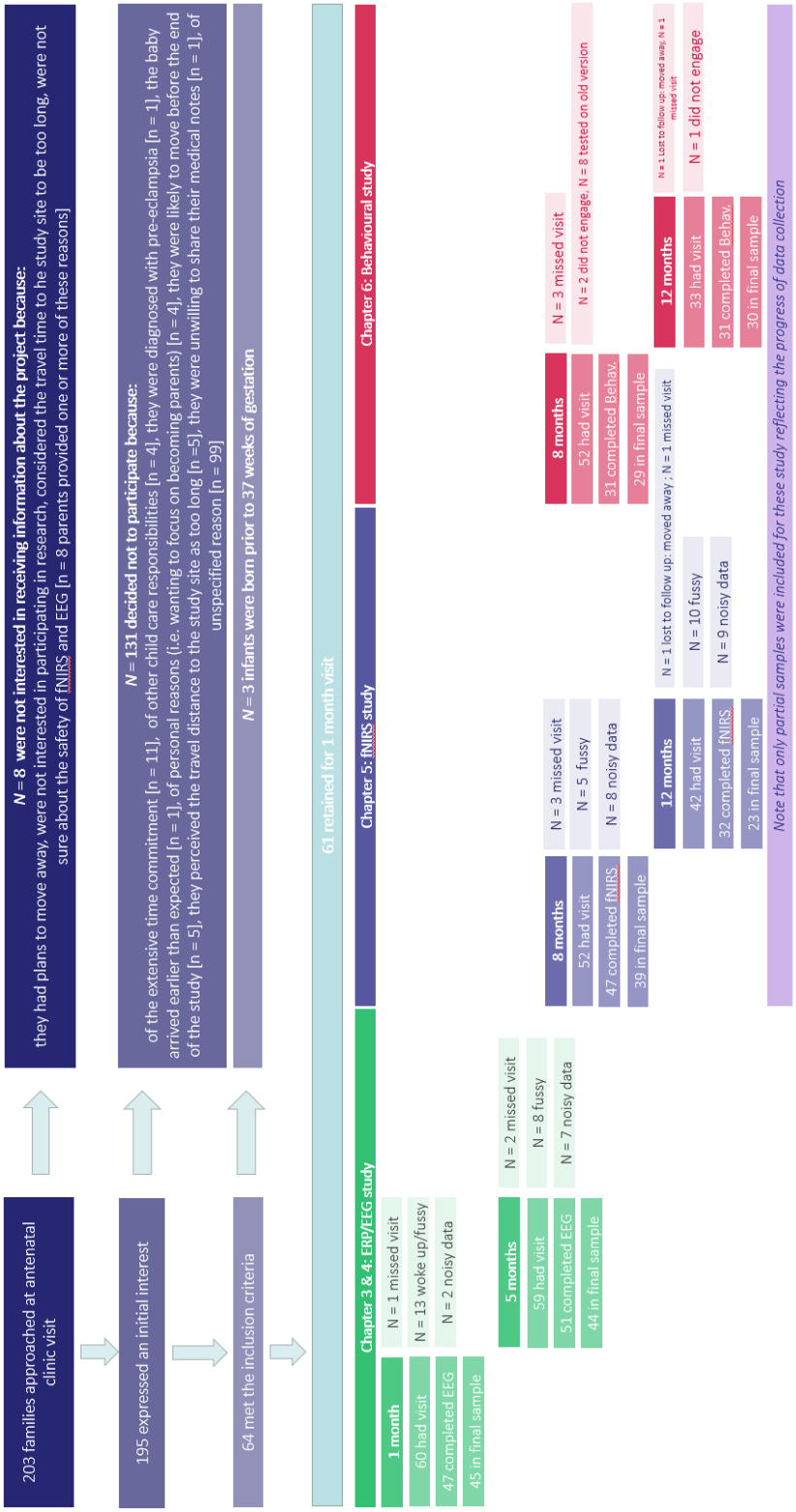


Figure 2.2. Numbers of participants approached, consented and retained at Cambridge site for each study in this thesis.

2.3 Participant characteristics

While some demographic data such as age on the day of testing is provided in context of each of the studies, some background will be provided here regarding the socioeconomic status of the enrolled families. Demographic and socioeconomic status data was collected at the 7-14 day study visit, and where possible maternal, paternal and infant characteristics were collected. The exact questions asked differed between study site, as they were in each setting based on meaningful indicators proposed by previous studies (Watson et al., 2018). Summary statistics can be seen in Tables 2.1-2.3.

Table 2.1 *Cambridge parental characteristics*

	Age at infant birth	Highest educational level obtained			Number of children (including participant)	Occupation Hours employed per week
	X ± SD	Secondary	Undergraduate	Postgraduate	X ± SD	X ± SD
Maternal	32.66 ± 3.01	9.8%	36.1%	54.1%	1.31 ± .53	36.6 ± 8.03
Paternal	34.45 ± 3.94	18.0%	29.5%	52.5%	1.31 ± .53	37.76 ± 5.31

Table 2.2 *Keneba parental characteristics.*

	Age at infant birth	Years of schooling	Number of children (including participant)	Occupation		
	X ± SD	X ± SD	X ± SD	Agricultural farming	Trade or employed	Unpaid work
Maternal	29.68 ± 6.89	3.25 ± 4.13	4.29 ± 2.67	62.4%	3.2%	34.4%
Paternal	42.18 ± 9.881	5.06 ± 5.295	7.2 ± 5.675	41.8%	52.7%	5.5%

Table 2.3 *Infant characteristics across both sites.*

	Gender	Mode of delivery			Gestational age	Birth weight
	% girls	Vaginal	C-section	Missing data	X ± SD	X ± SD
Cambridge	50	82.3%	16.4%	1.3%	40.3 ± 1.262	3.469 ± .483
Keneba	52.1	92.7%	1.2%	6.6%	38.6 ± 1.203	3.09 ± .364

Our sample in Keneba was found to be representative of the wider population in terms of maternal and paternal age, years of schooling, number of children and occupation. Our sample in Cambridge had elevated rates of parents with a higher educational degree, as was anticipated in the Cambridgeshire area as previously discussed. Rather than allowing a comparison between the two cohorts investigated in this thesis, this data is aimed to provide a better understanding of infants' family environment, which will help contextualise the findings provided throughout this thesis.

The data presented above were further examined to explore any potential differences between those infants that yielded valid data for inclusion in the final sample, and those infants that had to be excluded from analyses. Tables 2.4 and 2.5 show the sample characteristics separately for those infants that were included or excluded for each of the three studies presented in this thesis.

Table 2.4. *Keneba sample characteristics of infants included/excluded from analyses across the three studies.*

		Chapter 3 & 4: EEG/ERP study				Chapter 5: fNIRS study				Chapter 6: Behavioural study			
		1 month		5 months		8 months		12 months		8 months		12 months	
		Retained	Rejected	Retained	Rejected	Retained	Rejected	Retained	Rejected	Retained	Rejected	Retained	Rejected
Parental characteristics													
Age at birth	M	25.1	29.2	28.3	24.2	30.1	31.2	29.3	30.1	27.9	30.2	31.0	29.2
	P	37.5	39.2	37.1	41.1	40.1	38.2	42.3	36.1	37.2	41.1	43.4	40.1
Years of educati	M	2.9	3.1	3.7	4.1	2.5	3.8	3.7	4.1	2.8	3.1	3.5	3.1
	P	5.6	4.8	5.2	5.1	4.7	4.9	4.2	4.8	5.2	4.6	5.6	5.1
# of children	M	3.9	4.4	4.1	4.5	3.8	4.6	4.3	4.1	4.5	3.6	4.2	4.5
	P	8.1	7.6	6.9	7.2	7.6	6.8	6.9	7.5	7.2	6.7	7.4	7.1
Occupation	M	60.6 AG 2.1 TE 37.3 UP	59.8 AG 1.7 TE 38.5 UP	64.3 AG 3.5 TE 32.2 UP	61.3 AG 3.7 TE 35 UP	62.5 AG 4.2 TE 33.3 UP	59.8 AG 2.1 TE 38.1 UP	58.7 AG 2.5 TE 38.8 UP	61.4 AG 2.8 TE 35.8 UP	62.1 AG 2.5 TE 35.4 UP	59.2 AG 2.8 TE 38.1 UP	60.9 AG 3.4 TE 35.7 UP	60.7 AG 3.2 TE 36.1 UP
	P	36.7 AG 55.7 TE 7.6 UP	42.1AG 49.2 TE 8.7 UP	38.1 AG 54.1 TE 7.8 UP	42.1 AG 53.1 TE 4.8 UP	43.4 AG 55.8 TE 0.8 UP	40.8 AG 52.1 TE 7.1 UP	39.2 AG 54.1 TE 6.7 UP	42.8. AG 55.1 TE 2.1 UP	41.7 AG 51.2 TE 7.1 UP	40.1 AG 50.8 TE 9.1 UP	39.2 AG 49.8 TE 11 UP	41.9 AG 51.2 TE 6.9 UP
Infant characteristics													
Gender (% girls)		50	51	49	53	48	52	48	47	50	51	52	49
Gestational age		39.4	38.1	37.2	40.1	39.3	37.9	40.1	39.2	41.4	39.7	39.1	40
Birthweight		3.2	3.1	3.0	2.9	3.3	2.8	3.1	3.2	3.3	2.9	3.3	3.8

Note. M = Maternal, P = Paternal, AG = agricultural farming, TE = trade or employed, UP = Unpaid work.

Table 2.5. *Cambridge sample characteristics of infants included/excluded from analyses across the three studies.*

		Chapter 3 & 4: EEG/ERP study				Chapter 5: fNIRS study				Chapter 6: Behavioural study			
		1 month		5 months		8 months		12 months		8 months		12 months	
		Retained	Rejected	Retained	Rejected	Retained	Rejected	Retained	Rejected	Retained	Rejected	Retained	Rejected
Parental characteristics													
Age at birth	M	34.21	31.9	32.62	35.16	31.51	33.2	34.55	31.17	33.64	32.56	34.68	31.46
	P	32.57	35.68	35.68	34.01	33.67	35.26	32.16	35.25	36.58	32.76	35.9	34.02
Highest level of educational	M	10.7 SS 36.5 UG 52.8 PG	7.6 SS 36.4 UG 56 PG	10.2 SS 38.2 UG 51.6 PG	10.3 SS 35.1 UG 54.6 PG	8.4 SS 39.1 UG 52.5 PG	9.7 SS 35.2 UG 55.1 PG	10.6 SS 31.7 UG 57.7 PG	11.1 SS 34.2 UG 54.7 PG	12.8 SS 38.8 UG 48.4 PG	9.1 SS 34.9 UG 56.1 PG	10.7 SS 36.8 UG 52.5 PG	12.7 SS 37.2 UG 50.1 PG
	P	19.5 SS 27.3 UG 53.2 PG	16.9 SS 29.6 UG 53.5 PG	15.7 SS 26.2 UG 58.1 PG	19.5 SS 31.0 UG 49.5 PG	16.7 SS 26.2 UG 57.1 PG	17.6 SS 29.7 UG 52.7 PG	18.1 SS 30.1 UG 51.8 PG	17.8 SS 29.2 UG 53 PG	16.8 SS 29.1 UG 54.1 PG	19.1 SS 27.2 UG 53.7 PG	17.5 SS 29.7 UG 52.8 PG	18.6 SS 30.6 UG 50.8 PG
# of children	M	1.27	1.32	1.12	1.37	1.02	1.35	1.02	1.01	1.2	1.4	1.05	1.4
	P	1.27	1.32	1.12	1.37	1.02	1.35	1.02	1.01	1.2	1.4	1.05	1.4
Hours worked	M	35.7	34.2	38.1	35.5	37.1	36.2	38.1	32.3	31.1	39.2	31.6	33.4
	P	38.2	36.4	33.9	38.1	37.2	38.4	39.1	36.2	34.7	38.1	36.5	36.6
Infant characteristics													
Gender (% girls)		54	52	53	48	47	51	46	51	52	49	56	41
Gestational age		41.2	39.1	42.1	38.9	40.7	38.1	39.7	38.1	40.2	38.7	41.1	39.2
Birthweight		3.62	3.71	3.32	3.15	3.36	3.41	3.51	3.21	3.62	3.46	3.29	3.66

Note. M = Maternal, P = Paternal, SS = secondary school, UG = undergraduate, PG = postgraduate.

Participants lost to follow up. As can be seen in Figures 2.1 and 2.2 the loss to follow up to date has been relatively limited. As greater numbers of participant withdrawals are anticipated as the study progresses, steps will need to be taken to address potential bias resulting from this. Thus far, wherever participants were at risk of being lost to follow up, for example due to them moving out of the catchment area, it was prioritised to still collect those data that were not dependent on them coming into the lab, i.e. by sending questionnaires via post or with a visiting fieldworker, or by offering home visits, during which some of the assessments (i.e. neurodevelopmental scales) could be performed. This way, it will remain possible to compare participant characteristics between those that were retained and those that were lost to follow up and check for any potential bias.

Biases anticipated in context of this project. A few sources of bias were anticipated prior to the beginning of this study. These included gender imbalances across both neuroimaging modalities, due to the oftentimes longer hair and braids, which were anticipated to lead to a greater exclusion of girls compared to boys, particularly in Keneba. This was not found to be the case in the current investigation, but may become in an issue for older age groups and will thus be monitored. For the full sample, gender will be included as a factor in statistical analyses, using models that allow for weighing of responses in context of unequal sample sizes. Another issue that was anticipated was parents' ability to consistently attend all study visits. From conversations with parents in Cambridge during recruitment, it became clear that the vast majority of those who decided to participate were first time parents, which was in sharp contrast with parents in Keneba where all infants lived with one or more siblings. It was further expected that those parents in Cambridge with caring responsibilities for an older sibling might not be able to attend all study visits, which was found to be

apparent from the analyses performed here, as will be discussed in the following.

Differences in characteristics between those retained and rejected in analyses. As can be seen in Tables 2.4 and 2.5, participants retained and rejected were found to be similar across most demographic dimensions assessed. Exceptions included the number of children, which in Cambridge were found to be larger for those parents who's data was excluded from the final sample (t_{59} = 2.31, p = .024). This finding is in line with our expectation that those parents with additional care responsibilities might be less likely to come in for all visits. It was tried to counteract this issue by offering parents to bring in the sibling and ensure an additional researchers was present to entertain them, or when a visit was impossible to conduct a subset of assessments at a home visit. It should be noted though, that this difference only becomes apparent for one assessment (fNIRS) at one age point (8 month). This could be reflective of a transitional period during which many parents finish their parental leave, and may not have a fully established support system in place. This difference will be monitored for future analyses.

Some differences were further observed in Keneba, with regard to paternal levels of education. It was found that numbers of fathers engaging in unpaid work differed between those rejected and retained for the fNIRS assessment at 8 and 12 months. However, no uniform trend was apparent as a greater proportion of fathers amongst the rejected group worked as an unpaid worker (t_{89} = 6.808, p = <.001) of the 8 month assessment, but a smaller number at the 12 month assessment (t_{48} = 4.6, p = <.001). Due to the contrary directions of these results and because they occurred for only one assessment, it should be cautioned against strong interpretations, as differences may be reflecting a spurious difference.

Due to the approaches described above, some contextual information (i.e. on SES, behavioural development) should be available for most infants by the end of the study. Due to the substantial sample size and number of data points collected on each participant in this project, it will be possible to exploit this contextual information to some degree, and potentially consider imputation approaches where data is found to be missing at random.

The above findings give a first indication of variables that may come to play a role when repeating these assessments for the full study sample. It should be noted, that for the Keneba cohort no clear associations were observed between outcome variables of interest, such as birthweight, or maternal education. This is reassuring, given the importance of these outcomes in context of the aims of this research. It should further be noted that the comparison across a large number of SES indicators is not ideal and highly likely to yield spurious results. It is suggested that along with a more thorough characterisation of the Keneba cohort in particular, indicators most relevant to describe within group differences should be identified (i.e. by principal component analysis, as done in Krefis et al., 2010), and these should be used to make comparisons between infants retained and rejected in future analyses.

2.4 Ethical approval

Ethical approval was obtained for each study site separately. In The Gambia, the BRIGHT project was approved by the local SCC (project title 'Developing brain function for age curves from birth using novel biomarkers of neurocognitive function', SCC number 1451v2, on 13-01-2016, Appendix 2.5).

In Cambridge, ethical approval was granted by the NHS Health Research Authority (project title 'Developing brain function for age curves from birth

using novel biomarkers of neurocognitive function.', reference 15/EE/0202, project 178682 on 03-08-2015, Appendix 2.6).

This PhD project received R&D approval from the Institute of Child Health/ Great Ormond Street Hospital Joint Research Office (project title 'Imaging brain and social-cognitive development in British and Gambian infants', R&D Number 15NP01, on 02-02-2016, Appendix 2.7).

2.5 Implications for methodology

Working with young infants in a low- resource setting poses some constraints on the methods available for use. Three aspects need to be considered in particular:

1. Infant friendliness of the technology
2. Portability of equipment for use in a rural setting and robustness to last in such a setting
3. Objectivity of assessment across different cultures.

Previous studies seeking to assess cognitive development in remote rural settings primarily utilised behavioural assessments which typically have the benefit of being cost- effective, easy to transport in the field and well-suited for infant research (Abubakar et al., 2008). However, behavioural assessments have the disadvantage of only being able to measure development once any given cognitive process has reached a state of observable behaviour, thus subtle early stages of development are inaccessible to many behavioural assessment tools. Several other limitations arise, especially when using behavioural assessments in multicentre cross-cultural research. First, they require extensive training to ensure inter-rater standardisation. Secondly, in infants older than 12 months they can be very time consuming, limiting their potential for routine use in clinical practice. In terms of their implementation in different cultural contexts, the vast

majority of measures have been developed and normed in Western societies, limiting their ecological validity in non-Western settings or requiring prior collection of normative data. As the manifestation of overt behaviour is strongly influenced by social and cultural factors (Bradley & Corwyn, 2002), traditional assessment tools need to be substantially adapted for use in new cultural settings. Some groups have adapted developmental scales commonly used in western settings, such as the Bayley Scale of Infant and Toddler Development (Bayley, 2006) or the Mullen Scales of Early Learning (Mullen, 1995) to make them appropriate for use other cultural contexts (i.e. for use in Kenya, Abubakar et al., 2008). However, these culturally specific adaptations still prevent the scales' more wide-spread use across a variety of cultural settings. With regard to predicting later outcome, behavioural assessments have been shown to be limited when implemented during in the first year of life, especially when used to assess at-risk populations, such as infants with very low weight at birth (Hack et al., 2005) or infants with familial risk of autism (Elsabbagh & Johnson, 2009; Lloyd-Fox et al., 2013).

As part of this thesis, one behavioural study paradigm was adapted, which measures the extent to which infants can imitate and remember novel action sequences. The deferred imitation paradigm has been used extensively to characterise infant's memory development from the second half of the first year of life (for a review see Jones & Herbert, 2006). While using this well- established study paradigm, items were designed specifically with the goal of being novel to infants in both studied populations, as will be discussed in more detail in Chapter 6.

To enable more objective measurements across cultures and in even younger infants, the primary methodological focus of this thesis lay in the implementation of neuroimaging technology. A useful categorisation of the most commonly used neuroimaging techniques with regards to both their

spatial and temporal resolution as well as their degree of infant-friendliness has been developed by Lloyd- Fox, Blasi and Elwell (2010) and is shown in Figure 2.3. Out of the various imaging techniques that are available to research to date, only few meet the criteria of being usable with infants, as well as being transportable in rural remote settings. Most imaging measures used in adult research cannot be easily used in awake infants as they (a) do not allow for movement by the participant (functional magnetic resonance imaging [fMRI], magnetoencephalography [MEG]), or (b) are invasive and therefore not suited for use in infants and non-clinical populations (i.e. positron emission tomography [PET], single photon emission tomography [SPECT]). Additionally, all of these methods require instrumentation that is not easily portable to remote areas and require highly trained staff to operate the equipment.

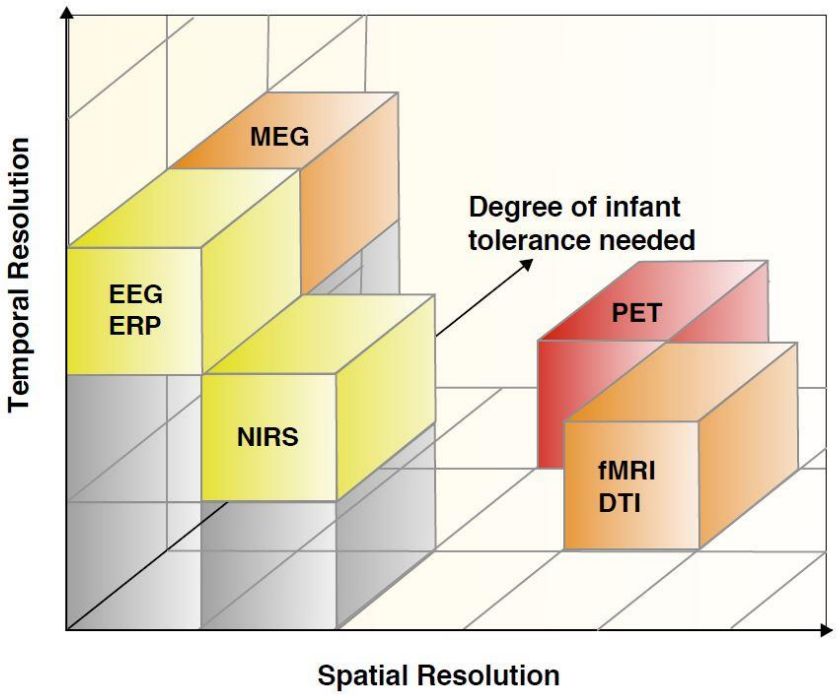


Figure 2.3. Reproduced from Lloyd- Fox et al. (2010). Commonly used neuroimaging techniques in context of their temporal and spatial resolution and their degree of suitability for infant research. EEG = electroencephalography, ERP = event related potentials, MEG = magnetoencephalography, NIRS = near infrared spectroscopy, fMRI = functional magnetic resonance imaging, DTI = diffusion tensor imaging, PET = positron emission tomography

The two techniques most widely used in infant research and implemented as part of this project are EEG and fNIRS. The properties of these methods are complementary in that EEG allows measurements of rapid changes in neuronal activity, whereas fNIRS is able to measure slower but more localised changes of the cortical haemodynamics. A summary of the properties of both neuroimaging measures and a comparison to behavioural paradigms can be seen in Table 2.6.

Table 2.6. *Strengths and weaknesses of methods available for use in infant research.*

	EEG	fNIRS	Behavioural
Corruption of data by movement	Yes	Some	No
Cost effective	Yes	Yes	Very
Portable	Yes	Yes	Yes
Staff training for data acquisition	Little	Little	Extensive
Staff training for data analysis	Extensive	Extensive	Moderate
Temporal precision	Excellent	Good	NA
Spatial precision	Low	Good	NA
Potential for cross cultural use	Yes	Yes	Limited
Use with newborns/ young infants	Yes	Yes	Limited

Note. EEG = electroencephalography, fNIRS = functional near infrared spectroscopy.

As both fNIRS and EEG directly measure infants' brain responses while they are passively watching or listening to stimuli, these methods lend themselves to the study of young infants at a point in time at which they cannot yet perform complex behaviours. Both methods use comfortable headgear which usually is well-tolerated by the infants and can be worn while infants sit comfortably on their parent's lap or while they are asleep.

EEG and fNIRS have been used to elucidate the neural correlates of infant brain and cognitive development in different ways. Both methods come with hardware that is portable enough to take to remote areas and use in non- standard lab settings and can be operated with only minimal training by non-expert staff.

2.6 Measures implemented in this thesis.

2.6.1 Electroencephalography.

Since their first implementation by Hans Berger in the late 1920's (Berger, 1929), EEG recordings have been extensively used in the study of cognitive functioning in adults, children and infants. Acquisition systems are more cost effective, easier to transport than hardware associated with other imaging modalities (i.e. MRI, MEG), while data is additionally less affected by participant motion. As the technique is relatively easy to implement, it has been used in infant research from as early as the 1930's (Lindsley, 1939) and is now one of the most commonly used tools in developmental research. ERP's have been used to study the whole spectrum of cognition in adults (i.e. attention, Astheimer et al., 2014; processing speed, Bieniek et al., 2015; memory, Maratos, Allan & Rugg, 2000; language, Sullivan et al., 2014; social cognition, de Haan & Carver, 2013) and similarly have been invaluable in understanding infant development in varied domains such as attention (Xie, Mallin & Richards, 2018), memory (Cowan et al., 1993), social cognition (Striano & Reid, 2006), sensory- (Sokol, 1978), emotional- (Leppänen et al., 2007), and language- (Bishop, 2007) development. Due to their applicability across wide age ranges ERP's have also been used to study specific developmental processes in infancy, such as perceptual narrowing (Maurer & Werker, 2014) or the development of visual expertise (de Haan, Pascalis & Johnson, 2002).

EEG recordings can be regarded as a transcription of electrophysiological activity, originating primarily in the brain. Synchronous electrical activation

generated by large populations of neurons in cortical brain regions can be measured by electrodes placed on the surface of the scalp (de Haan, 2013, Taylor & Baldeweg, 2002). An EEG primarily reflects activity from cortical pyramidal cells. These cells have a high density within the cortex and are aligned perpendicular to the surface of the scalp (Luck, 2014). As these cells are comparably large and uniformly aligned, their activity becomes strong enough to be read out by electrodes, placed on the head on scalp level (Cohen, 2014). Figure 2.4 illustrates the EEG cap placements in infants assessed for this thesis.

Figure 2.4. Electrode cap placement in the current study. Shown are a sleeping 1 month old (left) and a 5 month old infant (middle), wearing the electrode cap only. The right panel shows a 1 month old with both the electrode cap and an additional cap holding in place headphones through with sounds are presented.

The EEG signal can be analysed in a number of ways to better understand brain functioning and cognitive processes. Hereby, the continuous EEG can be analysed with regard to changes in the oscillatory activity within different frequencies contained in the EEG signal (Cohen, 2014). By decomposing the signal into the frequency spectrum and examining the interplay between frequencies additional information can be extracted from the EEG signal. A

detailed discussion of these techniques and their application to the data of this project can be found in Chapter 4.

The by far most commonly implemented analysis method of infant EEG data however lies in the extraction of ERP's in which the continuous EEG is subdivided into event related epochs, changes during which can be used to examine rapid changes in electrical activity generated in the brain across repeated stimuli (Luck, 2014). By averaging tightly time-locked responses across trials, noise can be reduced and changes that consistently occur in response to a stimulus can be isolated (Luck, 2014). The ERP can then be examined in a number of ways. Most commonly, the different components, that is positive and negative deflections within an ERP waveform are assessed. Both the component's magnitude (measured by a component's amplitude) and the timing of its occurrence (measured by its latency) can be compared across conditions and study groups.

Paradigm. ERP's are an excellent means to assess the early development of neural responses related to memory processes. This project implements a widely-implemented ERP paradigm, which taps memory in form of the response to novel versus a familiar stimuli. In studies of this kind, familiarity is either achieved by comparing stimuli that are inherently familiar to the infant to stimuli that are not (i.e. by comparing infants mother's face or voice to that of a stranger, Mash, Bornstein & Arterberry, 2013), or by inducing familiarity during a habituation phase in the study itself (Nelson, 1986). Habituation paradigms allow for more standardisation in that infants are exposed to identical stimuli while also eliminating some of the confounding factors of mother- stranger paradigms (i.e. time spent with infant, possible other caregivers). As caring practices differ across cultures, with infants being cared for by a larger group of people in The Gambia, it was decided to adopt an auditory oddball paradigm, which is reliant on task induced familiarity.

Oddball paradigms have been widely used to study cortical processes related to habituation and memory (Cycowicz, & Friedman, 2007). Classically, participants are presented with a series of identical auditory or visual stimuli that repeat for the majority of presentations. On occasion, however, this stream of frequently occurring, identical stimuli is intercepted by a novel, unfamiliar stimulus. The paradigm can be extended to not only examine responses to one infrequent stimulus, but to a range of stimuli. Kushnerenko et al. (2007) devised a paradigm in which participants were presented with frequent, infrequent and trial unique stimuli. Infrequent tones were the same at each presentation, whereas trial unique sounds encompassed a range of stimuli, each only presented once during the study. While initially implemented in adults and newborns (Kushnerenko et al., 2007), the paradigm has since been used to study infants at two (Otte et al., 2013) and four months of age (van den Heuvel et al., 2015) leading to a well-defined developmental trajectory over the first months of life. As will be discussed in Chapter 3, this ERP task will be used in this project when infants are 1 and 5 months old, allowing for a replication of previous studies, as well an examination of possible divergence from the previously defined trajectory.

2.6.2 Functional near infrared spectroscopy.

fNIRS has emerged more recently as a method of measuring brain functioning and has been optimised considerably over the last twenty years for infant use (Lloyd-Fox, Blasi & Elwell, 2010). Since initial studies in adults, which investigated primarily cortical activation associated with simple motor tasks in proof-of principle studies, the method has now been used in developmental research to study a wide range of cognitive functions such as language emergence and word learning (Benavides- Varela et al., 2012), social cognition (Lloyd-Fox et al., 2009) and emotion processing (Blasi et al., 2011). fNIRS offers a means of measuring functionally localised haemodynamic responses in

populations that cannot usually be assessed using imaging modalities such as infants and patient populations. It also offers novel approaches to study non-patient adult participants in more naturalistic ways. fNIRS hyperscanning studies have exploited the fact that fNIRS headgear can be worn during naturalistic interaction of multiple subjects, elucidating social processes in a more ecologically valid manner (Baker et al., 2016). Others lines of research have exploited its portability in tasks of spatial navigation, allowing participants to move freely outside the lab (Pinti et al., 2015). The technological development of fNIRS is still progressing, and future studies will benefit greatly from smaller sensors that can be applied on the head with high density and can be worn by participants in everyday tasks (Pinti et al., 2015).

The fNIRS technique uses light to measure changes in blood oxygen levels in the cerebral vasculature (Elwell, 1995). As neurons activate, blood flow in surrounding areas increases providing cells with additional oxygen. fNIRS exploits the fact that biological tissue is relatively transparent to light from the near-infrared spectrum, allowing it to shine through the skull and tissue. Near infrared light is primarily absorbed by compounds solved in the bloodstream, such as haemoglobin, whose absorption of light depends on the amount of oxygen it contains. Thus, depending on the levels of oxygenation of the blood, a proportion of the emitted near infrared light is absorbed while the remaining light continues to travel through the cortex.

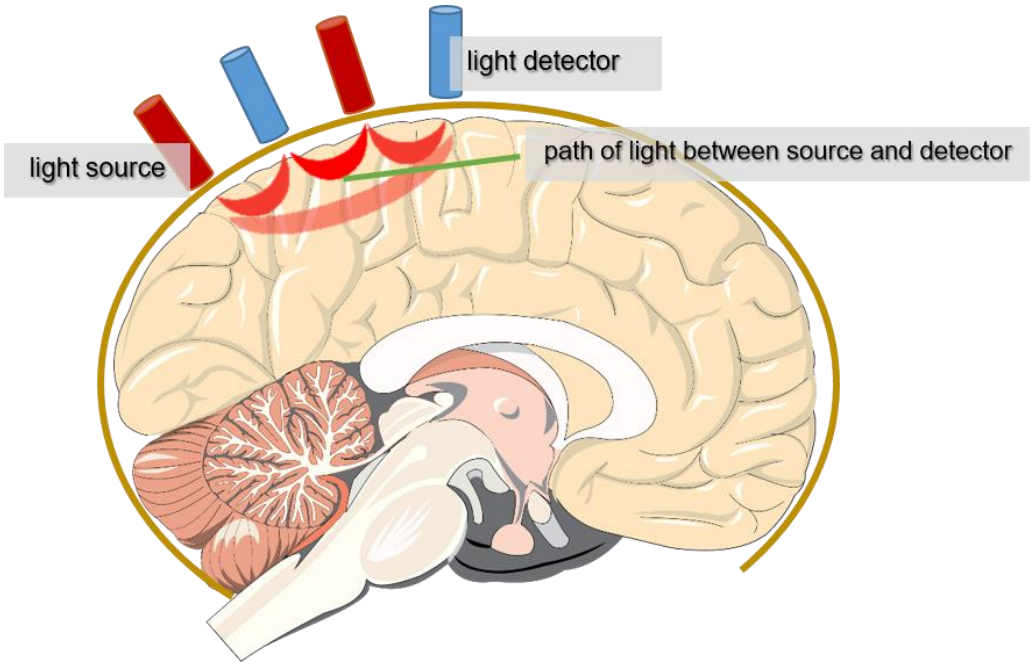


Figure 2.5. Schematic of functional near infrared spectroscopy. Sources emit light that is picked up by detectors as it travels through the cortex. As indicated, activation can be decoded between adjacent source-detector pairings, yielding a higher signal to noise ratio of activation in more superficial areas of the cortex. Activity can also be assessed in deeper regions by pairing farther away sources and detectors, usually yielding noisier estimates of activation.

Figure 2.5 provides a schematic of fNIRS measurements. By placing pairs of light emitting sources and light sensitive detectors in close proximity of one another on the participant's head, light absorption within the space between each source and detector pair can be deduced. By placing a greater number of these optode pairs over different regions of the cortex, region specific changes can be inferred.

Figure 2.6. Reproduced from Katus et al., (under review) Upper panel; fNIRS headgear worn by infants in the BRIGHT project in the UK and The Gambia. Lower panel; optodes and fibres (far left), sensor arrays with clip-on optode holders (left), source (red) and detector (blue) fibres clipped into array (right) and headband and optode array combined (far right).

Measurements obtained from fNIRS are similar to the fMRI blood oxygen level dependent (BOLD) response, capturing changes of oxygen saturation in cortical blood vessels. While fMRI utilises the magnetic properties of deoxygenated blood, fNIRS relies on the differential optical properties of oxy and deoxyhaemoglobin. As in fNIRS light is typically emitted at two different wavelengths, it enables measurements of both oxygenated and deoxygenated blood, yielding a richer measure of neural activation.

Having been extensively used to study infants on a diverse range of tasks, fNIRS has more recently been shown to be an effective method in rural Gambia, in terms of transportability, cost effectiveness, robustness of equipment, being accepted by the local community and yielding good quality data (Lloyd- Fox et al., 2014). Further, it has been shown that the fNIRS technique is able to measure infants of a wide age range, lending itself to longitudinal analyses (Lloyd-Fox et al., 2017).

fNIRS data most frequently is analysed by extracting peak values of the time course of the haemodynamic dynamic response function (HRF), which can then be modelled to compare conditions and groups.

Paradigm. A passive visual paradigm was be presented, tapping early WM development via an object permanence task. As will be described in more detail in Chapter 5, the tasks relies on infants watching a video of an actor lifting up an object, looking directly at the infant while holding the object and vocalising (i.e. saying 'Ooh!') and subsequently placing the object inside (experimental conditions) or on top of a box (control condition). The two experimental conditions were further divided in a short delay condition, in which the object remained hidden for 3 seconds and a long delay condition in which the object remained hidden for 6 seconds. The object was then retrieved from the box and again placed in the original position next to the box. This paradigm was originally developed in a previous phase on the BRIGHT project (Begus et al., 2016), and was further refined for use in this thesis. Changes in the paradigm are illustrated in Figure 2.7. As can be seen, only the two experimental conditions were presented in the Phase 1 paradigm, making it harder to attribute observed neural activation to a WM process. Further, trial lengths differed between the long and the short delay condition, thus not only altering memory load but also length of visual input. Lastly, the paradigm so far was implemented in 12-14 month old infants in London and in Keneba. As the task is reliant on object permanence which is said to emerge around 9 months of infant age (Baird et al., 2002), it was decided to administer the task both in at the 8 and the 12 month age points, to capture potential developmental changes.

Figure 2.7. Overview of the Phase 1 paradigm (top panel, Gambia version) and the Phase 2 paradigm (bottom panel, UK version). As can be seen, no control condition was presented in Phase 1, and trial lengths differed between conditions. In Phase 2 all trials have the same length and a control condition in which the object remains visible is presented.

2.6.3 Behavioural deferred imitation study. In the study of infant memory development, imitation paradigms have a long-standing tradition (Jones & Herbert, 2006). In particular, the ability to observe, hold in mind and enact a sequence at a later time has been used extensively to study how infants become progressively better able to perform actions they have observed from others. Studies typically take the form of a play based assessment in which infants are shown a new action by a demonstrator and then allowed to perform said action after a delay. Studies have varied the paradigm with regard to several key features such as number of demonstrations, length of delay and complexity of sequence (Jones & Herbert, 2006). For the given study, it was crucial to adapt the task by introducing stimuli that were as novel to infants at both sites as possible. We further introduced a condition contrast in which infants were allowed to imitate the action immediately after the delay for some of the presented items. This enabled an assessment of differences in imitation behaviour in context of duration over which the action had to be retained for. It also allowed for an assessment of potential practice effects. The study was implemented at 8 and 12 months, in order to assess developmental trajectories on this task.

2.7 General study design

The study design was determined as part of the Brain Imaging for Global Health project (The BRIGHT project, globalfnirs.org/the-bright-project), which longitudinally studies infant cohorts in Cambridge, UK and in Keneba, The Gambia, West Africa. Sample sizes differed between study sites with 61 infants enrolled in Cambridge and 225 infants enrolled in Keneba.

Statistical power. The BRIGHT project was powered based on previous infant studies indicating a minimum sample size of 40 infants tested was needed to detect a small effect between experimental conditions.This is common in infant neuroimaging studies, and takes into account data rejection due to anticipated data loss due to high noise and attrition of the longitudinal sample. The sample studied in Keneba was larger as more demographic and health variance between infants was anticipated in this population, especially with regard to nutritional status, the effect of which on cognitive development is one key objective of the BRIGHT project. The full sample recruited into the BRIGHT project is comparatively large in context of neurodevelopmental research, particularly the sample of 225 infants tested in Keneba. The larger sample in Keneba was determined for three reasons: 1. to allow longitudinal modelling of the neurodevelopmental outcomes in context of a large range of risk factors, 2. to enable these models despite known drop out and noncompliance rates in infant research, and 3. to allow analyses on a large enough group of infants anticipated to be affected by undernutrition. In Keneba, previous studies have shown that 25-30% of infants exhibit growth faltering over the first year of life (Nabwera et al., 2017), meaning that with 225 infants tested approximately 50-60 would be affected, thus enabling a thorough investigation of the effects of undernutrition on early brain development.

The smaller sample in the UK was determined in light of: 1. previous literature on fNIRS studies available for similar settings, indicating the detection of effects in cohorts of between 20-40 infants, 2. The assumption that infants in this cohort would be more homogenous with regard to their environment and response, 3. overall funding constraints of the project that necessitated careful allocation of resources, which in this case was decided to be allocated to the more novel, Gambian study context.

For the BRIGHT project, families were recruited antenatally as described above and then seen when the infants were 7-14 days, 1, 5, 8, 12, 18 and 24 month old. At each visit, an extensive protocol was administered encompassing different neuroimaging, behavioural and biological measures.

The protocol of the BRIGHT project is summarised in Table 2.5, and encompasses a wide variety of data points, including neurodevelopmental data, but also biological samples and background information of the familial context of participants. The outcome variables of the BRIGHT project can be broadly grouped into three categories: first, the core outcomes are the neuroimaging and eye tracking data, which are the main focus of this project and also deemed to be most appropriate in capturing developmental changes cross-culturally. Secondly, data on known risk factors are collected, including biological samples and growth measures to infer nutritional status, obstetric data etc. Thirdly, data is collected on mediating factors (i.e. parent interaction, family structure). Depending on the theoretical model taken, these data can be regarded as stand-alone risk/resilience building factors (i.e. maternal engagement with infant), but in the context of the analyses conducted in the BRIGHT project serve primarily for the examination of mediating relationships between the poverty related risk factors and neurodevelopmental outcome.

For this thesis, I use a subset of outcome measures and age points collected as part of this project. Specifically, I use EEG data collected at 1 and 5 months of age, as well as fNIRS and behavioural data collected at 8 and 12 months of age. All of these assessments are currently, or will be administered at additional age points, data on which was unavailable 6 months prior to the anticipated submission date of this thesis. For the same reason, the reported analyses do not include the total sample, but rather the proportion of infants tested by the time of the analysis phase of this PhD. A larger proportion of the final sample could be included for the younger age points, whereas fewer infants had been tested at the later age points, leading to relatively smaller samples in the studies conducted at 8 and 12 months.

Due to the BRIGHT project's focus to implement neurodevelopmental measures that have not been used in this setting before, the primary assignment of tasks within the team was guided by having a dedicated team member overseeing each of the neurodevelopmental assessments. Some of the secondary data (i.e. on growth, SES, obstetrics history) are well established measures that have been implemented in many previous studies in The Gambia, and were therefore planned to be collected with minimal ongoing support, with curation and analysis happening after the completion of data collection. For this reason, none of this data was available at the start of the write up of this thesis. Some SES data was made available during the write up phase of this thesis, which was included to aid description of the two study cohorts in Chapter 2. Other data that are anticipated to bear relevance to the described neurocognitive measures in this thesis, such as parent child interaction, infants' exposure to spoken language, infants' sleeping patterns and family structure are being collected but are still undergoing data curation. As it was beyond the scope of this thesis to complete the data curation on these additional outcomes, they are

not included here. The overall lack of secondary data led me to carry out analyses aimed at an in-depth description of the neurodevelopmental profiles encountered in both study cohorts. It is my goal to complete these analyses both in terms of including the whole sample as well as additional age points in future analyses, as is laid out in Chapter 7. The remainder of this thesis will focus on the description of methods relevant to this thesis which were collected when infants were 1, 5, 8 and 12 month of age (as highlighted in Table 2.5). Specifically, this PhD project includes the EEG data collected at 1 and 5 months, fNIRS data collected at 8 and 12 months and behavioural data, also collected at the latter time points. Samples presented in this thesis are limited to data available at the beginning of the analysis phase for this thesis. All analyses will be repeated once the full samples are available.

Table 2.5. *Overview of BRIGHT project protocol.*

	Type of measure	7-14 days		1 month		5 months		8 months		12 months		18 months		24 months	
		GM	UK	GM	UK	GM	UK	GM	UK	GM	UK	GM	UK	GM	UK
Neuroimaging															
fNIRS	audio-visual social vs non-social stimuli paradigm			x	x	x	x	x	x	x	x	x	x	x	x
	auditory habituation paradigm			x	x	x	x	x	x	x	x	x	x	x	x
	audio visual social vs non- social functional connectivity paradigm			x	x	x	x	x	x	x	x	x	x	x	X
	audio- visual working memory paradigm							x	x	x	x	x	x	x	X
	live interaction task: deferred imitation							x	x	x	x				
EEG	passive auditory oddball/novelty paradigm			x	x	x	x					x	x		
Behavioural assessment															
Eye Tracking	social vs. non-social preferences, saccadic latencies, visual search strategies, cognitive control and habituation					x	x	x	x	x	x	x	x	x	x
Mullen Scales of Early Learning	behavioural assessment					x	x	x	x	x	x	x	x	x	x
Parent Child Interaction	video of parent interacting with infant			x	x	x	x	x	x	x	x	x	x	x	x
Neonatal Behavioural Assessment Scale	behavioural assessment of reflexes, social interaction and state regulation	x	x												
Infant Tablet Task	tablet based assessment of rule learning and fluid intelligence											x	x	x	x

Language environmental analysis	auditory recording of words spoken by and around infant									x	x	x	x	x	x
Questionnaire data																
Obstetric history	recording any complications during pregnancy and birth	x	x													
Socioeconomic Status	recording household income, parental education and employment (site specific)	x	x							x	x			x	x
MacArthur-Bates Communicative Development Inventory	caregiver- rated receptive and expressive language									x	x	x	x	x	x
Sleep Diary	carer completed diary of sleep behaviour			x	x	x	x	x	x	x	x	x	x	x	x
Edinburgh Postnatal Depression Scale	parental depression	x	x	x	x	x	x			x	x				
Perceived Stress Scale	parental stress	x	x	x	x	x	x			x	x				
Positive and Negative Affect Schedule	parental mood	x	x	x	x	x	x			x	x				
Nutrition and Growth																
Anthropometry	length, weight, mid upper arm circumference, knee to heel length, head measurements	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Biological Samples	infant: blood, stool, urine, maternal: blood, breast milk			x		x		x		x		x		x	
Food Diary	carer completed diary on infant food intake				x		x		x		x		x		x

Note. Measures collected per time point. Marked in dark blue are data points considered in this thesis. fNIRS = functional near infrared spectroscopy, EEG = electroencephalography, UK = United Kingdom, GM= The Gambia.

2.8 Statement of involvement

2.8.1 Personal involvement. During the first 24 months of my PhD, I have been mainly involved in data collection at the Cambridge study site. I was involved in initial training and piloting at CBCD Birkbeck, London, where I conducted 48 study visits and collected fNIRS, EEG and eye tracking data, as well as data on behavioural measures (Mullen Scales of Early Learning, parent child interaction) with at least one other researcher present. When preparing the deferred imitation task, I also conducted 11 pilot visits with 8 and 12 month old infants at CBCD Birkbeck together with a placement student. Thereafter, during the main phase of the BRIGHT Project, I contributed to data collection at the 1, 5, 8, 12 and 18 month lab visits. During the initial phases of the study, I was also involved in conducting home visits in the Cambridge area to perform the Neonatal Behavioural Assessment Scale with 30 of the families as part of the 7 – 14 day assessment. As part of the training for the NBAS, I assessed 25 newborns and was given feedback by trainers. For the lab based data collection, initially at the Rosie Hospital in Cambridge and later at new lab facilities at the University of Cambridge, I conducted 34 1 month, 28 5 month, 26 8 month, 12 12 month and 16 18 month visits together with another researcher.

During four trips to The Gambia, I helped to set up the lab space and trained the local researchers in preparation for the 1, 5 and 8 month study visits. During the first trip, I also conducted NBAS home visits for reliability checks with the local researchers.

2.8.2. Data collected by other team members. In this thesis, I will be using data that was collected by other team members based in Keneba and Cambridge. In Cambridge, some of the EEG, fNIRS and behavioural data which I will be using was collected by our research assistants Nathan Hayes

and Maria Rozhko, as well as our placement students June Pastor, Dominique Taylor and Sophie Yelland, Marta Perapoch, our postdoctoral researchers Bosiljka Milosavljevic, Malen Crespo-Llado and Anna Blasi and one of our principal investigators Sarah Lloyd- Fox. I received help from our Cambridge based team (Marta Perapoch, Maria Rozhko, Malen Crespo-Llado) in coding the looking time for the visual fNIRS paradigm for videos from both study sites. I further had help coding videos for the behavioural imitation study, from a placement student coding infants' engagement with the objects.

The vast majority of the data from The Gambia was collected without my personal contribution by the locally based higher scientific officers Sophie Budge and Sam McCann and our field workers Saikou Drammeh, Ebrima Mbye, Muhammed Ceesay, Mohammed Camara, Ebou Touray as well our intern Laura Steiner.

Chapter 3. Deviance and Novelty Detection in Early Infancy

Study 1: ERP study at 1 and 5 months

3.1 Introduction

The ability to detect novel stimuli in the environment is a fundamental process of early cognitive development. The bias to preferentially attend to stimuli that have not been encountered before serves several crucial functions. From an evolutionary perspective, preferential attendance to stimuli that do not match repetitive environmental noise aids identification of potentially meaningful signals, while not expending energy on recurrent but inconsequential stimuli (Eisenstein et al., 2001). Novelty or deviance detection is reliant on both a discriminatory orienting response to the novel stimulus, as well as habituation to, and possibly retention of, previously encountered stimuli. On a cognitive level, diminished responses to a repetitively presented stimulus have been interpreted as successful encoding of the given stimulus, a process representing one of the most basic forms of memory formation and learning (Rovee-Collier & Cuevas, 2008).The process has been proposed to unfold through a formation of so called engrams, or representations of a just encountered stimulus into short term memory (Sokolov, 1963). Subsequently encountered stimuli are thought to be compared against this representation, and a neural or behavioural response will be elicited or modulated by the degree of similarity or dissimilarity with the previously encountered input. A highly dissimilar stimulus will therefore elicit a larger neural or behavioural orienting response, while repetitive stimuli will elicit weaker and weaker responses across trials.

With regard to infant development, it can be argued that a bias to orient to novel input facilitates exposure to a larger variety of sensory stimulation, which is crucial during critical periods of sensory development. Infants' ability to habituate to repetitive, and to orient towards novel, stimuli has been widely examined since the early beginnings of neurodevelopmental

research. For decades, a vast literature of behavioural studies has accumulated, aimed at elucidating the extent to which infants can remember visual or auditory stimuli in repetitive sequences or after a habituation phase. While studies utilising stimuli such as infants' mother's voice or face and comparing it to an unknown voice or face frequently find that infants preferentially attend to their mothers over the first months of life (Field et al., 1984, Houston-Price & Nakai, 2004), studies that habituate infants to one of two neutral stimuli, find larger behavioural and neural responses to the novel, rather than to the habituated stimulus (Nelson & Salapatek, 1986). One of the most influential paradigms in this context is the widely used 'visual paired comparison task', which exploits the fact that infants tend to look longer at a novel stimulus than at one which they were previously exposed to (de Haan, 2007). During visual paired comparison studies infants are typically presented with an image for a fixed duration of time or until they show a marked decrease in their attention to the stimulus compared to when they first encountered it. In a second phase, both the habituated and a novel image are presented to the infant again and it is measured whether they preferentially look at the novel stimulus. Overwhelming evidence has established that infants usually orient preferentially to the novel image. Paradigms can be varied along several parameters, including length of habituation, similarity of stimuli and delay between habituation and test phase. The visual paired comparison task has been widely accepted as a measure of infants' visual recognition memory (Richards, 1997, Pascalis et al., 1998). Due to the suitability of the paradigm to a broad age range, it can also easily be used to study the development of infants' abilities to discriminate between and retain different stimuli. Over the first year of life, infants become better able to discriminate more similar images (Courage & Adams, 1990), and to retain images in memory for longer delays (Rovee-Collier & Cuevas, 2008), even in the presence of

distractions in the retention interval (McCall et al., 1977) and changes in the context the stimuli was presented in (Jones et al., 2011, Shields & Rovee-Collier, 1992).

Recognition memory has also frequently been assessed in the auditory modality. While visual acuity undergoes rapid development over the first year of life but is not particularly well-developed in the new born, auditory stimuli can be processed in even the youngest infants (Slater, 1999). Even before birth, infants are able to perceive and retain auditory information into the postnatal period, as is for example evidenced by the differential response of newborns to nursery rhymes which were read to them by their mothers during the last trimester of pregnancy compared to novel nursery rhymes (DeCasper et al., 1994). Due to the earlier onset of exposure, auditory acuity is farther developed at birth, with visual processing lagging behind. This means that while both visual and auditory paradigms have yielded invaluable information about early infant cognitive development, there is a justified preference for the implementation of auditory tasks in neonatal and very young infant populations. While it is relatively easy to determine whether participants orient more towards novel stimuli in the visual modality, a mere directional orienting response cannot easily be used as an indicator in auditory paradigms. Some behavioural studies have relied on assessing changes in the rate of infants' non-nutritive sucking in response to novel or old auditory stimuli. In these studies, infants are given a dummy connected to an apparatus to determine the frequency and intensity of their sucking response. This response has been shown to be modulated by the degree of novelty of the stimulus (Mehler et al., 1988). The paradigm was also adapted to contain an operant conditioning component. In this modification of the task infants can influence whether they are exposed to novel or familiar stimuli by either increasing or

decreasing their rate of sucking. The paradigm can then be varied to either play, for example, a familiar or a novel nursery rhyme in response to an increased sucking rate. Using this paradigm, Spence (1996) reported a consistent preference for novelty and successful modulation of sucking rate in infants as young as 1 month. Other studies have relied more on physiological changes, such as changes in infant heart rate and their gross motor movements (Rovee-Collier & Cuevas, 2008) or hormonal levels such as cortisol (de Haan et al., 1998). Beyond that, many studies have relied on the direct measure of neural responses.

3.1.1 Implementation of ERP paradigms to study deviance detection.

In order to assess neural underpinnings of observations made based on behavioural paradigms, many groups have examined neural response patterns to repetitive vs novel stimuli using ERP's. ERP designs have been used extensively over the past decades to elucidate the development of recognition memory and novelty preferences in infancy. Paradigms fundamentally rest on the assumption that habituation will occur to identical repeated stimuli on the one hand, and a discriminatory mismatch response (MMR_{ERP}) to novel stimuli on the other.

In the classical version of the paradigm, a class of standard stimuli is presented, usually at a probability of 70-90% of trials, which is interspersed with deviant stimuli, presented for the remainder of trials. The resulting ERP waveform is characterised by components of stronger magnitude to the deviant compared to the standard trials. While MMR_{ERP} paradigms have been used to study cognitive processes across sensory modalities and could be shown to occur for tactile (Kuchenbuch et al., 2014) and olfactory (Krauel et al., 1999) stimuli, the vast majority of infant studies have used either auditory or visual paradigms. While these primarily offer insight into the development of habituation and dishabituation processes, the paradigm has

been adapted to answer more specific research questions, such as differential processing of speech sounds in children with dyslexia or language impairments (Baldeweg et al., 1999, Bishop et al., 2010), children exposed to environmental adversity and disease (Kihara et al., 2010) or developmental disorders such as autism spectrum disorder (Näätänen & Kujala, 2011) or Down's syndrome (Vieregge et al., 1992). When it comes to the study of very young infants however, the auditory oddball paradigm is by far one of the most frequently implemented studies used to assess infants at different ages. In this paradigm, frequently occurring stimuli are interspersed with infrequently occurring sounds which in infant studies are passively presented to participants.

While MMR_{ERP}'s have primarily been elicited by differing sensory stimuli (i.e. sounds differing in pitch, images of two different faces), some groups have tried to alter other parameters of stimulus presentation to elicit an MMR_{ERP}. Otte et al. (2013) reported MMR_{ERP}'s in response to deviant auditory stimuli which differed from the standards not in their pitch but rather the temporal sequence at which they were presented. Whereas standard tones were presented at an inter stimulus interval (ISI) of 300ms, ISI deviants were presented with an ISI of only 100ms. Although the effect was more subtle than those elicited by frequency deviants, this study provides some evidence that alteration of other parameters than merely stimulus quality can elicit MMR_{ERP} response. In fact, it has been shown that not only deviant stimuli but also stimuli missing from an otherwise repetitive sequence can elicit an MMR_{ERP}. In a study of 6 month old infants Nelson et al. (1990) presented infants with a series of pictures, each preceded by a tone. On some trials however, no picture was shown after the tone, resulting in an MMR_{ERP}.

The MMR_{ERP} has been employed extensively to understand different typical and atypical populations as well as different age groups. Further, the paradigm has been manipulated in a number of ways to understand subtle effects of stimulus properties and contrasts on the MMR_{ERP}. For this reason, the MMR_{ERP} lends itself for the study of a new, previously understudied populations allowing comparisons to previously reported developmental patterns.

3.1.2 The MMR_{ERP} waveform.

The MMR_{ERP} response has been well characterised under different conditions in adults and across infant and child development. In its most typical form, the infant MMR_{ERP} waveform is a three component complex, consisting of an early negative component (N1), a mid- latency, large amplitude positive component (P3) and a late latency negative component (Nc). These components are ascribed different functions. While the early occurring N1 has been associated primarily with processing of the incoming auditory information, the P3 component has been shown to be consistently modulated by the stimulus condition in novelty paradigms. The Nc has been interpreted as the neural equivalent of the orienting response, following a distraction due to an infrequently occurring stimulus.

In adults, the ERP waveform elicited by mismatch paradigms has been described to consist of two early occurring component (N1, P2) associated with sensory processing of an incoming stimulus, as well as the longer latency P300 component which is modulated by the novelty of the stimulus compared to preceding stimuli and a subsequent Nc associated with a reorienting response. A schematic of the MMR_{ERP} waveform elicited by frequent and infrequent stimuli is shown in Figure 3.1. A large body of literature from adult populations has shown a relatively stable occurrence of these components in tasks consisting of both frequently and infrequently

occurring stimuli. The modulation of the P3 in context of mismatch paradigms has hereby received the majority of the attention and is therefore primarily used to quantify mismatch responses.

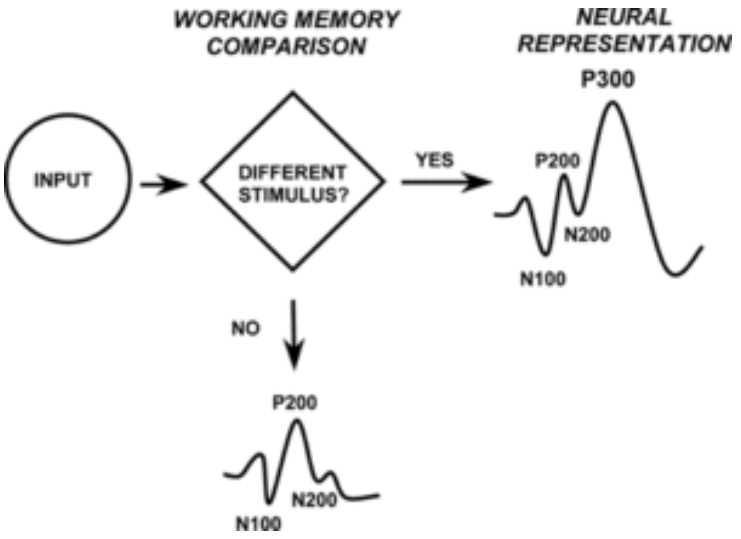


Figure 3.1. Reproduced from Polich (2012). Schematic of MMR_{ERP} waveform to frequent and infrequent stimuli in adults. As can be seen, the P300 is most strongly modulated by stimulus condition.

In infant studies, the N1 component has sometimes been difficult to reliably measure, due to greater variance in latency and the larger amplitude of the adjacent P3 component, leading the relatively smaller N1 to be masked at group level (Cheour et al., 1998), especially when newborns were tested (Kushnerenko et al., 2002). Kushnerenko et al. (2002) further argued that the P3 properties measured even in young infants seem to map well onto the P3a observed in adults, which led them to argue that the P3 measured in infants might provide a useful tool to examine maturation of the MMR_{ERP}.

From a theoretical perspective the P3 has been regarded in terms of the Context Updating Theory, which states that the magnitude of the component indicates revision of the mental representation elicited by the incoming stimulus, rendering it to be larger for more dissimilar stimuli

(Donchin & Cole, 1988). As such, the P3 has also frequently been regarded as an electrophysiological marker of the orienting response (Soltani & Knight, 2000).

In infants, the MMR_{ERP} waveform shows a more variable morphology that undergoes substantial changes across development. While the early auditory components are discernible even in young infants, they are much more variable and therefore not as reliably elicited compared to older infants (Kushnerenko et al., 2001). While some studies have inconsistently found distinct negative components at around 100ms in infant populations (Kushnerenko et al. 2007, Otte et al. 2013, Kihara et al., 2010b), others did not consistently measure this component (van den Heuvel et al., 2015). Further, significant condition differences for this negative component were not evident consistently across studies. While Kushnerenko et al. (2007) found condition differences in this first negative component for some stimulus contrasts, others did not find it to distinguish between frequent and infrequent sounds (Otte et al., 2013), or between clinical and non-clinical populations (Kihara et al., 2010b). Assuming functional similarity between the early negative components observed in infants and those observed in adults, it might be the case that the component carries information primarily on the sensory processes indifferent to stimulus condition, rendering the P3 the more reliable marker of stimulus novelty for infant studies.

Similarly, the Nc has been less reliably elicited in infants. Some studies have reported its occurrence and found it to be modulated by stimulus condition in a subset of condition contrasts (Kushnerenko et al., 2007). Due to its late occurrence in infants at around 500-600ms post stimulus onset the Nc cannot always be reliably elicited and has not been measured in other infant studies (Otte et al., 2013, van den Heuvel et al., 2015). The Nc could not be

reliably assessed in this study, as parameters of stimulus presentation led the Nc to the previous stimulus to be masked by the onset of the next stimulus. For this reason, it will primarily be focused on the P3 component.

3.1.3 Developmental changes of ERP mismatch response.

ERP waveforms change considerably during infancy, both due to brain development and physiological growth not directly related to brain development. In infants, ERP amplitudes usually decrease as a function of increasing skull thickness, especially in the early months during which the fontanelle closes (Jing & Benasich, 2006). Within the brain itself, the progression of axonal myelination leads to more efficient neural processing as reflected by a decrease in peak latencies or ERP responses (Cheour et al., 1998, Jing & Benasich, 2006). Implementing well-defined condition contrasts is thus crucial to disentangle developmental changes due to purely physiological growth (i.e. skull thickening) and neurocognitive aspects of development. Some studies using mismatch paradigms have defined stimulus contrasts which map onto developmental parameters known to change in the age group under investigation. In young infants and newborns, there is a known bias to preferentially respond to stimuli of higher intensity (Kushnerenko et al., 2007, 2013). For this reason, a series of studies (Kushnerenko et al., 2007, Otte et al., 2013, van den Heuvel et al., 2015) have implemented both infrequently occurring white noise segments, known to elicit large P3 responses due to the fact that a broad range of frequencies is covered, as well as a class of so-called trial unique sounds. The former occurred infrequently but were repetitive, while the latter consisted of a range of different sounds, each only presented once during the study. Past research has shown developmental P3 amplitude modulations to these deviant stimuli in the first six months of life (Kushnerenko et al., 2013). Using condition parameters that introduce a spectrally rich sound, such as a short

burst of white noise as well as a category of trial unique sounds, it has been shown that P3 amplitudes to the white noise sounds decrease over the first months of life, whereas the trial unique sounds are preferentially processed as indicated by larger P3 amplitudes from around 2 months of life (Kushnerenko et al., 2007, Otte et al., 2013, Kushnerenko et al., 2013). This has been interpreted as indicative of a maturational process, by which auditory responses are triggered primarily by high intensity, broad spectrum sounds in newborns, before the emergence of a preferential response to stimulus novelty. The maturational process hereby can be argued to be the result of more efficient habituation to repeated sounds, even when habituation to the infrequently occurring sounds is interrupted by the occurrence of the frequent sounds.

In a study aimed at disentangling the degree to which the MMR_{ERP} response was affected by stimulus properties (i.e. intensity, broad frequency spectrum vs harmonic pure tone) and presentation category (frequent, infrequent, trial unique), Kushnerenko et al., (2007) presented sleeping newborns and awake adults with different combinations of frequent and infrequent tones. They used a class of trial unique sounds, deviants that had a different frequency to the pure tone standard (frequency deviants), deviants of the same frequency that differed in loudness (intensity deviant) and white noise segments (white noise deviants). They found that adults for the majority of contrasts showed larger amplitudes to deviant sounds, regardless of which stimuli were presented. The tested adults reacted as much to novel sounds as they did to frequency deviants. This was true even when white noise segments were used as standard sounds, which usually elicit rather large responses even in adults. Adults also showed a larger response to frequency deviants when contrasted with intensity deviants, further corroborating the notion that ‘deviance’ rather than ‘intensity’

drove the effect. Lastly, adults showed a differential pattern when contrasting white noise deviants with trial unique sounds. The results obtained in the adult participants were in sharp contrast to those obtained in the newborn population. While a clearly larger P3 could be elicited to trial unique sounds when contrasting them with pure tone frequency deviants, this novelty preference seemed to be overridden in other conditions. A large amplitude MMR_{ERP} was observed in response to the intensity deviant, as well as to the white noise deviant. Both of these differed from the frequent tones in loudness and the width of the auditory spectrum that was covered. The notion that stimulus intensity holds the potential to override the preferential response to novelty is further supported by the fact that in the last condition by far the largest P3 response was elicited by the white noise sounds, even though they were presented as the frequent stimuli. The study demonstrates that stimulus properties greatly matter in newborn studies and stimuli thus need to be contrasted carefully. They further make the point that the preferential response to novelty emerges across development.

Some studies have applied similar paradigms in a range of infant age groups. Otte et al., (2013) examined a cohort of 2 month olds infants using different deviants while the infants were awake and asleep. Using the same stimuli as Kushnerenko et al., (2007), they observed stronger ERP amplitudes to their trial unique as compared to the white noise deviants. This is evidence that the progression from preferential response to stimulus intensity seen in newborns, matures to preference for stimulus novelty very early in infant development. Van den Heuvel et al. (2015) again implemented white noise as well as novel sounds and found that while 2 month olds showed a slight preference for novel over noise sounds, the ERP morphology between the two types of deviant changed considerably with more distinct components

observable at the 4 month time point. This was taken as support for the maturational perspective put forward by Kushnerenko et al., (2013). The response shift between the two infrequent conditions can therefore be utilised as an indicator of maturation in neurocognitive processing.

3.1.4 Neural generators of the MMR_{ERP}. While some attempts have been made to localise the neural generators of the MMR_{ERP}, particularly of the P3 component, in both adults and animal models, less is known about development of neural generators during infancy. In adult humans, two different parts of the P3 can be measured, each of which are associated with different neuropsychological functions (Polich, 2007). A distinction is made between the P3a, which is typically elicited in response to passive paradigms and the P3b which is related to task demands (i.e. pressing a button in response to a target sound). Different neural generators have been proposed for these two components. The P3a which is most similar to the P3 elicited in passive infant studies, has been associated with frontal lobe functioning (Polich, 2007). It was first shown to be reduced in adults with frontal lobe lesions, while the P3b was unaffected in these patients (Knight, 1990, 1996). More specifically, the P3a component has been found to be attenuated in patients with lesions of the dorsolateral prefrontal cortex (dlPFC, Knight, 1984, Baudena et al. 1995).

Some studies have further implicated temporal lobe involvement in P3a generation, as the component was found to be reduced in patients with temporal lobe lesions (Kotz et al., 2007). Studies using depth electrodes implanted into the hippocampus first suggested its involvement in P3b occurrence (Halgren et al., 1980, McCarthy, 1989). More recent work looking at hippocampal volume and correlating it with P3 amplitude found the two to be highly correlated, which was interpreted as a mediating role played by the hippocampus and surrounding tissue in novelty detection

(Polich, 2004). Another area implicated in deviance detection in adults is the temporal-parietal junction (TPJ, Knight et al., 1989, Verleger et al., 1994). Polich (2003) proposed for there to be a circuit between frontal and temporal-parietal areas activated by deviance detection. Neuropsychologically, it is assumed for incoming alerting stimuli to elicit a top down response reflecting attention allocation, controlled by frontal areas and related more strongly to the P3a. This is hypothesised to be followed by a second process involving MTL structures, relating to the comparison between current and previous sensory input (Polich, 2012). Lastly, it is hypothesised for the TPJ to be most strongly associated with the organisation of a response to target stimuli, and therefore being more related to the P3b component (McCarthy et al., 1997, Soltani & Knight, 2000, Potts et al., 1996). In infants, the P3 can be understood more like the adult P3a, as it is elicited in absence of specific task instructions. It can therefore be understood as an orienting response which draws attentional resources involuntarily towards deviant stimuli. This process can therefore be hypothesised to draw on both frontal and medial temporal parts of the circuit described by Polich (2007).

In a study aimed at devising a developmental model of novelty preference in infant monkeys, Bachevalier et al., (1993) showed that monkeys did show a robust novelty preference in a visual paired comparison task from around 15 days of age. However, this preference disappeared in monkeys with bilateral amygdala and hippocampal lesions. Interestingly, lesions in anterior inferior parts of the temporal cortex did not impair novelty preference in the infant monkey, even though the same lesions severely impaired adult monkeys on the same task. This was taken as some evidence that different neural generators could be supporting this function in adults and infants. While the direct evidence as to which brain areas the MMR_{ERP} originates

from is sparse, it can be speculated that the developmental change seen in the MMR_{ERP} and particularly the P3 is associated with the rapid developmental change of the MTL during infancy (Utsunomiya et al., 1999), as well as the more protracted development of the frontal lobes (Nelson, 2000), accounting for changes of the measures components that reach well into late childhood.

3.1.5 MMR_{ERP} response and habituation.

The reduced amplitudes to recurring stimuli seem to be at least partly driven by habituation (Romero & Polich, 1996). In a study with 9-month olds infants, Wiebe et al. (2006) exposed infants to novel action sequences in the first testing session. In the second session infants were then presented with images of the action sequence they had been shown before, images of another action that was presented infrequently, as well as trial unique images of other action sequences. ERP's were analysed separately for each condition; early and late session trials were compared, hereby focusing on the Nc component. A habituation effect was observed in which early trials were associated with a larger Nc than late presented trials for the familiar and infrequent sequences, while no such difference between early and late trials was found in the trial unique condition. The measured habituation effect is noticeable in context of MMR_{ERP} studies, even though the setup of this study differed from standard mismatch paradigms, in that complex action sequences rather than simple perceptual input was presented. While the degree of response decrement over the course of a recording session could give information on the efficiency of habituation, not many MMR_{ERP} studies have examined this for prominent components such as the P3 across infant development. Habituation effects will be examined in the current study and compared between age groups and sites.

3.1.6 ERP mismatch response and memory. There has been some debate around the specific function associated with the ERP components elicited in novelty paradigms. While in adults' successful memory encoding has been linked to larger P3 amplitudes (i.e. in word learning tasks, Karis et al., 1984), paradigms reliant on memory report cannot be used in infant studies. For this reason, novelty studies have been criticised on the basis that they are inconclusive as to what is being reacted to is novelty or merely infrequency of stimulus occurrence. To tease this apart, Nelson & Salapatek (1986) performed a series of studies in which infants first were habituated to an image of a face and then presented with the same or a different face at different probabilities. Study 1 followed a classical oddball set up presenting infants with the habituated face for 80% of trials and a different one for 20% trials. In order to rule out that this elicited a reaction purely to the rarity of the infrequent face, in Study 2 they repeated the habituation phase and then presented each face with 50% probability. In Study 3 they then presented both faces with 50% probability, but with no prior habituation phase. The discrimination elicited by the novel face in both Study 1 and 2, as well as the lack thereof in Study 3 can be read as evidence that novelty, rather than merely infrequency was driving the ERP response.

3.1.7 Mismatch ERP response and infant state. While the MMR_{ERP} can be difficult to elicit during drowsiness and sleep in adult populations (Raz 1999, Nakagome et al., 1998), it has been successfully elicited in infants during wakefulness, active sleep and deep sleep (Cheour et al., 2002, Martynova, Kirjavainen & Cheour, 2003, Hirasawa, Kurihara & Konishi, 2002). While some studies reported no differences in amplitude or latency for the MMR_{ERP} between sleep and wakefulness (Hirasawa, Kurihara & Konishi, 2002), others reported modulations to the ERP waveform. Otte et al., (2013) addressed this issue further by examining the impact of infant state on

different types of deviants. A group of 2-month-old participants was presented with frequent tones, white noise deviants, trial unique stimuli, and stimuli that did not differ from the standard in any way but their temporal occurrence after the preceding standard (ISI deviant). All classes of stimuli were presented to infants once while they were awake, and once while they were asleep. The resulting ERP's revealed that the different stimuli across conditions were affected by the state change. They did not however find differential effects for any condition in particular, but rather what seemed to be a relatively uniform change across conditions between states.

As can be seen in Figure 3.2 ERP morphology differed slightly, with a more flattened out peak in the sleeping infants. These flattened peaks can be speculated to reflect the higher prevalence of slow wave activity in the EEG during sleep.

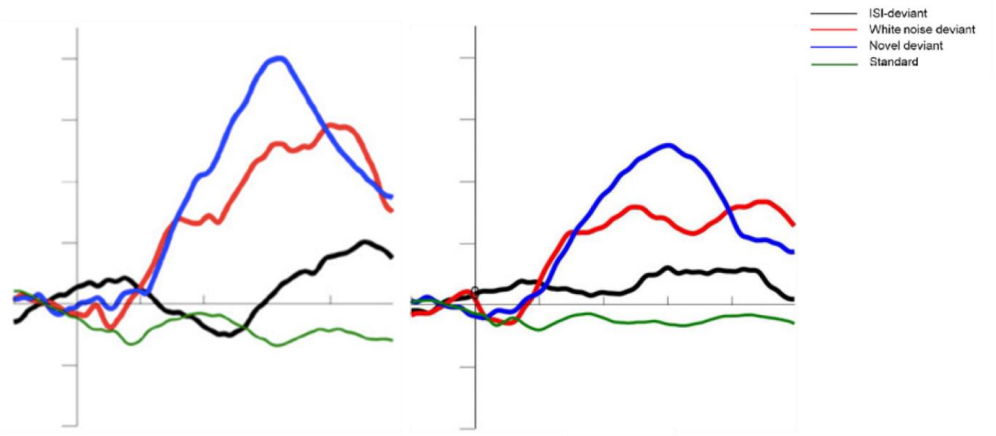


Figure 3.2. Reproduced from Otte et al. (2013). ERP's elicited to auditory stimuli in awake (left) and sleeping (right) two month old infants.

As discussed in Chapter 2, infants in this study were assessed during sleep when they were 1 month old and in a wakeful state when they were 5-month-old. When considering the results, this state change needs to be

kept in mind. However, it has been shown that even though the ERP in mismatch paradigm might be affected by state changes, it is unlikely to affect the relation between the responses across conditions. As the main focus of this project is to compare responses between sites and infant state was kept constant within time points the comparison should be unaffected by this difference. To assess whether ERP morphology would be affected by infant state, we took advantage of the fact that a subset of the Keneba cohort fell asleep during the 5-month studies. We assessed these infants and compared them with a subset of awake infants. From previous literature, a possible amplitude modulation was expected between sleeping and waking infants, but not an interaction of infant state and stimulus condition.

3.1.8 Deviance detection in clinical populations. Several advances have been made to use the MMR_{ERP} as a marker of atypical brain function and neurodevelopment in clinical populations, which has greatly contributed to the knowledge base on its potential for use in clinical practice. The MMR_{ERP} holds utility as a clinical tool due to its well documented waveform and its prior use in a range of populations, such as minimally conscious patients (Morlet et al., 2014), patients suffering from Alzheimer's disease (Polich et al, 1997a, Bashore 1990) and those with psychiatric diagnoses such as psychosis and schizophrenia (Shelley et al., 1991) and bipolar disorder (Takei et al., 2010, Andersson et al., 2008), developmental disorders such as autism spectrum disorder (Näätänen & Kujala, 2011) and dyslexia (Baldeweg et al., 1999) and acquired disorders such as aphasia resulting from stroke (Aaltonen et al., 1993). With regard to infant studies, novelty paradigms have been used to study the impact of specific risk factors, such as preterm birth (Cheour-Luhtanen et al., 1996) or chronic maternal conditions such as diabetes (Siddappa et al., 2004, Burden et al., 2007).

Advances were first made using MMR_{ERP} designs in the study of cognitive decline in populations of older adults. Studying older adults with and without Alzheimer's dementia, Polich (1997a) and Bashore (1990) both reported attenuated amplitudes and elongated latencies of the P3 response elicited by an auditory oddball task. In a review of the utility of the P3 components in different clinical groups, Goodin (1990) noted that P3 latency measures can help distinguish dementia caused by neurological decline from pseudo- dementia brought about as a co-occurrence of psychiatric disorders such as depression. In terms of predictive power, Goodin et al., (1983, 1990) found that the atypical P3 characteristics could be identified in a subset of asymptomatic patients infected with HIV, which has potential to distinguish those at greater risk of later developing HIV caused encephalopathy.

The MMR_{ERP} has also been used as an index of cognitive functioning in a range of psychiatric disorders, which have been linked to symptom severity (Takei et al., 2010, Andersson et al, 2008, Shelley et al., 1991). P3 characteristics, particularly latency measures, have therefore been proposed as a means of identifying which patients were likely to go on to showing particularly severe symptom patterns, which, if identified at an early stage, could facilitate early intervention in those most at risk of severe disease progression (Belger et al., 2012). From these studies, it was concluded that while a diagnosis can at least be greatly aided by the examination of the P3 in appropriate clinical contexts, clinically relevant stimulus contrasts need to be implemented to make predictions within populations known to be at risk of a particular deficit (Goodin, 1990).

Due to the known links between auditory discrimination and language acquisition, many studies have examined mismatch paradigms using language contrasts in populations with known deficits in this area. Studying

a population of stroke patients with aphasia, Aaltonen et al. (1993), found the mismatch negativity (MMN) component of the MMR_{ERP} to frequently be absent, and the magnitude of the component to be correlated with the degree of cognitive impairment. Similar observations have been made in developing populations. Profiles of children with dyslexia and specific language impairments showed a greatly reduced MMN (Baldeweg et al., 1999, Benasich et al., 2006, Bishop et al., 2010), as did children with autism spectrum disorder who often show delayed language development (Näätänen & Kujala, 2011). Interestingly, cohorts with autism spectrum disorder have repeatedly been shown to have typical or even enhanced MMR_{ERP}'s in response to non-speech sound contrasts, highlighting the usefulness of this paradigm to identify very specific deficits (Näätänen & Kujala, 2011).

Some studies have examined the impact of maternal factors on infant development. Siddappa et al. (2004) and Burden et al. (2007) examined the effects of iron deficiency, caused by maternal diabetes on infants' visual and auditory recognition memory using ERP paradigms. They observed differential responses in the affected infants compared to controls. Siddappa et al. (2004) found significantly shorter latencies in infants of diabetic mothers who were iron deficient compared to those not showing an iron deficiency. This was interpreted as an absence of sustained neural activity in areas associated with memory processes. Burden et al. (2007) compared infants with an iron deficiency to a control group and found reduced discrimination between a familiar and a novel image in the iron deficient group of infants.

From the literature using mismatch paradigms in clinical populations it becomes apparent that the MMR_{ERP} holds potential for use in at-risk populations. While clinical expertise is needed to determine which stimulus

contrasts are best suited for the population under investigation, it has been shown that differences in the MMR_{ERP} can be observed in at-risk populations even before the onset of overt symptoms of cognitive decline and can even help identify on an individual level those participants who are most likely to have severe impairments.

3.1.9 ERP findings from resource poor settings.

In addition to their use in clinical contexts ERP responses have been used in the context of populations exposed to different forms of adverse environmental factors. A range of studies has assessed children who were institutionalised at a very early age (Nelson, 2014). Due to the high numbers of children housed in these institutions these infants and children spent their early months and years in, individuals frequently did not receive adequate sensory input during infancy and did not have their emotional needs met. When tested at age 11 on ERP measures tapping executive control and response inhibition it was found that children who had been institutionalised as infants showed significantly lower ERP amplitudes in response to these tasks (Nelson, 2014). Interestingly, children who were placed into foster care before the age of 2 showed higher amplitudes than those children who remained in institutions beyond their second birthday. Both groups however had reduced amplitudes compared to children raised with their families within the general community. Though these children were tested on ERP measures different from those employed in the current project, the mechanistic explanations put forward to account for their findings apply to electrophysiological measures across a range of tasks. The reduced ERP amplitudes were hypothesised to be a result of slowed maturational processes of the brain. Two hypotheses were put forward in particular for this population. First, it was suggested that the brains of children spending their early years in institutions were chronically under aroused and failed to

respond strongly to sensory input. The second explanation was that the lack of early sensory experiences had led to an over- pruning of synaptic pathways in the brain, leading to weaker functional connections within the cortex resulting in reduced amplitudes.

With regard to novelty detection in particular, some advances have been made in the context of studying children in sub Saharan Africa. While ERP's have not previously been used to study infant development in Africa, some studies by Kihara and colleagues have examined novelty detection in children in rural Kenya. These studies examined the MMR_{ERP} to a novelty paradigm in typically developing Kenyan children, as well as those formerly affected by one of several types of Malaria. In the first of these studies (Kihara et al., 2010a), normative data was collected on a sample of n = 178 children from the community aged 4-12 years. It was found that early ERP components to visual and auditory novelty tasks decrease with age. The same was true for the later components, with exception for the auditory and visual P3a components, which were both found to increase. Building on these normative findings, Kihara et al. (2010b) then moved on to study a cohort of 6-7 year old children affected by several types of malaria and compared them to age matched community controls. They observed different profiles of changes to the MMR_{ERP} morphology in their different patient groups. Children formerly affected by more severe types of the disease, such as malaria accompanied by seizures as well as those suffering from malaria with prostration were neither found to show different P3 responses to novel over familiar images, nor to novel over familiar sounds. The findings were interpreted in terms of damage to the frontal-temporal network thought to underlie these processes. In a further study Kihara et al., (2012) examined a sample suffering from pneumococcal meningitis, a condition associated with a range of cognitive deficits. They observed longer

latencies for the novelty P3, as well as an apparent absence on group level of a P3 to novelty, compared to a control sample. These three studies illustrate that MMR_{ERP} effects can be elicited in resource poor settings and offer an objective, culturally neutral tool for assessment. They further demonstrate that differential profiles can be obtained from different clinical populations, exposed to conditions frequently encountered in resource poor settings. While the current study assesses typically developing infants, rather than clinical populations, some of the effects reported by Kihara and colleagues are still expected to be seen within the Gambian populations. This is due to the fact that despite being a representative, typically developing sample of the community in the rural Gambia, infants in this cohort are still exposed to vastly higher rates of risk factors such as undernutrition and infections and a lack of sanitary facilities, which as laid out in detail in Chapter 1 can all adversely affect neurocognitive development. These systemic issues are expected to be reflected in differences in neural responses similar to those reported above in context of atypical development in LMIC's.

3.1.10 Rationale for use in the current study.

The implemented ERP paradigm holds great potential for use in this project for several reasons. As discussed above, the response to infrequent and trial unique tones has been well-characterised in different infant cohorts in the past, enabling predictions as to what may indicate typical or atypical responses. The same is true for the developmental change that is expected between the studied age points. With regard to the set-up of the study, its implementation is facilitated by the fact that infants are listening passively, rather than being required to attend to visual stimuli. This is especially important given that it somewhat reduces the overall novelty of the testing situation for the Gambian infants, as they can face a parent or play with a toy of their choice.

Auditory paradigms further have the advantage to be useable in very young, sleeping infants. As newborns are asleep for up to 80% of the time (Maurer & Maurer, 1998), measurements during sleep are usually more likely to yield high quality data. With regard to cross- cultural use of this paradigm, it has the advantage that stimuli consist of pure tones, clicks or white noise which are not inherently specific to a particular culture. This helps to reduce bias, which may be introduced if language specific stimuli, such as different syllables had been used as these have different frequencies of occurrence in different languages and depend on infants' exposure to language. Lastly, detection of stimuli that have not previously been encountered before is crucial for many areas of learning and therefore relevant to measure in relation to at-risk populations. As valid, robust instruments which can be used cross- culturally are currently quite rare but much needed (Kihara, 2013), this study also serves to establish whether ERP measures in general and novelty paradigms in particular could potentially fill a gap in the study of global infant development.

3.1.11 Hypotheses.

In line with the discussed literature, the following hypotheses will be tested in the context of this study.

Experimental manipulation:

1. Amplitudes will differ between conditions, with both infrequent conditions eliciting larger amplitude responses than frequent tones.
2. There will be a habituation effect to sounds in the frequent and infrequent condition. Both will show a decrement in the P3 mean amplitude over the course of the session. This effect will be absent from the Trial Unique condition.

Developmental change:

3. It is expected that peak latencies will decrease from 1 to 5 months.
4. In line with previous studies in newborns (Kushnerenko et al., 2007), larger amplitudes are expected to be elicited by the infrequent sounds at the 1-month time point compared to both other stimulus conditions.
5. In line with van den Heuvel et al. (2015) and Otte et al. (2013) we expect trial unique sounds to elicit the largest responses compared to both other stimulus conditions at the 5-month time point.

Differences between sites:

6. The change in latencies is expected to be less pronounced in the Keneba cohort, reflecting a reduced developmental change between age points.
7. In line with the work by Kihara et al. (2010b, 2012), P3 amplitudes are expected to be reduced in the Keneba cohort compared to the infants tested in Cambridge.
8. As infants in The Gambia are exposed to a greater variety of environmental risk factors, this is expected to be reflected in the inter-individual variance in ERP amplitudes and latencies, with higher variance in the Keneba cohort at both time points.

Difference between states:

9. It is expected that 5 month old infants tested asleep will show similar ERP responses across conditions compared to 5 month old infants tested awake.

3.2 Method

3.2.1. Participants. Infants took part in the EEG study as part of the BRIGHT project when they were 1, 5 and 18 months old. Here data from the 1 and the 5 month age point is presented. Data is provided on a partial sample for which data was available at time of the analyses. Information of participants included in analyses can be found in Table 3.1. A breakdown of data quality and rejection is provided in Figure 3.4.

Table 3.1. *Sample sizes and age in days for 1 and 5 month time points for participants completing the EEG assessment.*

	1m		5m	
	N (girls)	Age in days X ± SD	N (girls)	Age in days X ± SD
Keneba	83 (46)	35.27 ± 6.02	44 (21)	159.24 ± 8.39
Cambridge	45 (22)	33.29 ± 6.25	38 (17)	154.59 ± 5.24

3.2.2 Stimuli and Design. Auditory stimuli of three different categories (Frequent, Infrequent, Trial Unique) were presented during this study. Sounds were presented through wireless Sony TMR-RF810R headphones, at a fixed sound level of 60 dB SPL. Sounds of three different categories were presented. The frequent sounds consisted of 500Hz pure tones, presented for 80% of trials, infrequent sounds were made up of white noise segments presented for 10% of trials and trial unique sounds were presented for another 10% of trials. The trial unique sounds were each only presented once during the study and consisted of a range of clicks, tones, digitised bird vocalisations and syllables (adapted from Kushnerenko et al., 2007). Each sound was presented for 100ms with a 5ms ramp up and down period. ISI's were jittered between 650 and 750ms with a mean of 700ms. At the 1 month age point, a total of 750 trials were presented, resulting in 600 frequent trials, 75 infrequent and 75 trial unique trials. Due to the increased movement artefacts in the data at the 5 month time point, it was decided to present a larger number of trials, so a total of 1000 trials was presented, with 800 frequent, 100 infrequent and 100 trial unique trials. The stimulus presentation, electrode montage and study setup are displayed in Figure 3.3.

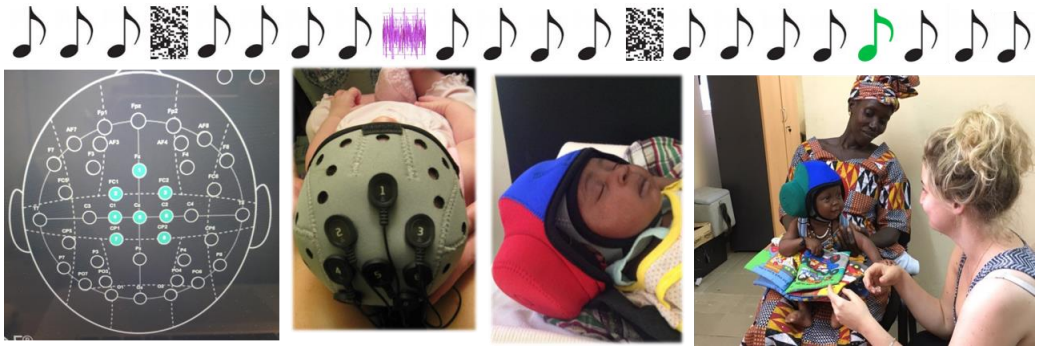


Figure 3.3. Schematic of stimulus presentation (top row), electrode montage (bottom left), electrode arrangement on infants head (mid left), infant during assessment at 1 month (mid right) and infant during assessment at 5 months (right).

3.2.3 Apparatus and Procedure. Data was collected using a wireless Enobio8 system from Neurolectrics (Enobio, Neurolectrics, Barcelona). The system implemented in this project used eight electrodes, which were localised at positions Fz, FC1, FC2, C1, Cz, C2, CP1 and CP2 according to the 10-20 system. Conductive electrolyte gel was used to make contact between electrodes and scalp. Electrodes were placed in defined locations in a neoprene cap and connected to the electrical fibres. The electrodes were then gelled immediately prior to cap placement. One researcher placed the cap onto the infant's head, checking that each electrode made contact with the scalp and ensuring the chin strap of the cap was fastened to prevent movement of the cap during the study. The reference and the ground electrodes were placed unilaterally onto the infant's left mastoid and data was recorded in reference to this position. Secondly, another cap holding in place headphones was placed on top of the electrode cap, making sure both ears were fully covered by the headphones.

For the 1 month studies, EEG data was collected during sleep, immediately after fNIRS data collection. If the infant was in a deep sleep, the fNIRS cap was taken off and replaced by the EEG cap. Data was then collected with the infant placed on a researcher's lap, their head resting in the researcher's hands. If the infant started to wake up or became fussy at any point, this setup allowed for the researcher to either wrap the infant without causing too much disturbance, or gently rock their knees from side to side to minimise artefact while trying to prevent the infant from waking up.

For the 5 month studies, infants were assessed while awake so a second researcher interacted with the baby during cap placement. Once both caps were in the correct position, the study was started usually with the infant sitting on the parent's lap while researchers silently interacted with some quiet toys, bubbles or simple gesture games. For both age points, sessions

were video recorded and reviewed after the session during data analysis to identify long periods of movement or fussing. The standard operating procedure as provided to all testers can be found in Appendix 3.1.

3.2.4 Data pre- processing and analysis. Data was pre-processed within Matlab 2015b (The Mathworks, Inc, 2015) using a customised, semi-automated analysis pipeline generated as part of this thesis. Raw data was imported into Matlab using the neurolectics pop_easy function (Neurolectrics, 2014) and converted from nano- to microvolts. Data was then bandpass filtered between 0.5 and 30Hz using a Blackman filter (Widmann, 2006) with a filter order of 5500 minimising the effect of any frequencies beyond the filter cut offs. In the context of infant research, ERP's are most commonly filtered somewhere within a passband of .1 and 30 Hz. Depending on the recording conditions and research question, these cut offs vary between studies. It is generally recommended to high pass filter data between 0.1 and 1Hz and filter out frequencies above 20-30Hz (Hoehl & Wahl, 2012). In the context of this study, some low frequency artefact was recorded due to the hot climate in which one of the infant cohorts was tested in, leading them to sweat and therefore to cause subtle movement of the electrodes. These drifts could be corrected using a high-pass filter setting of 0.5Hz, which is within the recommended range for infant ERP research (Hoehl & Wahl, 2002).

A timing test revealed a constant delay of 32ms between the event marker being sent in the data and the stimuli being presented over the headphones. To account for this, 32ms were added to each event marker. Epochs were then created with a 200ms pre-stimulus baseline and an 800ms post stimulus response window. For artefact rejection, the distance between the minimum and maximum value in each epoch was calculated and trials with a distance larger than 200µV were discarded from further

analyses. To discard trials that were collected after the cap had been removed quickly due to the infant getting fussy, a flat-line detection was introduced which discarded trials that showed a difference smaller than .1 μV the minimum and maximum points. In cases when the study was terminated early, the final recorded epoch was too short for further analysis, so length of each epoch was checked and epochs short than 800ms were discarded.

To ensure equal trial numbers in each condition, a random sample of epochs was chosen for each condition based on the number of trials in the condition with the fewest trials. Datasets with fewer than 15 trials in each condition were discarded from further analysis, which is conservative compared to previous recommendations of 5-10 artefact free trials for ERP analysis in infants (DeBoer, Scott, & Nelson, 2007; Stets et al., 2012). At the 1 month time point, valid datasets had between 23-74 trials (maximum number 75), at the 5 month time point, between 15 and 89 trials were included (maximum 100).

3.2.5. Data quality. Using the above pre-processing parameters and rejection thresholds, the following inclusion rates were obtained. In Cambridge 74% of the 1 month olds and 75 % or the 5 month olds who came in for a lab visit were included in the final sample. In Keneba 62% were included at 1 month and 47% at 5 months. The most common reason for exclusion included infants waking up during the capping process or waking up and fussing soon after the beginning of the study (at 1 month). At 5 months some infants did not allow for the cap to be placed on their heads or became fussy soon after capping. The higher rates of rejection in Keneba were primarily due to equipment malfunction, specifically the fact that the EEG acquisition system broke and stopped recording from some channels. Even though this was reported immediately, delays in obtaining a replacement device and having

it taken to Keneba caused disruption of data collection. As data collection is still ongoing, the inclusion figures are expected to improve. Inclusion numbers and reason for data exclusion are summarised in Figure 3.4.

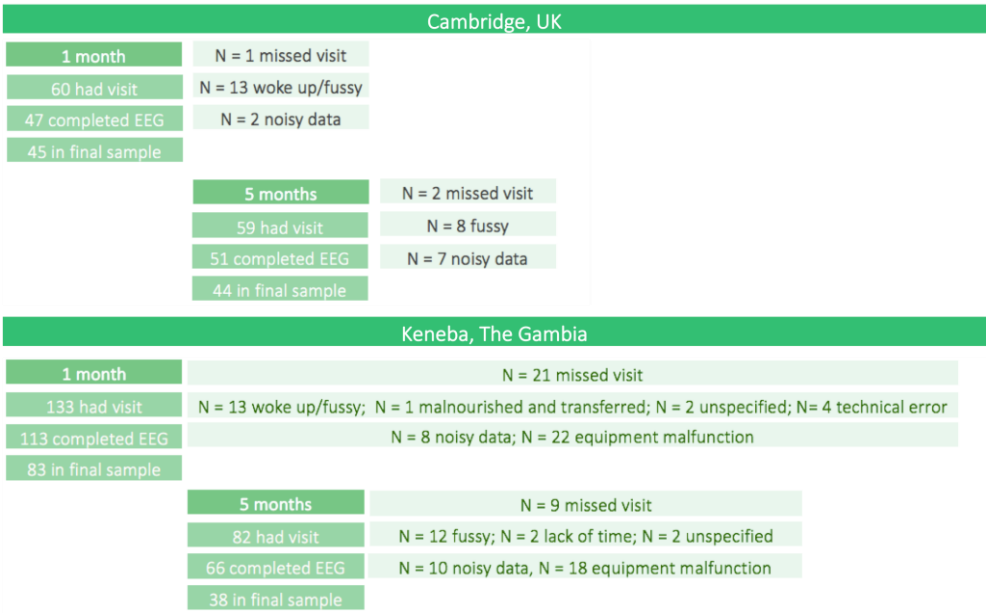


Figure 3.4. Overview of data rejection and retention for both sites and time points.

3.2.6 Statistical analysis. Based on recommendations for infant EEG data, mean- rather than peak amplitudes were used as an outcome measure (Picton & Taylor, 2007; Luck, 2014). Peak amplitudes are obtained by finding the local maximum in a time window of interest, whereas for the mean amplitude, data is averaged across a specified time window. While peak amplitudes can be artificially distorted by noise present in the data, mean amplitudes yield a more robust measure of neural activity (Luck, 2014). This is illustrated in Figure 3.5.

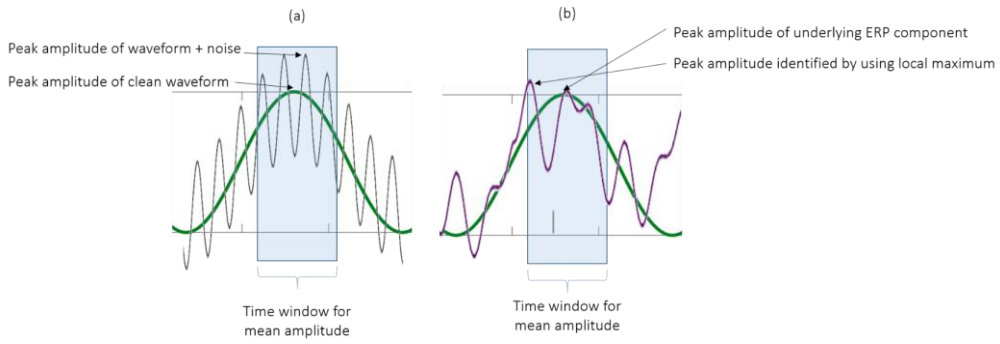


Figure 3.5. Schematic of peak amplitude distortion by noise. Different types of noise can distort peak amplitude and latency estimates. High frequency oscillatory artefact (panel a) may result in relatively accurate latency estimates but may lead to inflated amplitude estimates. In case of more irregular artefact (panel b) both estimates of peak and of latency can be distorted by measuring the peak waveform. Note that in both cases mean amplitudes in the highlighted time window produce the same estimates. Note also that centring the analysis time window on the peak of the measured signal will lead to very different estimates in panel a compared to panel b.

As our ERP paradigm has been extensively used in the literature, time windows over which these mean amplitudes were calculated, were based on previous studies in comparable age ranges. Additionally, plots for all individual participants were inspected blindly with regard to group membership (i.e. Keneba or Cambridge) and peak latencies noted to ensure the time window chosen captured the majority of these peaks. This method was chosen for three reasons. First, I wanted to avoid artificial inflation of the effects by defining a time window based on the grand average. Secondly, I wanted to ensure the same components were measured across participants, which could have not been guaranteed by taking an approach purely driven by individual peak latencies, due to the aforementioned issues with noise. Third, we wanted to avoid inclusion of only those infants which showed the expected ERP waveform on the individual level, as deviance from the expected waveform could be an indicator of differences in neural processing and therefore an important outcome for assessment. While it is

difficult to balance rejection of noise and detection of deviance in a population with suspected atypical development, the combination of reliance on prior literature as well as the careful examination of individual data without focus on group membership provided the most appropriate way of analysing this data. The chosen time windows can be found in Table 3.2. Due to the decrease in ERP latencies during early infancy (Moore & Guan, 2001; Edgar et al., 2015), the time windows for the later occurring P3 component were centred differently for the 1 month and 5 month time point.

Table 3.2. *Time windows during which the N1 and P3 components of our waveform were assessed at the 1 month and the 5 month time point.*

	N1	P3
1m	50-150ms	250-450ms
5m	50-150ms	200-400ms

The mean amplitude during the defined time windows was entered into repeated measures mixed effects models. Separate models were generated for the N1 and the P3 component of the ERP. As the electrodes we recorded from were closely spatially aligned, we did not enter electrode site into the model as a factor but rather focused our analyses on the Fz location which is the principal location for the novelty response (Polich, 2007). It was considered to average Fz and its surrounding channels to reduce noise levels, however this was not pursued for two reasons. First, responses were all strongly correlated at the 1 month time point, presumably due to the open fontanelle, meaning that noise levels were not significantly affected by

averaging across electrodes. The response was however much more localised at the 5 month time point, with similar condition response patterns between electrodes but attenuated amplitudes at posterior electrodes. Using an average of several electrodes would therefore have resulted in artificially small amplitudes at the 5 month but not the 1 month time point.

Age point (1 month/ 5 month) and Condition (Frequent/ Infrequent/ Trial Unique) were entered as within subject factors, Study site (Cambridge/ Keneba) was entered as a between subject factor.

3.3 Results

3.3.1 ERP morphology. Figure 3.6 shows ERP grand averages for both age points and study cohorts for all conditions. Results are displayed for electrode Fz. The morphology of the ERP is in line with previous studies using similar paradigms with an early negative (N1) and a high amplitude positive component at around 300ms (P3). The 5 month time point, the N1 component is more variable compared to the 1 month time point. While the N1 will be considered for some of the following analyses, the P3 has been shown as the primary indicator regarding condition differences in mismatch paradigms, which is why the majority of analyses will examine this component.

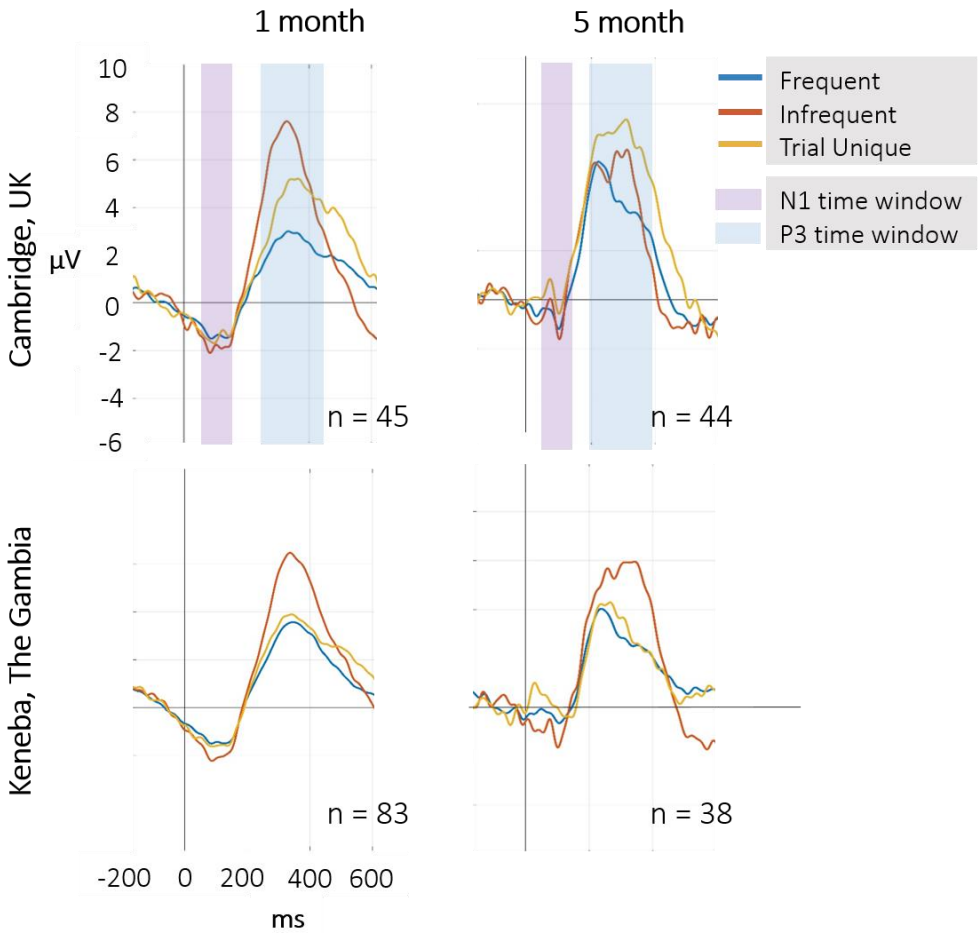


Figure 3.6. ERP's for infants in Cambridge (top) and Keneba (bottom) tested at 1 (left) and 5 months (right). ERP amplitudes (μV) are plotted over time (ms). The three different conditions are plotted separately.

3.3.2 Statistical analysis. Descriptive statistics for the N1 and P3 mean amplitudes can be seen in Table 3.3.

Table 3.3 *Descriptive statics of ERP mean amplitudes of N1 and P3 components for Cambridge and Keneba at 1 and 5 months.*

		1 month		5 month	
		N1	P3	N1	P3
		$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD
Cambridge	Frequent	-2.380 ± 5.09	1.317 ± 4.25	0.991 ± 5.15	3.594 ± 8.59
	Infrequent	-1.443 ± 4.50	8.765 ± 8.47	2.137 ± 6.87	5.409 ± 10.4
	Trial Unique	-1.458 ± 4.46	5.468 ± 2.32	.956 ± 5.30	8.157 ± 9.07
Keneba	Frequent	-1.554 ± 4.38	2.319 ± 5.23	-1.441 ± 5.66	0.319 ± 9.29
	Infrequent	-1.201 ± 4.72	8.137 ± 6.97	-.888 ± 7.61	6.249 ± 8.39
	Trial Unique	-1.074 ± 4.65	4.491 ± 5.82	.555 ± 8.15	5.291 ± 7.80

To examine the data taking into account all factors, a repeated measures mixed effects ANOVA (RMANOVA) was fit, using Age (1 month / 5month) and Condition (Frequent / Infrequent / Trial Unique) as within subject factors and Site (Cambridge / Keneba) as the between subjects factor. Prior to fitting the model, assumptions of normality, homoscedasticity and sphericity were tested separately for the N1 and P3 components. It was found that all subgroups were approximately normally distributed. Levene's tests for subgroups of the repeated factors revealed that the assumption of

homoscedasticity of variance was met, with p-values all ranging between $.065 < p < .974$. The assumption of sphericity, which only applies to within subject repeated independent variables with more than two levels, was met for both the factor of Condition (Mauchly's $W_{N1} = .97$, $p_{N1} = .68$, Mauchly's $W_{P3} = .99$, $p_{P3} = .77$), as well as the interaction of Condition * Age (Mauchly's $W_{N1} = .99$, $p_{N1} = .78$, Mauchly's $W_{P3} = .995$, $p_{P3} = .88$).

As all parametric assumptions were met, mean amplitudes of the N1 and P3 components were entered into separate RMANOVA's. Results are summarised in Tables 3.4 and 3.5. For the N1 only the factor Age was found to be statistically significant ($F_{1,53} = 16.418$, $p < .001$). Despite this Age effect, the analysis of the N1 component was not taken forward as it was not found to discriminate between stimulus conditions. As discussed above, the P3 component is best suited to discriminate between frequent and infrequent conditions in mismatch paradigms and will thus be focused on for the remainder of analyses.

For the P3, mean amplitudes were found to significantly differ by Condition ($F_{2,106} = 15.728$, $p < .001$). Post- hoc comparisons for the factor Condition (Table 3.6) revealed that P3 mean amplitudes were higher for the infrequent and trial unique when compared to the frequent condition. Infrequent tones did not differ significantly from trial unique sounds.

There were no main effects for either Age ($F_{1,106} = .001$, $p = .987$) or Site ($F_{1,53} = 2.232$, $p = .141$). The interaction terms of Condition * Age ($F_{2,106} = 3.980$, $p = .022$) and Condition * Age * Site ($F_{2,106} = 3.776$ $p = .026$) were found to be significant. The three way interaction is illustrated in Figure 3.7, and shows similar results across Sites and Age points for the frequent sounds. There is a stronger decrease in P3 amplitude in the Cambridge cohort but no apparent change in the Keneba cohort for the infrequent

sounds. As expected developmentally, there was an increase in response to trial unique sounds when collapsing across cohorts. However, this trend was found to be driven by the Cambridge cohort as can be seen in Figure 3.7. All of these results will be discussed in more depth in the context of the formulated hypotheses.

Table 3.4. *Analysis of variance for N1 mean amplitude with Age, Condition and Site as independent variables.*

IV	*F*	*df*	*p*	η_p^2
Condition	2.017	2,106	.138	.037
Age	16.418	1,53	<.001*	.237
Site	.075	1,53	.785	.001
Condition * Age	.392	2,106	.677	.007
Condition * Site	1.824	2,106	.166	.033
Age * Site	1.524	2,53	.222	.028
Condition * Age * Site	1.960	2,106	.146	.036

Note. IV = Independent variable, df = degrees of freedom, η_p^2 *= partial eta squared.*

Table 3.5. *Analysis of variance for P3 mean amplitude with Age, Condition and Site as independent variables.*

IV	*F*	*df*	*p*	η_p^2
Condition	15.728	2,106	<.001*	.229
Age	.001	1,53	.987	<.001
Site	2.232	1,53	.141	.040
Condition * Age	3.980	2,106	.022*	.070
Condition * Site	.465	2,106	.629	.009
Age * Site	.177	2,53	.676	.003
Condition * Age * Site	3.776	2,106	.026*	.066

Note. IV = Independent variable, df = degrees of freedom, η_p^2 *= partial eta squared.*

Table 3.6. *Post hoc tests for factor Condition.*

	Mean Difference	*p*
Frequent – Infrequent	-4.345	<.001*
Frequent – Trial Unique	-4.643	<.001*
Infrequent – Trial Unique	1.969	.987

Note: significance level of α = .05 Bonferroni corrected.

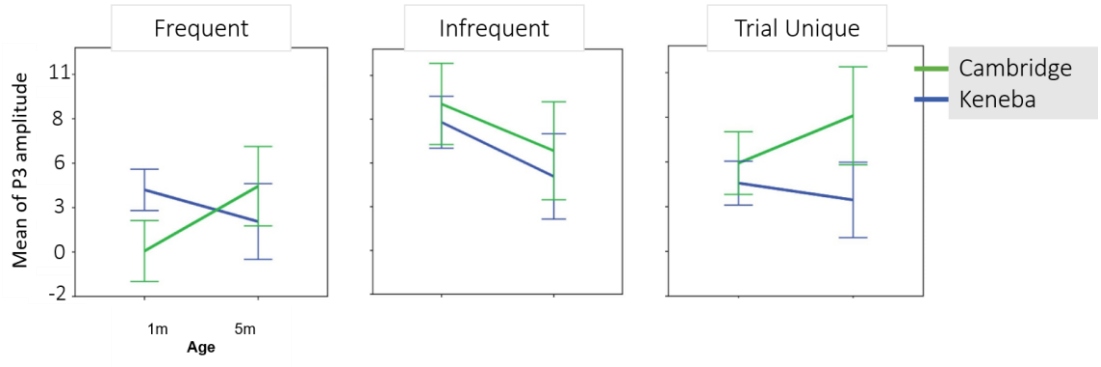


Figure 3.7. Interaction effect of factors Condition, Age and Site on P3 mean amplitude. Displayed are results from Cambridge (green) and Keneba (blue). Error bars indicate 95% confidence intervals.

3.3.3 Experimental Manipulation. *Hypothesis 1.* It was expected that frequent tones would elicit a smaller P3 response compared to both other conditions. As can be seen in Table 3.5, data were in line with this hypothesis, as evidenced by a significant main effect for Condition ($F_{2,106}$ = 15.728, p < .001). Posthoc comparisons revealed that when collapsing across Age and Site, Infrequent tones elicited larger amplitudes than Frequent tones (p <.001) and Trial Unique tones (p < .001).

Contrary to the hypothesis, it can be seen from the ERP waveform (Figure 3.6) that at the group level, there does not seem to be a discrimination between frequent and trial unique sounds in the Keneba cohort at either age point. To explore this effect, the same model as above was fit for Cambridge and Keneba separately, leaving out the factor of Site (Table 3.7). In both models there was a main effect for condition as well as a significant interaction term for Condition * Age. The main effect for condition was followed up by post hoc comparisons (Table 3.8), which showed similar results across sites. In both cohorts, Infrequent and trial unique sounds differed significantly from frequent sounds, but not from each other. Notably, the difference between Frequent and Trial Unique sounds in

Keneba is marginally significant (p = .046), indicating a smaller effect in this comparison.

Table 3.7. *Results per study site- analysis of variance for P3 mean amplitude with Age and Condition as independent variables, fit separately for Cambridge and Keneba.*

	Cambridge				Keneba			
IV	*df*	*F*	*p*	η_p^2	*df*	*F*	*p*	η_p^2
Condition	2,70	9.399	<.001*	.212	2,36	6.717	.003*	0.008
Age	1,35	.050	.824	.001	1,18	0.15	.703	.272
Condition * Age	2,70	5.696	.005*	.14	2,36	6.717	.003*	.087

Note. IV = Independent variable, df = degrees of freedom, η_p^2 = partial eta squared.

Table 3.8. *Post hoc tests for factor Condition for Cambridge and Keneba.*

	Cambridge		Keneba	
	Mean Difference	*p*	Mean Difference	*p*
Frequent – Infrequent	-3.769	.009*	-5.436	<.001*
Frequent – Trial Unique	-4.615	<.001*	-4.697	.046*
Infrequent – Trial Unique	-.846	.978	0.739	.987

Note: significance level of α = .05 Bonferroni corrected.

Hypothesis 2. It was expected than when comparing P3 amplitudes over the session, we would find habituation effects for both the frequent as well as the infrequent sounds. Trials unique sounds were expected to not show an amplitude decrease, as they are unique by definition and therefore should not elicit habituation. For this analysis, each dataset was subdivided into three bins, grouping early, mid and late occurring trials for each session. ERP's were then averaged across each of those three epochs. Results of this epoch analysis can be seen in Figure 3.8. As can be seen from the figure, infants showed habituation effects to the frequent tones at the 1 month time point. In Cambridge, this was most prevalent between the first and the second epoch and no further decrement could be observed between epoch 2 and 3. In the Keneba group, amplitude decreases occurred linearly across the entire session. Habituation was also expected to occur for the infrequent tones. At the one month time point, habituation was observed across all epochs at both sites.

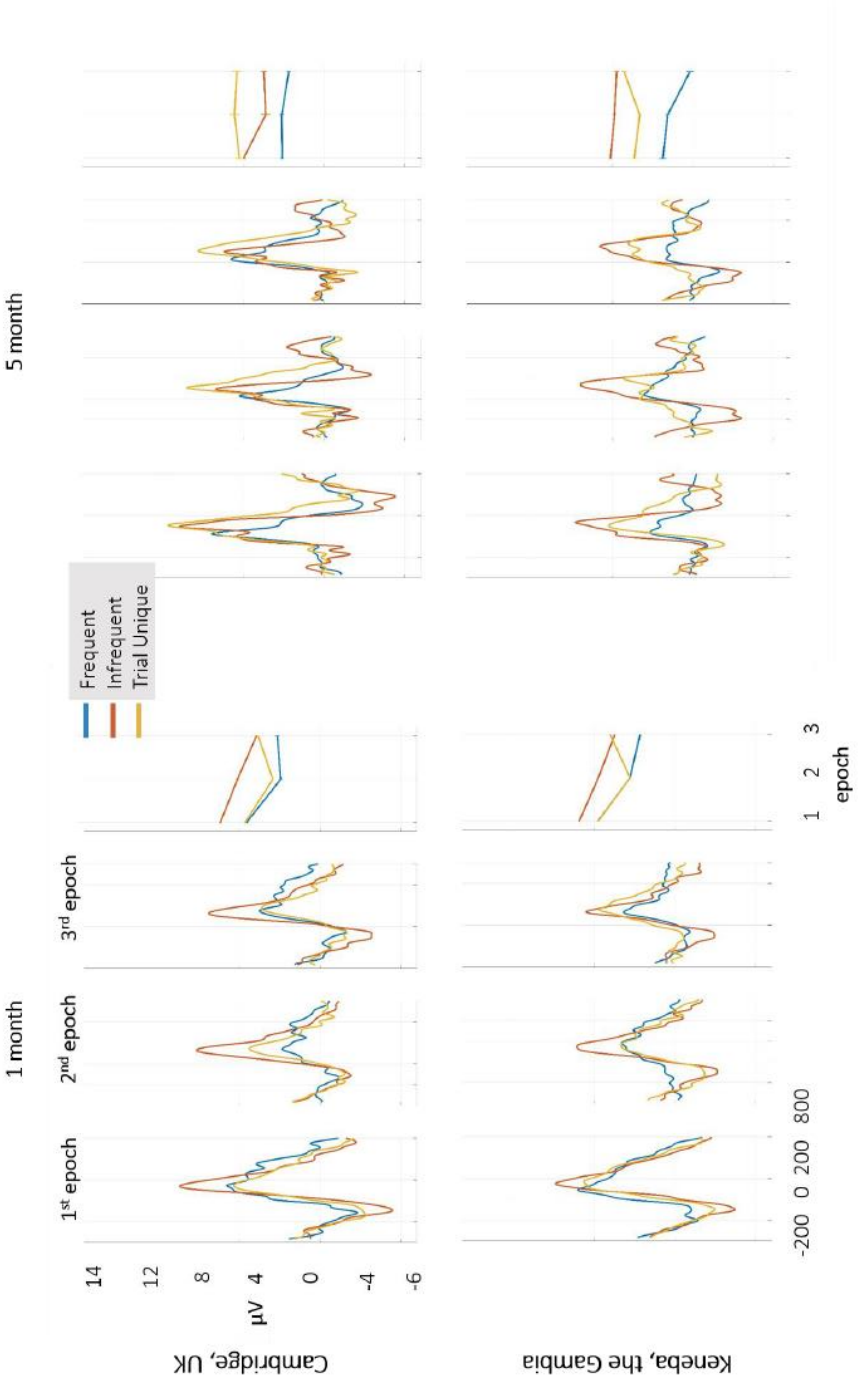


Figure 3.8. ERP amplitude changes over first, second and third epoch of the recording. Results are displayed separately for Cambridge (top) and Keneba (bottom) at the 1 month (left) and 5 month (right) time point.

With regard to the trial unique sounds, it was expected that habituation would not occur. It was found however, that there were amplitude decrements in response to these sounds between the first and second epoch. Interestingly, amplitudes then increased again between epoch 2 and 3. This was interpreted as a late occurring contrast effect, by which the habituation to tones from both other conditions made the trial unique tones more salient against the habituated background. Notably, no differences in habituation processes were observed between Cambridge and Keneba at the 1 month time point. Habituation responses differed between sites at the 5 month time point. No habituation effects were observed in response to the trial unique tones in the Cambridge cohort, whereas amplitudes to both the frequent and infrequent conditions decreased across epochs. Interestingly, both infrequent and trial unique tones elicited amplitudes of similar magnitude during the first third of the session. By the end of the session, there was a clear discrimination in response between the two conditions, which seems to have been driven by habituation to the infrequent tones.

In Keneba however, a different response pattern was observed. In contrast to the Cambridge group, responses to the infrequent sounds were highest during the first epoch, indicating that infants still predominantly reacted to stimulus intensity, rather than novelty. Further, the response decrement observed for the infrequent tones was found to be relatively minor. Habituation effects were observed in the frequent and infrequent tones, but not for the trial unique tones. Even though trial unique sounds did not seem to have been preferentially processed at this age point the contrast effect to the trial unique sounds towards the end of the session was visible, leading to amplitudes of similar magnitudes for both infrequent conditions in the latter epoch. Interestingly, when splitting the session into epochs, it

becomes apparent that there is discrimination between the trial unique and frequent tones, which gets larger towards the end of the session as infants habituate more and more to the frequent tones. On a whole session level however, this is not visible in the ERP waveform. Overall, two factors seem to be driving the ERP grand average waveform: first, infants initially react more strongly to infrequent sounds, which signals immature intensity over novelty preference. Second, the habituation they show to the infrequent sounds is minimal, meaning that despite the presence of a saliency effect to the trial unique sounds during later trials, this is not strong enough to override the lack of habituation to the infrequent sounds. Taken together, lack of habituation and decreased novelty preference indicate a less mature processing style within the Keneba cohort at 5 months, which will be discussed later on.

To examine these effects statistically, mean amplitude of the P3 components were entered into a repeated measures mixed effects ANOVA using Epoch (1^{st}, 2^{nd}, 3^{rd}), Condition, Age and Site as independent variables. The factor Epoch was found to be a highly significant predictor of P3 mean amplitude ($F_{2,106}$ = 14.119, p <.001). Post-hoc comparisons revealed that mean amplitudes for the P3 measured in the second epoch were significantly lower compared to the first epoch (p <.001), but there was no further decrement in amplitude between the second and third epoch (p = .674). Further, the interaction between Condition * Epoch was found to be significant ($F_{4,106}$ = 3.387, p = .012), indicating differential habituation effects across conditions.

3.3.4 Developmental Change.

Hypothesis 3. It was expected for peak latencies to decrease between age points. Due to the generally higher noise levels in data obtained from developing populations as well as the less clearly defined, more plateau-like peaks in infant data, it can be misleading

to focus on the peak within a response window alone (Thierry, 2005, Hoehl & Wahl, 2002). Several methods have been proposed to more robustly estimate component latencies, however they are rarely used in developmental studies (Hoehl & Wahl, 2002). In fact, even the noise levels in relatively clean single subject data obtained from adult populations have been found to not be ideal for accurate latency measurements for some ERP measures more prone to higher signal to noise ratios (Miller, Patterson & Ulrich, 1998). To obtain a more robust measure of changes in peak latencies, a jackknife approach was taken. In this approach which has been proposed for ERP data by Ulrich and Miller (2001), the grand average across n-1 subjects is taken and the peak latency is determined for this average. This procedure is then repeated, until n grand averages are obtained each leaving out one subject. The resulting averages are then statistically treated like single subject peak latencies and analysed as such. The individual waveforms as well as the n grand averages obtained from the jackknife procedure are illustrated in Figure 3.9.

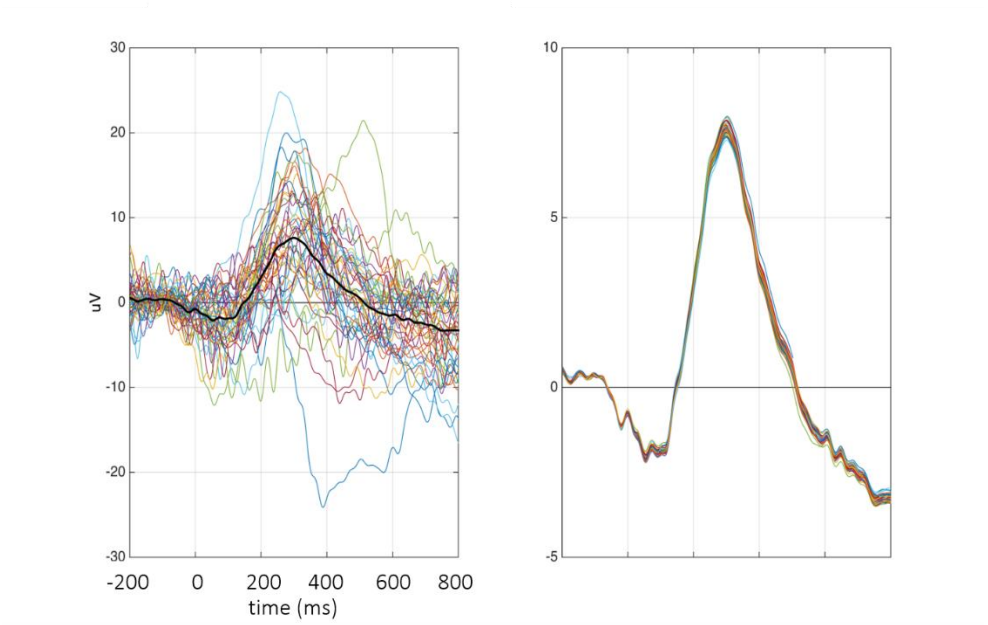


Figure 3.9. Illustration of jackknife procedure for peak latency analysis. Individual waveforms overlain by grand average (left) and jackknife subsamples (right).

Descriptive statistics for the latencies obtained through the jackknife procedure per site and age point can be found in Table 3.9. As for the amplitude measures above, is was first checked whether assumptions of normality, homoscedasticity and sphericity (for Condition and Condition * Age) were met. All subgroups were found to be approximately normally distributed. The assumption of homoscedasticity was met, as evidenced by Levene's test result ranging between $.186 < p < .975$. The assumption of sphericity was met for Condition (Mauchly's W = .99, $p = .78$), as well as the interaction of Condition * Age (Mauchly's W = .996, $p = .89$).

Table 3.9. *Descriptive statistics of latencies across grand averages obtained from jackknife procedure for Cambridge and Keneba at 1 and 5 months and group totals.*

	1 month	5 month	Total
	$\bar{X}$ ± SD	$\bar{X}$ ± SD	
Cambridge	307.61 ± 13.52	247.52 ± 41.07	291.16 ± 37.54
Keneba	309.29 ± 4.02	256.95 ± 47.47	278.14 ± 42.69
Total	308.77 ± 8.73	252.29 ± 44.59	

Latency measures were entered into a RMANOVA model with Condition (Frequent / Infrequent / Trial Unique) and Age (1 month / 5month) as within-, and Site (Cambridge / Keneba) as between subject factor. As jackknife procedures lead to inflated test statistics, due to the highly reduced error variance, the resulting t or F-values need to be adjusted, as is laid out in detail in Ulrich and Millner (2001). This is achieved by adjusting the (in case of an ANOVA) *F* statistic by the number of participants in each cell ($F_{corrected} = F/(n\text{-}1)^2$). Since the degrees of freedom do not need to be adjusted, the corrected p values can simply be determined using $F_{corrected}$. The model can be seen in Table 3.10. A significant main effect for age was found. This is in support of the well- documented effect of decreased latencies in older infants. Differences in the decrease between sites will be discussed in the context of Hypothesis 6.

Table 3.10. *Analysis of variance for P3 latency with Age, Condition and Site as independent variables.*

independent Variable	$F_{corrected}$	*df*	$p_{corrected}$
Condition	2.13	2,106	.124
Age	6.52	1,53	.014*
Site	4.13	1,53	.047*
Condition * Age	1.64	2,106	.199
Condition * Site	1.12	2,106	.330
Age * Site	7.87	1,53	.007*
Condition * Age * Site	2.16	2,106	.317

Hypothesis 4. It was expected that the infrequently appearing white noise segments would elicit the largest P3's in the 1 month cohorts, as this is what has been previously reported for newborn populations (Kushnerenko et al., 2007, Háden et al., 2013). As the full model showed a significant Condition * Age interaction effect, this effect was assessed further by fitting a model for each age point separately, thus examining factors Condition and Site but leaving out Age (Table 3.11). Data were in line with this hypothesis, as evidenced by a highly significant main effect for Condition at the 1 month time point. Post hoc comparisons (Table 3.12) showed higher amplitudes to infrequent than to frequent ($p < .001$) and to trial unique ($p < .001$) sounds.

Even though this preference has previously been mostly demonstrated in neonates, it was still clearly measurable in both 1 month cohorts.

Table 3.11. *Results of ANOVA examining factors Condition and Site separately for 1 and 5 months.*

	1 month				5 month			
IV	*df*	F	*p*	η_p^2	*df*	F	*p*	η_p^2
Condition	2,252	36.351	<.001*	.223	2,156	8.193	<.001*	.093
Site	1,126	.079	.779	.001	1,78	1.577	.213	.020
Condition * Site	2,252	0.921	.399	.027	2,156	3.639	.029*	.045

Note: IV = Independent variable, df = degrees of freedom, η_p^2 = partial eta squared.

Table 3.12. *Post hoc tests for factor 'Condition' for 1 and 5 month age point.*

	1 month		5 month	
	Mean Difference	*p*	Mean Difference	*p*
Frequent – Infrequent	-6.391	<.001*	-3.717	.013*
Frequent – Trial Unique	-2.868	<.001*	-4.751	<.001*
Infrequent – Trial Unique	3.523	<.001*	-1.034	.987

Note: significance level of α = .05 Bonferroni corrected.

Hypothesis 5. It was expected that trial unique sounds would elicit the largest P3 response at 5 months of age compared to both other stimulus conditions. This response pattern, which reflects a transition from reaction to intensity towards preferential processing of stimulus novelty, has previously been demonstrated in adults (Kushnerenko et al., 2007) and older infants (Otte et al., 2013, van den Heuvel et al., 2015). As the shift has been documented in 4 month old infants (van den Heuvel et al., 2015), it was expected to be observable in our 5 month cohorts. As for Hypothesis 4, a model containing Condition and Site was fit for the data collected at 5 months only (Table 3.11). In this model, the main effect for Condition was found to be significant. Posthoc comparisons showed differences between frequent sounds and both infrequent conditions, but not between the two infrequent conditions (Table 3.12). The interaction term of Site * Condition however was significant, indicating that condition differences were measured when controlling for the factor of Site. To explore this further, the factor of Condition was examined separately for the 5 month cohorts in Keneba and Cambridge in a univariate analysis of variance. A significant difference was found in both cohorts ($F_{Cambridge\ 2,84}$ = 3.674, $p_{Cambridge}$ = .030, $F_{Keneba\ 2,72}$ = 5.949, p_{Keneba} = .004). Post hoc comparisons (Table 3.13) showed that in Cambridge, the only significant difference that could be observed was between the frequent and the trial unique sounds, which is in support of the notion that infants at this site showed the strongest response to the Trial Unique sounds. In Keneba, significant differences were found between frequent and infrequent as well as frequent and trial unique sounds, but not between infrequent and trial unique sounds. This response pattern is very similar to that observed in the Keneba cohort at the 1 month age point, and is in contrast to the hypothesis. Overall these findings provide evidence indicating that trial unique sounds are eliciting the largest

response in the Cambridge cohort at 5 months, but not in the Keneba cohort.

Table 3.13. *Post hoc tests for factor 'Condition' at 5 month age point fit separately for Cambridge and Keneba*

	Cambridge		Keneba	
	Mean Difference	*p*	Mean Difference	*p*
Frequent – Infrequent	-1.815	.806	-5.929	.013*
Frequent – Trial Unique	-4.562	.028*	-4.971	.041*
Infrequent – Trial Unique	-2.747	.393	0.958	.987

Note: significance level of α = .05 Bonferroni corrected.

3.3.5 Differences between Sites. *Hypothesis 6.* As discussed above, changes in latencies can give an indication of maturational processes in the brain such as myelination of underlying tissue. In line with the hypothesis that development within the Keneba cohort would on a group level progress at a slower rate, including neurobiological development, it was expected that peak latencies would decrease less between the 1 and 5 month age points. This was found to be the case. As the results in Table 3.10 indicate, a significant interaction effect was found of Age * Site ($F_{1,53}$ = 5.48, p = 0.007). Figure 3.10 illustrates this interaction. It can be seen that at 1 month, both cohorts start out with similar peak latencies. The developmental change however is larger for the Cambridge cohort as indicated by a significant Age * Site interaction effect, and they display significantly shorter latencies at the 5 month time point (t_{53} = 2.105, p = .02).

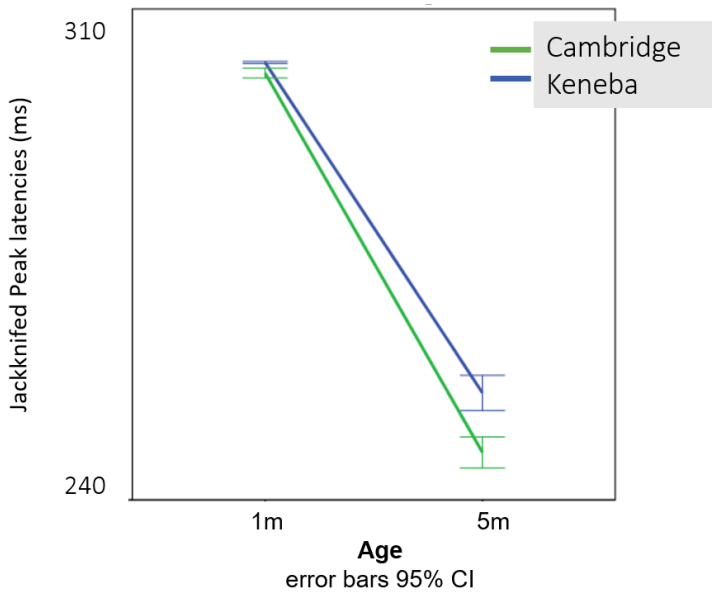


Figure 3.10. Latency changes from 1 to 5 months for Cambridge (green) and Keneba (blue).

Hypothesis 7. It was expected that P3 amplitudes to infrequent and trial unique sounds would be reduced in the Keneba cohort at both time points. To test this hypothesis, data from the infrequent and trial unique conditions were entered into a 2 by 2 repeated measures ANOVA, using Site (Cambridge / Keneba) as a between subject factor and Condition (Infrequent / Trial Unique) as a within subject factor (Table 3.14). Results show a significant main effect for Site, the directionality of which was verified through the descriptive statistics (Table 3.15).

Table 3.14. *Results of 2 by 2 ANOVA examining factors Condition and Site.*

	F	*df*	*p*
Site	4.287	1,206	.039*
Condition	31.825	1,412	<.001*
Site * Condition	1.664	1,412	.191

Table 3.15. *Descriptive statistics of P3 mean amplitudes separated between Sites and for each infrequent condition level.*

Site	$Condition_{Level}$	P3 mean amplitude
Cambridge	$Condition_{Infreq}$	$\bar{x}$ = 7.087
Cambridge	$Condition_{TrialUnique}$	$\bar{x}$ = 6.813
Keneba	$Condition_{Infreq}$	$\bar{x}$ = 7.193
Keneba	$Condition_{TrialUnique}$	$\bar{x}$ = 4.891

Hypothesis 8. It was expected for the variance within the sample to be larger in Keneba compared to Cambridge. This was expected to manifest in more variance in peak latencies and mean amplitudes. This was addressed using two univariate models, each fitting only the factor Site to in turn predict mean amplitude and peak latency. It was however found that the assumption of homogeneity of variance was not violated in either model, indicating similar variance between Cambridge and Keneba. Results of the Levene's test can be found in Table 3.16. While this effect could be by larger sample sizes in the Keneba sample, especially at the 1 month age point, the hypothesis of higher between individual variations does not seem to hold based on this data despite the fact that sample sizes are similar with exception of the Keneba cohort at the 1 month age point.

Table 3.16. *Test of homogeneity of variances for the P3 mean amplitude and component latency.*

	Levene's statistic	*df*	*p*
P3 mean amplitude	.436	1,643	.509
P3 latency	1.127	1,643	.289

3.3.6 Differences between states. *Hypothesis 9.* We analysed the ERP of a subset of infants from the Keneba cohort who could only be assessed asleep at their 5 month visit. Infants were generally found to be more tired and prone to fall asleep at this testing site which enabled us to obtain a sufficiently large sample of n= 10 infants. Infants assessed in Cambridge were all awake for the entire duration of the assessment, preventing us from making the same comparison at both sites. The subset of sleeping infants was compared to an equal sized subset of awake infants matched on number of trials included (±5 trials). The resulting ERPs can be seen in Figure 3.11. The effect of sleep on the ERP components was examined by entering State (asleep/awake) into two separate one-way ANOVAs to in turn predict N1 and P3 mean amplitude. ERP amplitudes did not differ between sleeping and awake infants for either the N1 ($F_{1,64}$ = .190, p= .664) or the P3 ($F_{1,64}$ = .033, p = .857) components. While this is not to say that EEG measurements are unaffected by participant state, it suggests that in light of this particular ERP study, measurements obtained from sleeping and awake infants did not differ significantly which increases confidence in the differences observed between age points.

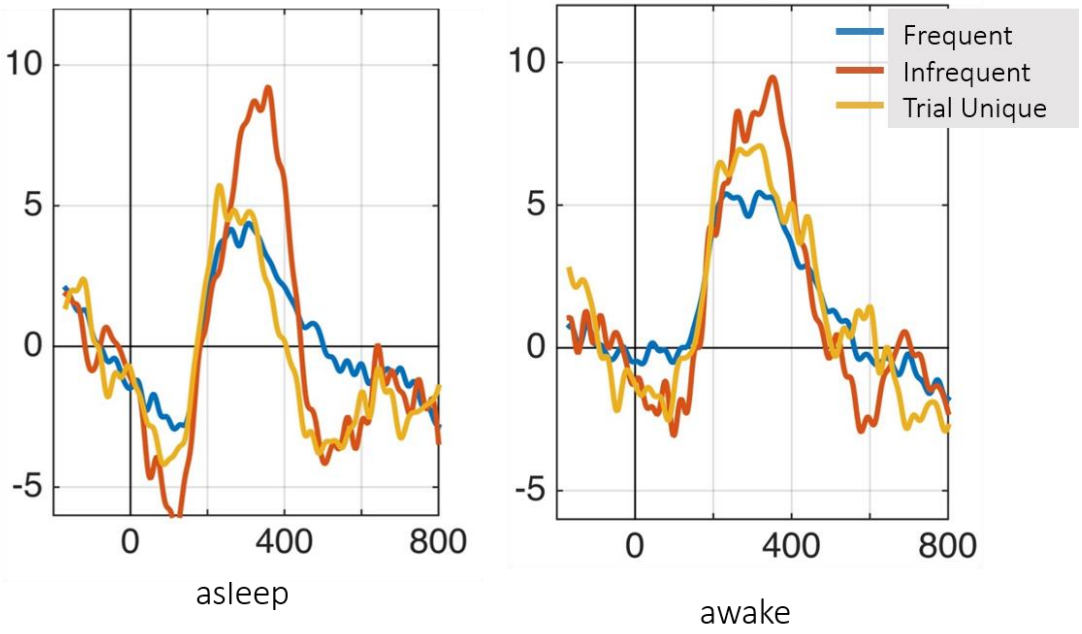


Figure 3.11. ERP from Gambian infants who were assessed asleep (left, *n* = 10) and awake (right, *n* = 10).

3.4 Discussion

The present study examined the development of deviance detection between 1 and 5 months in infant cohorts in Cambridge, UK and Keneba, The Gambia by implementing an MMR_{ERP} paradigm. Some of the developmental changes previously reported to occur between these two age points were replicated, whereas others were only evident in the Cambridge cohort. The presented data are indicative of a different developmental progression between sites and specifically suggest a slowed or delayed developmental change in the Keneba cohort.

3.4.1 Experimental manipulation. With regard to the premise of the study design, we successfully replicated the effect of heightened ERP responses to infrequently appearing stimuli, indicating the presence of deviance detection in both studied infant cohorts. We could also demonstrate that

this was partly driven by habituation to the frequent and recurring infrequent, but not trial unique stimuli.

3.4.2 Developmental change. The developmental change anticipated to occur between 1 and 5 months was partly observed. As expected, ERP latencies were shown to decrease between age points, at both study sites. It was also shown that at 1 month, infants reacted most strongly to stimulus intensity, rather than stimulus novelty, as evidenced by the largest ERP amplitudes occurring in response to the white noise sounds. The expected change to preferential processing of the trial unique sounds at 5 months however could only be observed in the cohort tested in Cambridge.

3.4.3 Differences between sites. Some other differences were evident between the studied cohorts. With regard to latencies, the anticipated decrease between age points was less pronounced in the infants tested in Keneba, and at 5 months they showed significantly longer latencies compared to the infants tested in Cambridge. We further observed reduced P3 amplitudes in the Keneba cohort and the absence of the developmental change towards novelty processing observed in Cambridge. Taken together, the diminished latency decrease, and the absence of change towards novelty processing point towards a diminished developmental change in the Keneba cohort. While data from a follow up time point will be needed to make definite statements about the further course of the developmental trajectories, the data collected from these two time points also strongly indicates a developmental delay, as infants tested in Keneba show responses resembling those of younger infants. While a slowed developmental progression seems likely from the presented data, this does not serve as an explanation for all findings. While ERP responses elicited at 1 month are more similar between sites than at the 5 month age point in terms of amplitudes to the different stimulus conditions and in terms of

component latencies, there are already some differences between sites, most notably in the reduced amplitudes to both infrequently recurring sounds. While without further analyses it cannot conclusively be determined what might have caused the observed differences, some risk factors implicated in previous work can be expected to have an impact and should be further investigated.

3.4.4 Limitations. Implications of the current study must be regarded in light of some specific limitations discussed in the following.

Novelty of testing environment. As for all neuroimaging studies conducted in rural settings, it needs to be considered how far the novelty of the testing environment affected the outcome. As this study was conducted during deep sleep at 1 month, the impact or novelty of the testing environment can be deemed negligible for this age point. As for the 5 month age point, this study has the advantage of neither requiring the infants to watch a screen nor relying on them having to interact with an unknown experimenter. Rather, infants were allowed to look at their parents, or play with a toy they found interesting. It further needs to be considered in how far prior exposure to stimuli similar to the ones used here might have contributed to the outcome. Many previous studies looking at novelty detection have used either faces or syllables as stimuli, which can be expected to be differentially processed by infants with different degrees of previous exposure to face to face interaction or infant directed speech. As this study used relatively neutral stimuli, cultural differences in those dimensions is unlikely to drive the observed effects. While it has been suggested that it is more likely for infants in Cambridge cohort to have more prior exposure to digitised sounds at even a young age, this does not suffice to explain the results of this study, given that that the additional novelty to digitised sounds could be expected to result in increased rather than

reduced amplitudes in the Keneba cohort. Similarly it can be argued that infants in rural Gambia are generally exposed to nosier environments, due to multiple family members living in close proximity, while infants in the UK are frequently shielded from noise. This would suggest infants in the Keneba cohort to potentially habituate more quickly to auditory input as an adaptive response, however this is not in line with data presented in the habituation analysis (Hypothesis 2), which shows diminished amplitudes even in the first couple of trials across both sites. It therefore is likely that cognitive factors, rather than stimulus properties, provide a better explanation for the observed data.

Study design. One limitation of this study is that stimuli were not randomly assigned to the frequent, infrequent and trial unique conditions for each baby which would have enabled conclusions more independent of stimulus properties and the potential for replication of previous studies who included such a counterbalanced protocol (Kushnerenko et al., 2007). However, adding a within subject manipulation would have either reduced available trials by fragmenting the session or added a substantial amount of testing time. The latter is not trivial given the short periods of time that infants are usually calm and alert and the extensive testing protocol that the BRIGHT project implements. Counterbalancing between participants would have fragmented the smaller sample enrolled in Cambridge too much and would have likely resulted in too small samples for each condition to draw meaningful conclusions.

State change between age points. A further limitation of this study is that data was acquired in different states (asleep at 1 month/awake at 5 months) which complicates conclusions regarding the developmental change. As discussed above in context of Hypothesis 9 of this chapter, it was tested whether ERP's differed between infants tested asleep or awake at the 5

month age point. While this was found not to be the case, it would be a consideration for future studies to be conducted with infants in the same state at all time points. While assessing infants asleep at both time points has advantages from a consistency and a data quality point of view, it needs to be considered that assessing infants during sleep becomes harder as they age and sleep less during the day. Longitudinal studies should therefore employ study designs containing meaningful condition contrasts to make them less affected by state changes. The reason for the decision to test the 5 month olds in an awake state, despite the fact that virtually all of them still had to take a nap during the testing day, was to not compromise this nap by the disruption of continuous auditory stimulation and therefore potentially preventing deep sleep. This could have led infants to be less well- rested, which in the context of the BRIGHT testing load would have risked data quality in other assessments.

Validity of Site and Age point comparison. In addition to the issues laid out above, some consideration needs to be given to the possibility how analyses might be biased due to a non-uniform developmental change at the two study sites. One issue lies in the definition of appropriate time windows over which to average data to obtain mean amplitude measurements. As laid out above, the latency at which components such as the P3 occur changes as a function of infant age, and mean amplitude time windows should therefore be adjusted for different age groups. However, due the non-uniform latency change in the two cohorts, with a stronger decrease in the UK compared to the Gambian cohort, using the same time window in both cohorts could be argued to bias the results. As described above, the time windows were chosen in guidance of both the data and previous literature, to maximise the validity of analyses. Particularly, all individual data for each age points were plotted irrespective of cohort membership and P3 peaks were

identified for these individual data. Time windows were then chosen to be inclusive of a majority of these peaks, which helped to eliminate some of the bias that would have been introduced by using an approach purely guided by prior literature. To be inclusive of as many different response patterns as possible, it was further decided to choose a very wide window over which to average. This was deemed the best approach, as fitting one time window per site might have led to a bias in results for the Gambian cohort, in favour of those infants showing elongated latencies, which could have artificially amplified hypothesised effects.

A similar issue arises for the early sensory components. As marked morphological changes occur between the two age points with differences in the components' morphology between the two sites, an analysis by mean amplitude is not ideal, and a comparison of the same outcome across age points not very informative. Future analyses of these components might focus on condition and cohort differences within each age point, and also may use peak amplitudes as this will avoid a cancelling out of a response over an average time window due to the window including two components of opposite polarity at one but not the other age point. For this latter approach, in order to yield a more stable estimate, a peak within a time window of interest could be identified, around which several data points will be averaged.

3.4.5 Future directions. The presented results form a basis for numerous follow up analyses. While ultimately they will be related to data on infants' exposure to environmental risk factors, they offer interesting new insights even before this final stage of analysis. As one of the fNIRS paradigms included in the BRIGHT project examined habituation and novelty detection, it is planned to consider results from both modalities together, to examine converging findings. Results from the fNIRS habituation and novelty

detection paradigm (Lloyd-Fox et al., 2019) show similarities with the presented habituation analysis in which infants at both sites show rapid habituation to auditory stimuli, whereas processing style at 5 months shows marked differences between sites, indicating either differential developmental trajectories, or for differences between sites to be modulated by infants' state. These similarities will be revisited once data collected of the final sample is completed.

In absence of data on risk factors for this thesis it was attempted to understand in greater detail the properties of the EEG measurements, to better elucidate possible mechanisms for the presented effects. This work will be discussed in the following chapter.

Chapter 4. Implementation of Spectral EEG Analysis Approaches

Further analysis of Study 1: EEG study at 1 and 5 months

4.1 Introduction

While there is a longstanding tradition in research of exploring systematic changes in EEG recordings through the extraction of ERP's, a range of other modes of analysis are available which can provide additional information on relevant electrophysiological changes. Even though some of the very first EEG studies examined associations between changes in the continuous EEG and cognitive processes (Berger, 1931), these approaches have only more recently gained more wide-spread traction. Especially in context of increased computational power and the development of more standardised analysis streams, these approaches can provide valuable information on specific features within EEG data which add to our understanding gained from ERP analyses (Isler et al., 2012).

This chapter details the research project undertaken during a UCL Bogue Research Fellowship at the Laboratories of Cognitive Neuroscience at Boston Children's Hospital/Harvard Medical School under supervision of Charles A. Nelson and his group. The primary goal of the visit was to learn about and apply advanced EEG analysis methodology. In the following I will first lay out the rationale for exploring novel EEG analysis tools and review methods currently available before presenting the results of their application to the data collected in this project.

4.1.1 Rationale for further analyses.

EEG data can be understood as consisting of a sum of oscillations occurring at different frequencies, as illustrated Figure 4.1 using a simulated EEG-like signal. Just as this simulation can be produced through summation of oscillations at different frequencies, the human EEG can be decomposed into the different frequency bands and analysed for the contribution of each frequency over a

given period of time, which forms the basis of many of the analysis methods described in the following.

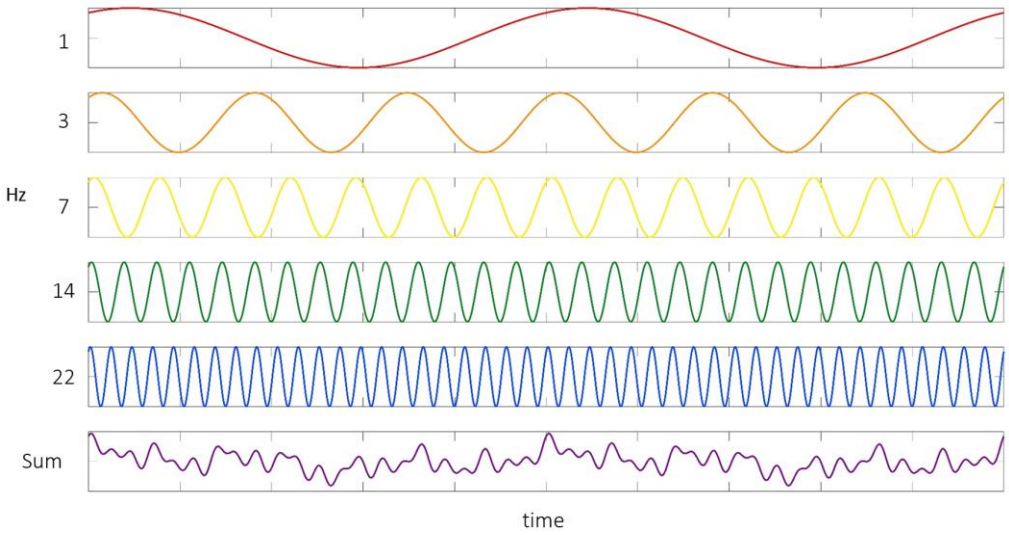


Figure 4.1. Simulation of several sinewaves of different frequencies and their sum.

Oscillations can be defined by three properties, namely their frequency, power and phase (Cohen, 2014; see Figure 4.2). The frequency describes the speed of oscillations contained in the signal in hertz (Hz). Power (defined by the squared amplitude of an oscillation) describes how much energy there is in any given frequency band, and thus how strong the signal is. Lastly, the signal's phase denotes the position of a given oscillation against the sine wave of the corresponding frequency, measured in radians of degrees referring to the alignment with the sine wave. Each of these three features can be useful in interpreting EEG data and holds potential about underlying neural mechanisms.

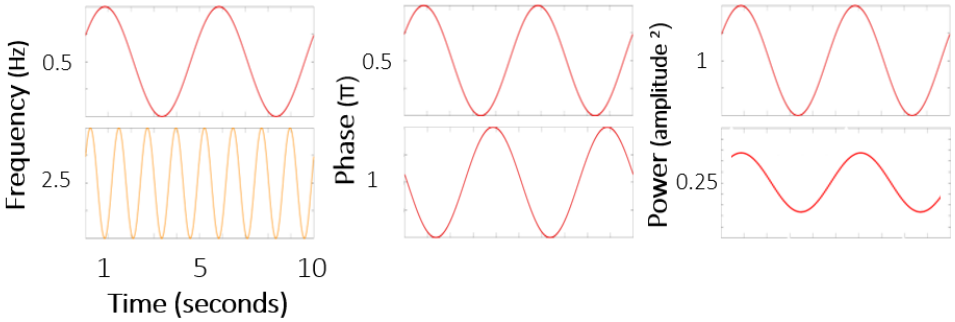


Figure 4.2. Sine waves differing in Frequency (a), Phase (b) and Power (c) with the respective other two properties kept constant.

In an ERP analysis, some of these properties are exploited whereas others are not contained in the ERP waveform. To obtain an ERP, micro voltage changes in the EEG are averaged across many trials, enabling a differentiation of signal from noise. During this process, not only noise but also any changes in the EEG that are not tightly phase locked to the stimulus are averaged out. ERP's are an excellent method to examine time-locked changes within the EEG, but by definition cannot depict any relevant non-phase locked changes. Above this, it is not possible to accurately infer from an ERP analysis the contribution of specific frequency bands at any given time to the resulting signal. These are the two main reasons why other analyses, particularly those assessing changes across the frequency spectrum which the EEG is composed of, might be useful to be used in combination with an ERP analysis to further refine our findings, and will be further laid out in the following.

Phase locked and non-phase locked power. A distinction is commonly made between phase locked (or evoked) and non- phase locked (or induced) power (Cohen. 2014). Phase locked power reflects the summed postsynaptic potentials elicited by an event, that are tightly time and phase locked across trials (Cohen, 2014). As such, phase locked power makes up the part of the EEG reflected in an ERP. Non-phase locked activity on the

other hand can be understood as a more continuous, oscillatory activity that is modulated by an event (Bastiaansen & Hagoort, 2003). It therefore is related to a stimulus, as it occurs in response to it, but varies in phase across trials and therefore averages out in an ERP analysis, despite being potentially meaningful to the ongoing cognitive process (Roach & Mathalon, 2008). Non- phase locked power has been ascribed a regulating function of interactions within neural networks and has been implicated in establishing and activating networks of cells in that are not spatially, but rather functionally aligned (Bastiaansen & Hagoort, 2003). For this reason, non-phase locked activity has been studied in the context of more integrative processes like feature binding and memory formation (Bastiaansen & Hagoort, 2003). In addition to these neuroscientific considerations, spectral power based analyses have also been proposed as advantageous from a clinical viewpoint.

Clinical utility. In addition to enabling the analysis of non-phase locked power changes in the EEG, there are some other advantages spectral analyses offer, particularly in context of developing or clinical populations. Power based measures offer an approximately 10 times better signal to noise ratio than ERP's (Pfurtscheller & Da Silva, 1999) meaning fewer trials are required which poses an advantage when seeking to apply a tool in routine clinical practice. Further, spectral analysis methods have sometimes been found to be predictive of behaviour when ERP's are not, such as in context of schizophrenia diagnosis, which further corroborates their potential for clinical applications (Dvey-Aharon et al., 2015).

For this reason, several groups have attempted to extract clinical markers based on spectral EEG changes. As laid out in Chapter 3.1, mismatch responses (MMR) have received a lot of attention as they reflect numerous processes relevant to cognitive development. Initially, the discovery that the

MMR based on the ERP (MMR_{ERP}) is present at birth (Alho et al., 1990) rendered it promising as an early marker of atypical development in at- risk populations. However, this work ultimately did not lead to routine clinical application for several reasons (Isler et al., 2012). First, the morphology of the MMR_{ERP} is more variable in developing populations than in adults, making it harder to detect and characterise (Fellman & Huotilainen, 2006). Secondly, different methods regarding data acquisition and processing (Bishop et al., 2007), subtle differences in the contrasts of stimuli (Kushnerenko et al., 2007) and the differences in chronological age and gestational age of participants (Leppanen et al., 2004) all have added to some inconsistencies in findings (for a review see He, Hotson & Trainor, 2007). In order for the MMR to be widely useable as a clinical tool, it must be possible to consistently detect the response in typical as well as atypical and at-risk populations. It has been suggested that spectral analyses of the MMR through offering insights into the oscillatory response underlying the MMR_{ERP} might hold potential to further elucidate reasons for the variable findings described above, and therefore could help generalizable predictions (Isler et al., 2012, Gilley et al., 2017). Spectral analyses have also been proposed as a means of gaining additional insights regarding mechanisms underlying an observed neural response as will be reviewed in the following.

Enhanced mechanistic insights. Attempts to better understand the significance of oscillatory response patterns have yielded differential associations between specific frequency bands and cognitive processes (Buzsaki & Draguhn, 2004). So far, a majority of research has been focused on the alpha, theta and gamma bands (Bastiaansen & Hagoort, 2003), which has added greatly to our understanding of basic cognitive processes.

The primary approach taken has been to define associations between activity in one or several frequency bands and cognitive processing. In the very first EEG studies conducted by Hans Berger in the 1930's he detected a prevalent rhythm in the continuous recording oscillating at around 7-10 cycles per second. He described oscillations within this frequency band, termed 'alpha rhythm', to be modulated by whether participants were assessed with opened or closed eyes (Berger, 1931). Since these studies, changes in the alpha rhythm have repeatedly been associated with changes in participants arousal state (Barry et al., 2007). Further, some groups have investigated the interplay and reciprocal modulation between oscillations at different frequencies. Hereby, it has been shown that high frequency oscillations, such as activity in the gamma band, are modulated by lower frequency activity such as in the alpha or theta bands (Voytek et al., 2010). Hereby, it has been shown that the low frequency oscillations support long range networks between cortical regions, and orchestrate localised, high frequency activity (Von Stein & Sarnthein, 2000).

The above illustrates what spectral analyses can add to our understanding of cognition and cognitive development. First, they allow for oscillatory activity to be understood in terms of their contribution to ubiquitous neural processes on synaptic, cellular and system levels which underlie a huge range of cognitive processes and therefore offer neural mechanistic insight into the cognitive process under investigation (Varela et al., 2001). To a degree, ERP's can provide insight into different aspects of a cognitive process, for example by utilising early components to elucidate sensory mechanisms associated with the tasks and later occurring components to understand higher cognitive components (Luck, 2014). While it is possible to derive a general functional meaning for some components and compare across studies, ERP's are less well suited to understand more basic mechanisms on a neural rather than a cognitive level.

Secondly, network based interpretations are enhanced by examining prevalence and strength of certain oscillations across different electrodes, which is not easily achieved in an ERP study. While it is possible to compare ERP morphologies across different electrodes, it is difficult to subsequently interpret differences between electrodes in terms of underlying cortical networks (Cohen, 2014). The mechanisms of synchronisation and desynchronization processes within oscillations across different brain areas however are better understood, and therefore provide a highly time resolved insight into dynamic networks established which are temporarily established in response to an event (Bastiaansen & Hagoort, 2003, Cohen, 2014).

A range of methods based on analyses of spectral changes in the EEG exist, which lend themselves to the exploration of diverse research questions. The most commonly used of these methods will be reviewed the following.

4.1.2 Available methods. Spectral analyses can inform the understanding of EEG data in several ways. Some methods lend themselves to the application to both event related and resting state data, whereas others can only be applied to one form of design. Another distinction can be made between those methods that allow for a degree of temporal resolution, thus allowing to observe changes in oscillatory power and those that reflect a given combination of frequencies averaged over a prolonged period of time. Table 4.1 summarises the methods most commonly reported in the literature.

Table 4.1. *Overview of most commonly implemented methods of spectral EEG analysis with regard to their application to event related and non-event related designs and the degree to which they offer an understanding of temporal dynamics.*

	Time resolved	not time resolved
Event related	Time frequency (TF) Inter trial phase coherence (ITPC)	Power spectral density (PSD) Inter trial phase coherence (ITPC) Cross frequency coupling (CFC) Phase amplitude coupling (PAC)
Not event related		Power spectral density (PSD)

Power spectral density. One predominant method to examine the contribution of different frequencies to the EEG signal lies in its decomposition across a range of frequency bands. The resulting power spectrum can then be analysed and compared across different states, developmental ages, or task conditions. The power spectral density (PSD) is obtained through a Fourier transform, which decomposes the time domain signal into the frequency domain. This is achieved via convolution of the signal with sine waves of different frequencies. The correspondence between sine wave and signal is determined yielding a measure of prevalence and magnitude of the various frequencies in the observed signal.

PSD analyses lend themselves to the study of baseline resting state designs, in which the power spectrum of a resting state EEG is extracted, features of

which can be related to a range of other measures such as performance on a behavioural task, or examined in isolation (i.e. hemispheric synchrony). Further, some studies have compared the power spectra associated with different states (i.e. infant attention) and examined changes within frequency bands of interest (Xie, Mallin & Richards, 2017). While task or state related changes can be reflected in different PSD profiles, this method does not offer insight into time resolved oscillatory changes occurring in response a stimulus or event.

Time frequency analysis. While it is impossible to make inferences on changes in the frequency domain from ERP's and on the time domain from the PSD, time-frequency analyses allow us to gain some understanding within both these domains concurrently. This is achieved through convolution of shorter segments of a sine wave (wavelets) with the signal at multiple adjacent points across the time series. This method provides an estimate of the correspondence between the signal and the convolution kernel over time.

Cross frequency coupling. Several methods exist aimed to assess the co-occurrence or modulation between oscillations of different frequencies (Canolty and Knight, 2010). All methods assess the degree of coherence between different frequency bands, for example by measuring the degree of whichoscillations across different frequency bands are in phase with one another or the degree to which power within one band can affect measures of power in another band. Most frequently, phase coupling measures are used to derive information on cortical networks, by examining similarities in the oscillatory response at non-adjacent electrode sites.

Inter trial phase coherence. Inter trial phase coherence (ITPC) is a frequency domain technique to measure the degree of phase locking of an oscillatory

response across trials. It can be obtained by assessing the phase of a given oscillatory signal across a number of identical trials. The degree to which a responses are consistent in its relation to one another across trials can then be calculated where ITPC = 0 indicates no phase coherence and ITPC = 1 perfect coherence over trials. ITPC can be extracted in a time resolved manner thus showing the degree of coherence over time, or extracted as a static measure per frequency and time bin.

4.1.3 Methods applied in this project. A subset of methods was selected deemed most informative in context of the current study. To further understand spectral as well as temporal dynamics of deviance detection in our populations, the time frequency spectrum was compared across ages and study sites. Additionally, ITPC measures were used to explore the degree of intra-individual variance within the electrophysiological response.

The PSD was not assessed as no resting state power was collected. Though PSD can also be assessed in an event related fashion, this method is more robust with longer segments of data. In the present project, which employed a fast-paced stimulus presentation, data segments were not long enough. Lastly, cross-frequency coupling was of interest for this project, but due to time constraints during the research fellowship, it could not be formally assessed.

In the following relevant evidence from both TF as well as ITPC investigations will be discussed, with particular focus on memory and change detection, in order to formulate hypotheses for the current project.

4.1.4 Oscillation based measures of memory and global neural functioning.

Oscillatory bases of memory functioning. A wealth of research has examined associations between EEG spectral changes and memory processes in

adults. Hereby, enhanced activity within the theta band over temporal-parietal areas has repeatedly been related to successful memory encoding, with particular importance being attributed to theta and gamma coupling effects in context of feature binding and memory formation (Fuentemilla et al., 2008, Hsiao et al., 2010, Ko et al., 2012). In a comparison of responding to a novel vs familiar stimuli in adults, it could be shown that novel trials were accompanied by an increase in theta power (Sauseng et al., 2007) which was interpreted as being brought about by heightened demands on both sustained attention and WM (Gomarus et al., 2006, Sauseng et al., 2007). This is further corroborated by work using intracranial EEG in adult epilepsy patients, which showed the utility of predicting recall during a memory task, from increased theta and gamma activity during the encoding phase (Sederberg et al., 2003). Examining word memory in children and adolescents, theta band power could be shown to increase over right frontal and temporal leads, which further was found to be correlated with successful recall (Sederberg et al., 2003). Some research has linked spectral properties of EEG recordings to cognitive functioning not only in children but also in infancy, as will be reviewed in the following.

Evidence from developing populations. Some research has examined associations between spectral properties of the EEG and memory development. In some of the earliest studies on this subject, Bell and Fox implemented a resting state PSD analysis and found associations between frontal alpha power and subsequent performance on an A-not-B task in 7-12 month old infants (Bell & Fox, 1992) and an object permanence task in 8 month old infants (Bell & Fox, 1997). In an event related design, Bell (2001) examined EEG power changes of 8 month old infants in response to a passive looking version of an A not B task. An increase of alpha power was found across frontal as well as posterior regions of the scalp only in those infants that performed high on the A not B task in terms of correct eye

movements and remembering over longer delays. Further, this heightened alpha activity during the looking paradigm was related to infants' behavioural performance on an A not B task (Bell 2002, Bell and Wolfe 2007) and could be used to predict errors on the task (Bell, 2002).

Another result of these early studies was to help begin to establish a convention for defining frequency bands in infants. As indicated above, different frequency bands are conventionally used that are primarily grouped together due to their functionality. These frequency bands are defined slightly differently in adults and infants (Table 4.2) for two main reasons. First, the power spectrum of infants contains a higher proportion of lower frequencies. Secondly, it has been demonstrated that there are functional overlaps between activity in adult frequency bands and oscillatory changes in slightly lower frequencies in infants (Lindley, 1938, Marshall, Bar-Haim & Fox, 2002, Stroganova et al., 1999). The conventionally used frequency bands utilised in adult and infant (Levin et al., 2018) research are summarised in Table 4.2.

Table 4.2. *Frequency band definitions for adults and infants.*

	adults	infants
gamma	30-100Hz	30-50 Hz
beta	13-30 Hz	13-30 Hz
alpha	8-13 Hz	High 9-13 Hz
		Low 6-9 Hz
theta	4-8 Hz	4-6 Hz
delta	<4 Hz	2-4 Hz

Some groups have examined task related changes in the context of infant deviance detection. In one of the first studies to examine spectral properties

of the mismatch response (MMR_{TF}), Isler et al. (2012) presented newborns with frequent and infrequent auditory stimuli. They observed greater power increases in both the theta and gamma bands in response to the infrequently than to the frequently occurring stimuli, suggesting that to a degree the functional role of the two frequencies can already be measured at birth. To further understand how different kinds of sound contrasts were reflected in the spectral properties of the EEG response Gilley et al. (2017) implemented a range of progressively more subtle stimulus contrasts. Neonates were presented either with a non-speech contrast (white noise/ tone), a vowel contrast (a/o) or a hard to distinguish consonant contrast (da/ba). They too observed a co-occurrence of theta and gamma activity, with two distinct theta burst and an early onset gamma feature. Further, as can be seen in Figure 4.3 responses subtly differed between the condition contrasts.

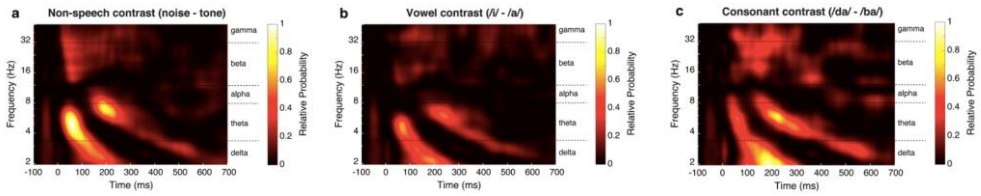


Figure 4.3. Reproduced from Gilley et al., (2017). Newborn responses to three different auditory contrasts. All contrasts show two distinct theta and a gamma feature, which differ between conditions.

The time frequency analysis implemented in the current project was aimed at further examining previous findings stipulating that the MMR_{TF} could be a viable candidate in defining and early marker of cognitive development in even the youngest infants, and even in those instances where the ERP might not be. As one long term goal of the work presented in this thesis is to establish early markers of atypical development, it was decided that an analysis in line with previous work (Isler et al., 2012, Gilley et al., 2017) could yield valuable additional information and should therefore be

pursued. Further, both groups found that MMR_{TF} responses in some instances picked up more subtle differences between the conditions than ERP alone. As some of the findings in the ERP's of this study indicate no mean differences between some of the conditions (i.e. no differences between frequent and trial unique sounds in Keneba cohort), it is of interest to explore whether differences are detected within the TF domain.

Measures of global neural integrity. Phase coherence measures have been proposed more recently as a potential index the efficiency of neural transmission (Russell et al., 2006). While it can easily be appreciated that the power within the frequency bands contained in the signal affects its morphology, Figure 4.4 illustrates the effect that phase synchrony has on the resulting signal. As can be seen, the degree to which responses across a number of trials are coherent in phase will affect the magnitude of the average across trials. From the averaged response alone it cannot be seen whether reduced power within an oscillation of phase asynchrony are at the core of a response. An investigation of the phase coherence, for example by measuring the ITPC therefore can add additional information regarding observations made both in the ERP as well as power analyses.

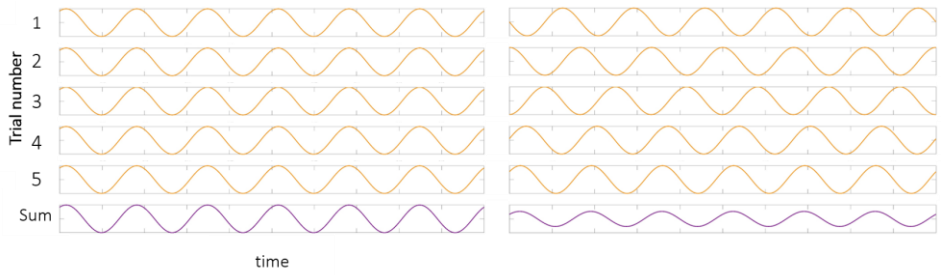


Figure 4.4. Illustration of the effect of phase coherence on the summed signal. As can be seen, oscillations coherent in phase in sum result in a signal of identical power in the given frequency (left). Oscillations of the same amplitude and frequency however sum to a lower power signal if phase shifts occur (right).

Having been primarily described in research on individuals with autism spectrum disorder (ASD), ITPC has been hypothesised that atypical responses stem from an impairment in glial astrocytes, which are crucial in orchestrating neuronal oscillatory responses (Russel et al., 2006). These impairments have been proposed as a potential cause for many findings associated with ASD including both variability between single trials of EEG tasks (Geurts et al., 2008, Milne, 2011), fMRI tasks (Haigh et al., 2014, Dinstein et al., 2012) as well as higher order behavioural response variance (i.e. in reaction time, Milne, 2011). Being well documented in ASD (Milne, 2011, Dinstein et al., 2012, Haigh et al., 2014) ITPC measures have not yet found a wider application within neuroscientific research. While it is not fully understood in how far the mechanisms underlying phase incoherence generalise across other medical and psychiatric conditions, higher response variance seems to be a feature in many cases of atypical neural development (Castellanos et al., 2005). ITPC therefore offers a way to further investigate intra-individual variance as a potential source of differential individual outcomes. In context of the current study it is of interest, as differences in ERP amplitudes were observed between study cohorts at even 1 month of age. While this was initially hypothesised to be in part due to inter-individual variance in peak latency this hypothesis did not hold when formally assessed. Intra-individual variance would lead to the same outcomes on group level and might therefore more accurately account for the reported results.

4.1.5 Hypotheses.

Based on the reviewed literature the following hypotheses will be assessed.

1. It is expected that a theta-gamma activation will be observed in response to the infrequent and trial unique sounds.

2. It is expected that differences in the MMR_{TF} will be visible between conditions even where the MMR_{ERP} was found to not distinguish at the group level. This is of particular interest in the comparison of the frequent and trial unique sounds in the Keneba cohort.

3. ITPC values are expected to differ systematically between Sites, with lower values observed in the Keneba cohort.

4.2 Method

As a reanalysis of previously acquired data was conducted, participants, apparatus and stimulus presentation parameters were the same as in Study 1, details on which can be found in Chapter 3, section 3.2. As the pre-processing parameters and the statistical analyses were unique to this reanalysis, they will be laid out in the following.

4.2.1 Pre-processing. Data were pre-processed within Matlab 2015b (The Mathworks Inc, 2015) using a customised, automated pipeline generated during the fellowship. Data was bandpass filtered between 1-45 Hz, using a Blackman filter (Widmann, 2006) with a filter order of 5500. The filter cut-offs differed between the ERP and spectral analysis for two reasons. First, while high high-pass filter will likely distort the ERP waveform, this is less of a concern in spectral analyses. Secondly, the frequency band under investigation was based on previous relevant studies examining frequencies up to 50Hz. However, in order to avoid contamination by line noise around the 50Hz frequency it was decided to use a 45Hz low pass setting rather than a notch filter at 50Hz which might have a distorting effect on the data. Particularly in event-related designs there is a lower limit of which frequencies can reliably be decomposed, as in order to obtain a reliable signal, a few cycles of an oscillation should have occurred within the epoch

that they are decoded in (Cohen, 2014). This means, that slower frequencies are most reliable in resting state or long designs or whenever long epochs are presented.

As in the ERP analysis, data was offset corrected by 32ms, before data were epoched. Epochs were created from 400ms before stimulus onset to 1200ms post stimulus onset for decomposition. These longer intervals compared to the ERP analysis were due to the decomposition via a moving window wavelet convolution, which is prone to distortions around the edges of an epoch. By running the decomposition over an extended epoch and subsequently trimming it, the edge artefacts have less of an impact on interpretation of the results. Epochs were rejected using the same threshold as in the ERP analysis of absolute values between minimum and maximum >200 μV, and a flatline correction was run removing trials in which minimum to maximum measures were <.1 μV. It was then corrected for potential early termination of the study by rejecting all epochs <1200ms post stimulus onset, thus rejecting the final trial of some study sessions. To equalise trial numbers, a random subset of epochs from each condition was chosen of size n, where n = number of trials in condition with fewest trials. All datasets with >10 trials were included for further analysis. This number was smaller than in the ERP analysis because spectral analysis usually have a better signal to noise ration and thus require fewer trials than ERP analyses (Pfurtscheller & Da Silva, 1999), and because the artefact rejection yielded more conservative results in light of longer epochs and thus fewer trials remained compared to the ERP analysis. At the 1 month age point, datasets had between 20-72 trials (maximum number 75), at the 5 month time point, between 10 and 69 trials were included (maximum 100).

As for the ERP analysis the TF and ITPC analyses were focused on the Fz electrode. Thus, all data remaining after artefact rejection were then

decomposed for the spectral analysis via wavelet convolution using the newtimef EEGlab plugin. Complex Morlet wavelets between 1-45Hz in 1 Hz intervals were used. While there are multiple ways to choose a kernel for this time resolved convolution (short Fourier transform, Hilbert transform), Morlet wavelets have been found to have certain properties making them well- suited for electrophysiological data (Samra, Bopardikar, Rao & Schwartz 1999, Csibra & Johnson 2007). Morlet wavelets are sine waves which are temporally weighted using a Gaussian distribution. This results in a convolution kernel which more heavily weighs the signal at the centre of the kernel than at its periphery, which reduces artefact. Morlet wavelets of different frequencies can then be convolved with the EEG signal, resulting in an estimate of dynamic changes in different frequency bands over time. Further, Morlet wavelets can contain varying numbers of cycles, meaning that temporal resolution is increased within higher frequency bands. For the 1600ms long epochs this led to a minimum of 1.6 cycles at 1Hz, with a linear increase towards the higher frequencies and 12.5 cycles at the high end of the frequency spectrum. After decomposition, epochs were trimmed to -200 – 800ms, and thus the same length as in the ERP analysis. Trials were then averaged per condition thus yielding an estimate of the total (that is phase locked and non-phase locked) power. ITPC values across the relevant time windows and frequencies were obtained via the newtimef function, for further statistical analysis.

4.2.2 Statistical analysis. While ERP's can be analysed in a number of ways, the introduction of an additional dimension (frequency) in spectral analysis means that spurious findings need to be controlled for even more carefully. The statistical analyses of this project therefore were targeted to answer three main questions:

1. Examine the spectral properties of the activation examined in the ERP analysis, particularly in those conditions that seemed to not show a difference on group level in the ERP analysis.

2. Examine whether previously reported findings on a theta-gamma coupling effect can be replicated, and how they might differ across conditions.

3. Examine whether differenced in ITPC across Age points and Sites, to see whether these differences might account for some of the flattened ERP peaks observed in the Keneba cohort.

Time-frequency analysis. For the time-frequency analysis, the whole power spectrum was extracted to examine changes in any frequency band from baseline. Subsequently, t-test were used to compare each time point in each frequency band against average baseline activation, corrected via FDR routines embedded in newtimef.

For further statistical modelling, data were then averaged across the relevant time windows and frequency bands. The decision on what frequency bands and time windows were going to be focused on was guided 1) by prior literature (Isler et al., 2012, Gilley et al., 2017) implicating co-occurring theta and gamma effects, 2) by findings of peak latencies in the preceding ERP analysis, showing occurrence of the N1 and P3 component over different time windows 3) by visual inspection of activity observed in the current study. Resulting power measures were then modelled by Condition, Age and Site.

ITPC analysis. ITPC values were extracted within the same time windows and frequency bands as in the TF analysis. Even though no amplitude differences were observed for the N1 in the ERP analysis, it was decided to

examine both components, as this could potentially enable an examination of whether ITPC differences were visible in ERP amplitude or not. Most previous studies have examined ITPC within the alpha band (Milne, 2011, Levin et al., 2018). However, in the context of change detection, alpha has been less strongly implicated than activity in theta and gamma bands. Further, in order for the ITPC analysis to be most informative of findings from both the ERP and the TF analyses, it was decided to examine ITPC across the same frequency bands and time windows chosen for statistical analysis of the TF analysis. ITPC was then modelled by Condition, Age and Site.

4.3 Results

4.3.1 Time – frequency analysis. In this analysis, the EEG signal was decomposed as described above, separately for each condition and age point. Results of this analysis are displayed in Figure 4.5. As can be seen in Figure 4.6, several of the observed features reached statistical significance against baseline.

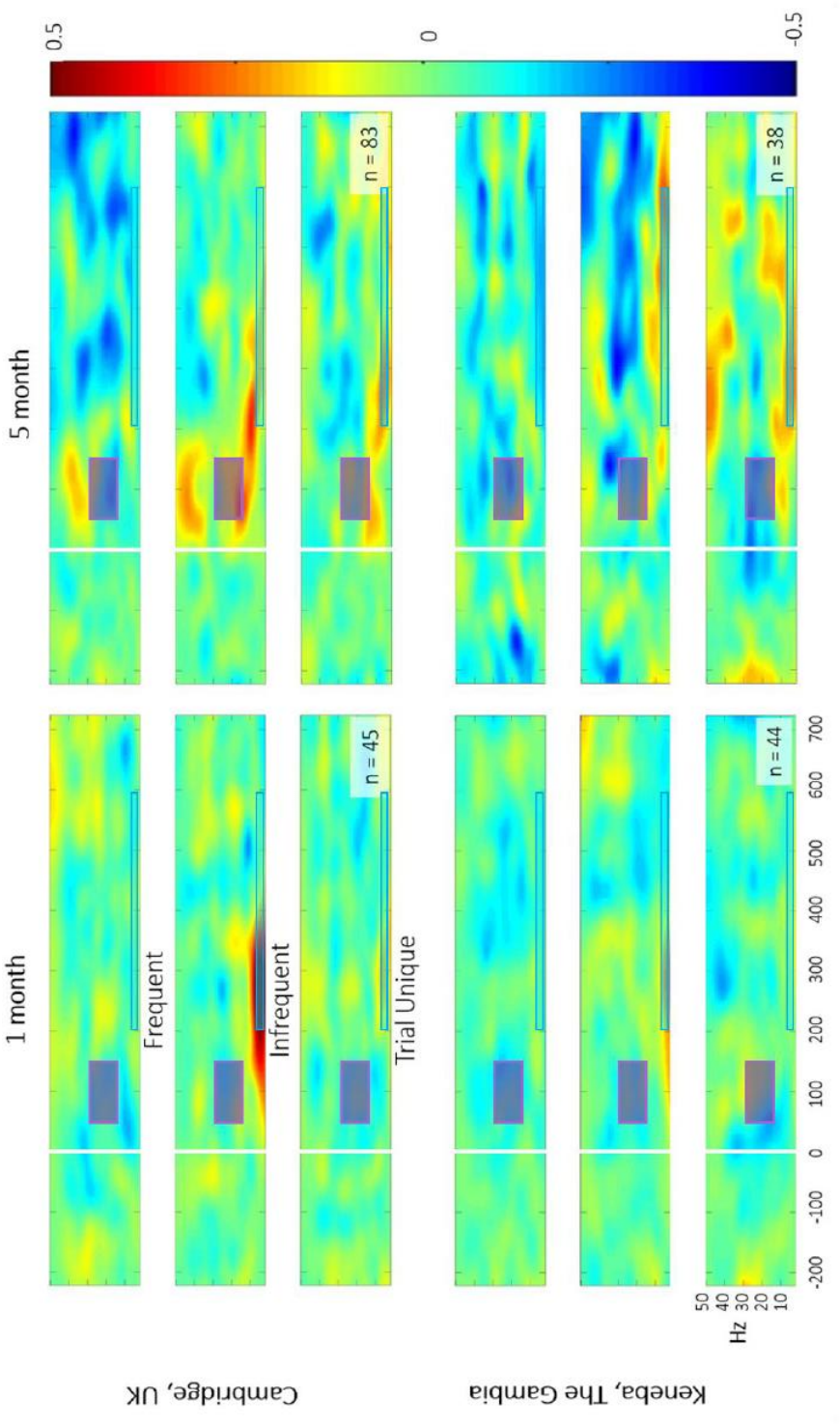


Figure 4.5. Results from time frequency analysis per Condition, Age and Site. Hot colours signify increases in power in the corresponding frequency band, cold colour indicate decreases against baseline.

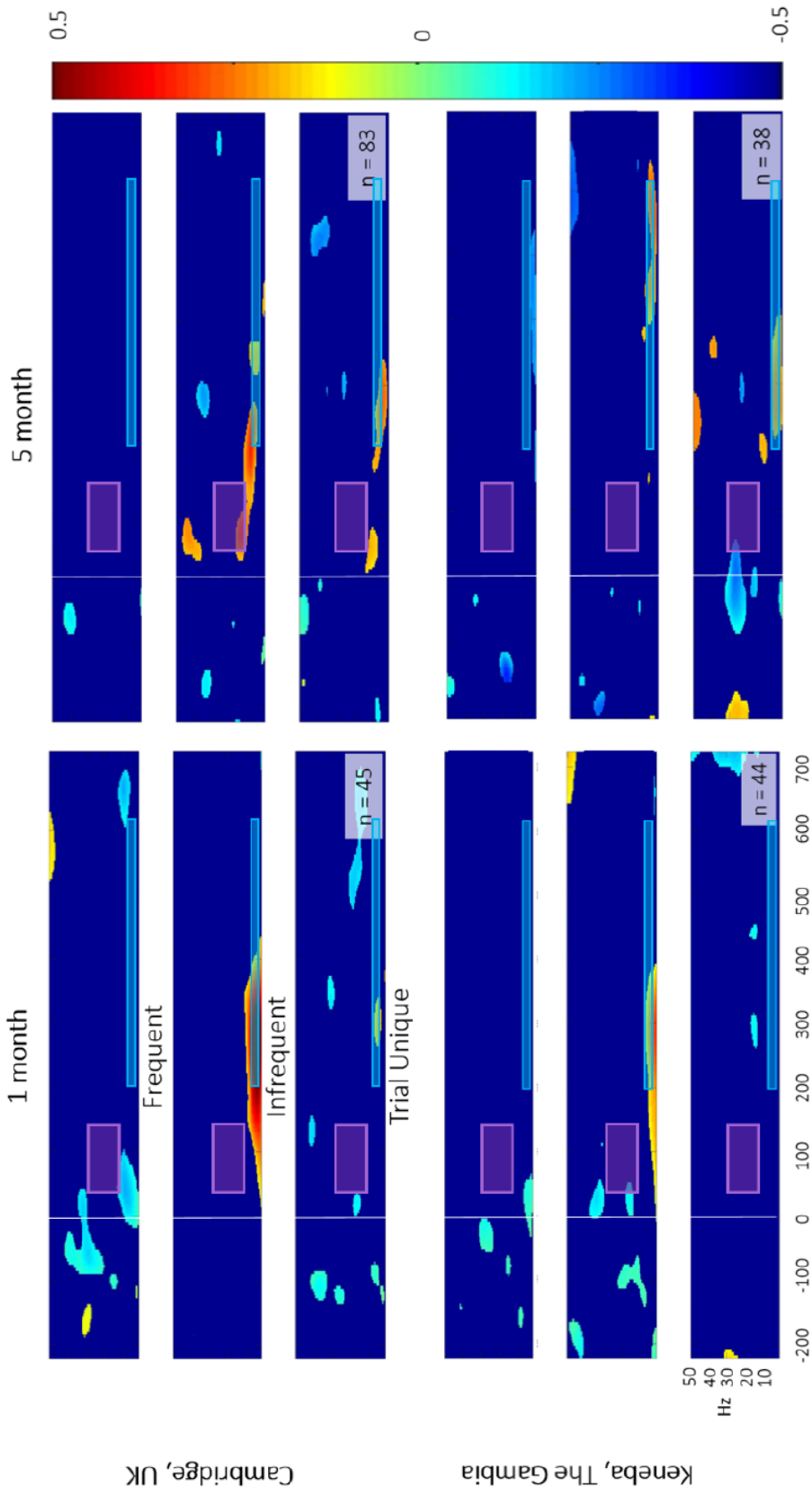


Figure 4.6. Results from time frequency analysis per Condition, Age and Site. Visible through the mask are all features reflective of a significant change from baseline (FDR corrected).

At the 1 month age point, the most prominent feature in both cohorts was an increase in theta band power between 200-400ms, which was absent for the frequent tones and most pronounced for the infrequent tones. A smaller magnitude feature was also apparent in response to the trial unique sounds in the Cambridge cohort only. These findings map onto the ERP findings obtained at the 1 month Age point. In contrast to previous studies, our findings did not show any significant gamma features at this age point, and also showed one drawn out theta component, rather than two temporally distinct features.

At the 5 month age point, patterns of activation differed between cohorts not only in magnitude, but also in timing of occurrence. In the Cambridge cohort, two distinct features were present which broadly mapped onto the previously observed ERP N1 and P3 peaks at 50-150 and 200-400ms for both the infrequent and trial unique tones. The N1 feature was found to occur in the beta range, whereas the P3 feature spanned the theta range. While features were found to be of similar magnitude, the P3 feature seems to be strongest at a later latency post trial onset in response to the Trial Unique tones. Additionally, an early gamma feature was observed only in response to the Infrequent sounds. In the Keneba cohort, features relating to the N1 were less pronounced, and did not reach statistical significance when compared against baseline. While a clear theta feature was apparent in the P3 time window to the trial unique sounds, a feature within a similar frequency range was observed at a much later latency for the Infrequent sounds. Contrary to the Cambridge cohort, the anticipated gamma feature only reached significance in response to the Trial Unique but not the Infrequent tones. Interestingly responses to the Frequent and Trial unique sounds show distinct spectral patterns in the Gambian cohort, counter to the observation of only minor amplitude differences in the ERP analysis.

In order to assess this statistically we extracted features as described above. While based on previous literature a separate investigation of theta and gamma band activity was anticipated, the absence of significance changes in the gamma band from baseline in all but two responses suggested for formal modelling to not yield additional insights. Instead, it was decided to focus analyses on activity in two time windows which broadly mapped onto the observed N1 and P3 ERP components. For the early time window (50-150ms), power within the beta range (13-30Hz) was averaged, reflecting the band of maximum baseline changes. For the later time window (200-600ms), data within the theta band (4-6Hz) was averaged, which was found to be inclusive of maximum theta power changes across groups. Prior to fitting the model, assumptions of normality, homoscedasticity and sphericity were tested separately for the early beta and late theta components and were found to be met.

Table 4.3 *Descriptive statics of mean power in early beta and late theta feature by Condition, Age and Site.*

		1 month		5 month	
		early beta	late theta	early beta	late theta
		$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD
Cambridge	Frequent	.029	-.035	-.207	-.201
	Infrequent	.067	.405	.448	.315
	Trial Unique	-.248	.261	.217	.396
Keneba	Frequent	.021	-.029	-.193	-.112
	Infrequent	-.087	.201	.082	.398
	Trial Unique	-.156	.126	.127	.301

Table 4.4. *Analysis of variance for mean beta power within early epoch with Age, Condition and Site as independent variables.*

IV	*F*	*df*	*p*	η_p^2
Condition	.112	2,98	.886	.002
Age	1.991	1,49	.165	.039
Site	1.328	1,49	.255	.026
Condition * Age	3.449	2,98	.036*	.066
Condition * Site	5.017	2,98	.008*	.093
Age * Site	.496	2,49	.485	.010
Condition * Age * Site	.301	2,98	.741	.006

Note. IV = Independent variable, df = degrees of freedom, η_p^2 *= partial eta squared.*

Table 4.5. *Analysis of variance for mean theta power within late epoch with Age, Condition and Site as independent variables.*

IV	*F*	*df*	*p*	η_p^2
Condition	6.231	2,98	.006*	.113
Age	.004	1,49	.948	<.001
Site	1.236	1,49	.021*	.062
Condition * Age	.564	2,98	.571	.011
Condition * Site	.019	2,98	.981	<.001
Age * Site	.184	2,49	.670	.004
Condition * Age * Site	.608	2,98	.546	.012

Note. IV = Independent variable, df = degrees of freedom, η_p^2 = partial eta squared.

For the early beta activity interaction effects were observed between Condition * Age and Condition * Site. Post hoc comparisons revealed that only the change within the beta power in response to the trial unique sounds significantly differed between Age points (t_{50} = -2.930, p = .005), whereas none of the other condition contrasts by Age or Site reached statistical significance.

The late latency theta analysis showed a significant main effect for Condition and Site. Post hoc comparisons showed a trend towards a difference between activity elicited by frequent and infrequent sounds (t_{124} = -1.774 , p = .078) and no differences between other condition pairs. A significant difference between Sites was found (t_{203} = -2.067, p = .02).

4.3.2 ITPC analysis. The ITPC was extracted across the same frequency bands and time windows as for the TF analysis above. Descriptive statistics are summarised in Table 4.6. Results from the statistical model are provided in Table 4.7 and Table 4.8. Prior to fitting the model, assumptions of normality,

homoscedasticity and sphericity were tested separately for the early beta and late theta components. All assumptions were found to be met.

Table 4.6 *Descriptive statics of ITPC means in early beta and late theta feature by Condition, Age and Site.*

		1 month		5 month	
		early beta	late theta	early beta	late theta
		$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD
Cambridge	Frequent	.043	.042	.065	.049
	Infrequent	.052	.063	.066	.056
	Trial Unique	.045	.049	.056	.068
Keneba	Frequent	.047	.046	.079	.063
	Infrequent	.057	.068	.086	.064
	Trial Unique	.049	.051	.066	.059

Table 4.7. *Analysis of variance for ITPC within early beta feature with Age, Condition and Site as independent variables.*

IV	*F*	*df*	*p*	η_p^2
Condition	3.103	2,98	.050	.060
Age	8.898	1,49	.004*	.154
Site	1.514	1,49	.224	.030
Condition * Age	3.689	2,98	.029*	.070
Condition * Site	.044	2,98	.957	.001
Age * Site	1.955	2,49	.168	.038
Condition * Age * Site	1.730301	2,98	.183	.034

Note. IV = Independent variable, df = degrees of freedom, η_p^2 *= partial eta squared.*

Table 4.8. *Analysis of variance for ITPC within late theta feature with Age, Condition and Site as independent variables.*

IV	*F*	*df*	*p*	η_p^2
Condition	2.011	2,98	.139	.039
Age	2.168	1,49	.147	.042
Site	1.547	1,49	.015*	.082
Condition * Age	2.099	2,98	.128	.041
Condition * Site	1.890	2,98	.156	.037
Age * Site	3.843	2,49	.056	.073
Condition * Age * Site	1.805	2,98	.175	.036

Note. IV = Independent variable, df = degrees of freedom, η_p^2 *= partial eta squared.*

Within the early beta feature a main effect for Age was found. Post hoc comparison via a paired samples t-test revealed for ITPC to become larger with age (t_{50} = -2.644, p = .011). There further was an interaction effect for the ITPC was for Condition * Age, as well as a trend for a main effect for Condition. When followed up, it was shown that the effect was driven by a significant difference in ITPC in response to the frequent (t_{50} = -3.276, p = .002) as well as the infrequency tones (t_{50} = -2.173, p = .035) between the 1 and the 5 month age point.

With regard to the theta feature, only a significant main effect was observed for Site, which as predicted was found to be higher in the Cambridge cohort (t_{127} = -2.656 , p = .009).

4.4 Discussion

This chapter documented analyses ancillary to the ERP work discussed in Chapter 3. Specifically it was focused on spectral features of the mismatch response with was examined via time frequency analysis and through an examination of the ITPC within prominent features. These analyses were aimed to specifically address some of the core findings of the ERP analysis that warranted further investigation. Specifically, it was assessed whether the MMR_{TF} would show differential responses between the frequent and trial unique sounds in the Keneba cohort which had shown a lack of difference in the ERP response. Response differences were indeed found between the two conditions, at the 5 month age point only, where a distinct theta feature was present which did not show in response to the frequent tones (Figure 4.6). This effect however was not apparent at the 1 month age point, and did not reach statistical significance when modelled in context of all other factors. The other question that this analysis was specifically aimed at answering was whether a lack of phase coherence could account for some of the amplitude differences observed in the Keneba cohort at the 1 month age point. Initially hypothesised to be partially explained by inter-individual variance in peak latency, this was not confirmed in the ERP analysis. However, the ITPC analysis showed differences in phase coherence by Site, specifically lower phase coherence in the Keneba cohort. This result is meaningful not only as it provides some explanation of the reduced amplitudes observed in the ERP, but also as it provides some evidence of ITPC to differ in an at-risk infant population outside the context of ASD.

Previous research suggests a higher sensitivity of spectral feature changes compared to ERP analyses (Isler et al., 2012).This sensitivity specifically has been suggested to help discriminate between responses where ERP's are not able to reliably show differences. In the current study, ERP responses revealed clear condition and group differences, however the spectral analyses added some additional understanding. First, this study extends the existing literature into the study of awake and slightly older infants, suggesting its utility for the definition of developmental trajectories. Secondly, the present data extend our understanding of the oscillatory dynamics in context of different stimulus contrasts. As has been proposed by Gilley et al. (2017), the interplay of the different features, particularly the difference between the two theta features can give an indication as to the degree of similarity between stimuli (as can be seen from their schematic in Figure 4.7). While in the current study, one unitary theta burst was observed at the 1 month age point, two distinct feature became apparent towards the 5 month age point, which were more clearly visible in the Cambridge cohort. The features were more clearly separated for the trial unique sounds, which can be regarded as in support of the view that the degree of separation of these bursts can give an indication of the degree to which a stimulus is perceived as novel. While Gilley et al. (2017) proposed this view based on the auditory characteristics of sound pairs, the current study while preliminary might indicate a similar pattern for sound categories, such as inherently more novel trial unique sounds.

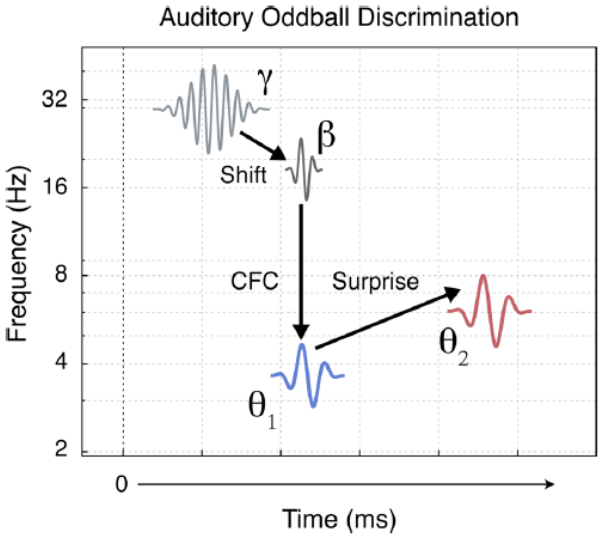


Figure 4.7. Reproduced from Gilley et al. (2017). Schematic of spectral-temporal dynamics of auditory oddball discrimination. In response to an incoming deviant stimulus is, there is an initial gamma response which shifts towards a beta range response if the auditory signal differs from the preceding signal. This gamma-beta complex coupled to an initial theta feature (blue), followed by a second theta feature (red) that occurs after a short delay to the gamma-beta complex. Gilley et al. (2017) propose that the degree of difference between the two theta features reflects the degree of difference of the incoming stimulus to the anticipated stimulus, which is interpreted as a neural correlate of deviance detection.

The findings presented here underline the potential benefits of implementing converging analysis techniques to understand the richness of electrophysiological data. This is particularly important in datasets which are rarely available and not easily replicable such as longitudinal data from developing populations. Further, this study follows on from previous work by exploring developmental changes within the MMR_{TF}. A main effect for Age was only observed within the ITPC analysis for the early beta feature, and not in regard with either of the TF measures. This might suggest that when seeking to determine developmental trajectories, established

developmental changes, such as shortening of peak latencies in an ERP analysis provide a more robustly measure.

4.4.1 Limitations and directions for future research. As this study originally was conceptualised as an ERP design, not all methods could easily be applied to the resulting data. First of all, time frequency based approaches generally require longer baseline windows, in order to successfully decompose even low frequencies. Ideally, the baseline period should be long enough to allow to for at least one full cycle of the lowest frequency to occur (i.e. if a delta frequency of 1Hz is of interest, the baseline would need to be 1 second long, 500ms for 2Hz etc.). This means that inter stimulus intervals ideally should be longer than in this study, so that the EEG can return to baseline a few hundred milliseconds before onset of the next stimulus. While the baseline period can be extended into the preceding epoch as was done here, this runs the risk of contamination from previous responses and thus longer ISI's would provide cleaner contrasts. This has to be weighed against the time it takes to administer a sufficient number of trials in each condition to achieve an optimal balance.

Further, some limitations were posed on this analysis due to the low channel count. While previous studies found theta and gamma activity to be strongest over frontal leads (Isler et al., 2012, Gilley et al., 2017), there is some indication that certain higher frequency oscillations appear more focally. For example Isler et al. (2012) reported on some beta features that were most clearly observed over temporal cortex. Other authors have even suggested for the gamma features to be most pronounces in areas other than the frontal cortex (Fuentemilla et al., 2008, Hsiao et al., 2010, Ko et al., 2012), suggesting that more coverage might have yielded a more robust gamma response. This limitation also bears relevance in the exploration of other advanced EEG methods, such as cortical source analysis, whereby the

data observed on scalp level can be reconstructed to infer cortical origins of the observed signal.

Due to the relatively good documentation in the literature and the resulting known developmental changes in the baseline power spectrum of infants, it is further recommended that future studies should try and acquire resting state data prior to the recording of any experimental paradigms, as this can yield a valuable measure of the more global electrophysiological response patterns in infant development.

Validity of site and age group comparison. Similar issues as have been described in context of the ERP analyses arise in the current chapter related to differences in response and developmental patterns across the two sites and age groups. Hereby, it needs to be noted that activity in the TF analyses was observed in slightly different frequency bands that have previously been reported in the literature, and that the latency of occurrence differed across study sites in some instances. To avoid a cohort bias which would have results from activity over the same time windows across both cohorts, longer windows were chosen, to include responses from a maximum number of infants that were assumed to be functionally similar. The issue of responses occurring in slightly different frequency bands across the groups could not fully be addressed. While one option might have been to take a more data driven approach and include data from a boarder spectrum to encompass responses across age points and study sites, this would have artificially inflated the effects and further made interpretations regarding the functional meaning of these responses challenging. For future analyses it will be considered to conduct a replication of the current findings in an independent subset of newly incoming data, to increase confidence in frequency and latency of the observed features, which will guide analysis of the entire sample.

In summary, both this and the preceding chapter illustrate the potential of electrophysiological assessment in the context of resource poor settings and provide two tools of examining early cognitive development. Both ERP and the proposed spectral analyses have yielded novel insights into the development of the infant novelty response, and it is therefore recommended to implement both modes of analysis in future investigations. In the following chapter, the focus will shift towards an assessment of the haemodynamic response in infants assessed during the second half of the first year of life.

Chapter 5. Development of Object Permanence and Working Memory

Study 2: fNIRS study at 8 and 12 months

5.1 Introduction

The ability to briefly retain a mental representation of incoming information is a crucial cognitive process, and fundamental to all forms of abstract thought. Working memory (WM) enables individuals to utilise information to guide prospective action, attain goals or solve a problem (Fuster, 2015). As such, WM plays a significant role in many cognitive processes, such as action planning, decision making, and strategy use. Due to its ubiquity in tasks of daily life, WM abilities have frequently been associated with a range of life outcomes, such as academic performance (Alloway et al., 2004) or quality of life (Goghari & Lawlor-Savage, 2017).

WM differs from other forms of memory with regard to two critical aspects: its limited capacity and duration of retention. Different theoretical models have proposed conceptualisations of WM, arguably the most influential of which has been drawn up by Baddeley and Hitch (1974). It holds that WM comprises different subdomains that allow for short term retention of input from the different sensory modalities as well as processes coordinating this input. The model has been revised and updated in light of empirical evidence, and in its most recent form (Figure 5.1) consists of four main components (Baddeley, 2010). An attentional control system, the central executive, is conceptualised as drawing together inputs from the three subdomains, the visuo-spatial sketchpad, the episodic buffer and the phonological loop. The visuo-spatial sketchpad is proposed as the primary processor of incoming visual information, whereas the phonological loop processes auditory input. These two processing modalities have more recently been suggested to be aided by a fourth component, the episodic buffer, the function of which is to draw together multidimensional information, which combines all sensory modalities. It further acts as a buffer in that it provides an interface for incoming sensory information and

activated long term memory, and thus thought to bind together present relevant input and past consolidated memory (Baddeley, 2010).

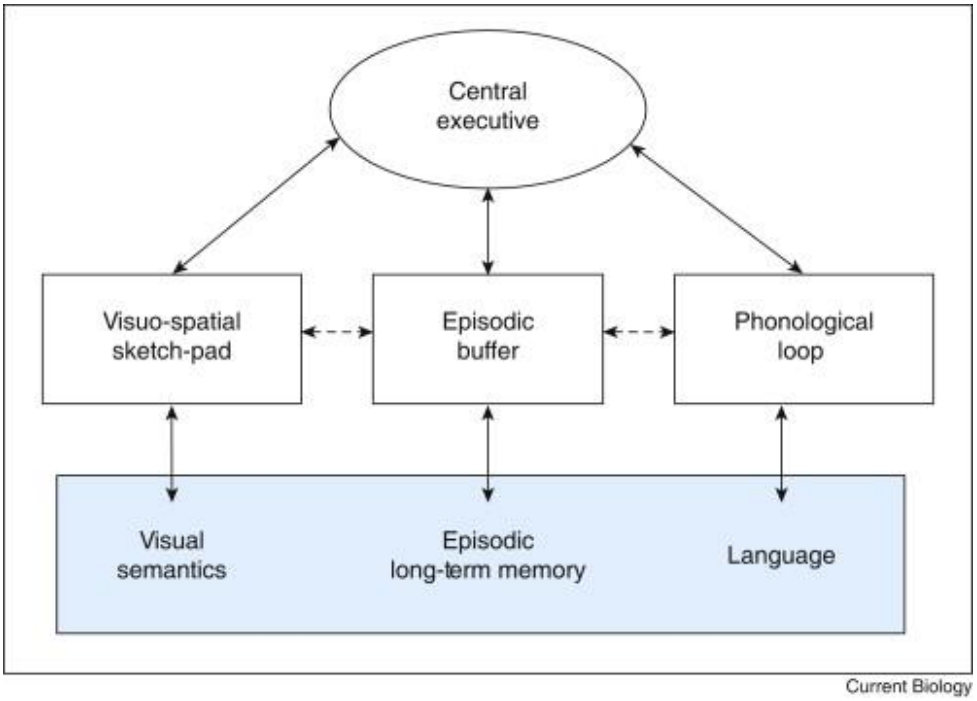


Figure 5.1. Reproduced from Baddeley (2010). Updated model of WM including visuo-spatial sketchpad, episodic buffer and phonological loop contained in a feedback loop with the central executive.

While this conceptualisation is useful in adults to disentangle modality specific WM processes, limitations of infant assessment have made it difficult to apply the same rigour of investigation to the early emergence of WM functioning. From infant and paediatric studies, it is known however that WM capacity increases vastly over the course of infancy, childhood, and indeed adolescence and early adulthood (Nelson et al., 2000). Despite this protracted development, early foundations of WM functioning can be observed to emerge during the infant period.

5.1.1 The development of WM in infancy. While WM encompasses a complex set of abilities and in adults has been assessed using a wide variety of tasks, only few assessment tools are available that are suitable for infant research. The majority of tasks used to study early WM abilities have thus

relied on paradigms eliciting object search. The tasks rest on the assumption that infants who can form an abstract representation of a hidden object will attempt to search for it, whereas those that have not yet developed this ability will fail to do so.

The most basic assessment of infants' early WM abilities is through the assessment of object permanence (OP). OP refers to the ability of knowing that an object continues to exist even though it is temporarily out of sight. As OP requires the online maintenance of visual information that is no longer present, it has been argued to be one of the first methods of assessing the development of WM (Lowe et al., 2015). Adults and children possess this capacity and therefore perceive an object that is temporarily out of sight and then reappears as having continually existed. Similarly, two objects that subsequently appear in two different locations are perceived as being different, unless movement from one location to the next was observed. Young infants have yet to develop this ability in order to perceive the world around them as a coherent whole. In classical task designs infants are presented with a salient object which is subsequently hidden from view. OP is hereby operationalised as infants searching for the object indicate awareness of its existence.

The development of OP has been theoretically conceptualised by Piaget (1954), who distinguished six stages of infant's object concepts. According to Piaget, in Stages 1 and 2, for approximately the first four months of life, infants do not have any concept of the continued existence of hidden objects. In Stage 3, from around 4 – 8 months infants begin to search for partially hidden objects, before reaching Stage 4 in which they are able to form a concept of a hidden object, and thus reliably engage in search behaviours. Stage 4 according to Piaget occurs when infants are around 8-12 months of age. He characterised development in subsequent stages as

taking into account visible (Stage 5) and invisible (Stage 6) displacements of objects to different locations.

Experimentally, the specific age points of OP emergence proposed by Piaget have been called into question. While many studies have demonstrated that infants indeed become reliably able to actively search for occluded objects at around 9 months (Baird, 2002, Bell & Fox, 1997), others have provided evidence for OP to be emerging at a much younger age (Hood & Willatts, 1986, Baillargeon, 1984, 1985, 1986). In a series of studies (Baillargeon, 1984, 1985, 1986) it has been demonstrated that infants begin to show the first signs of OP from around 4 months of age. In these studies, infants' looking time was measured while they were presented with both possible and impossible events. In one study, 6-8 month old infants were presented with a car driving down a ramp, with a 'roadblock' being either installed in front of the car, thus preventing it from driving down the ramp (impossible event), or behind the car (possible event) and thus not affecting its trajectory (Baillargeon, 1986). The ramp with the two types of road blocks was hidden behind a screen and the car drove down the ramp. Infants looked significantly longer at impossible compared to possible events. Studying even younger infants, Baillargeon (1984) presented 5 month old infants with a similar set up. An occluder was placed in front of the infant, and swung back and forth in a draw-bridge- like fashion. After a habituation phase, an object was placed behind the occluder, preventing it from moving through the entire 180 degree arc. In possible events, the occluder stopped when hitting the object, whereas in impossible events it continued to move as if the object had disappeared. Infants attended more to impossible events, indicating that a violation of their expectation of OP had occurred. The experiment was repeated in 3.5-4.5 month old infants (Baillargeon, 1985). A subgroup of infants, those who had habituated to the initial event quickly, looked at the impossible event significantly longer than at the

possible event. This suggests that in a subset of infants OP begins to emerge as early as 4 months of age, however with substantial inter-individual variance.

More complex paradigms assessing early infant WM include the A-not-B task, in which an object is hidden in one of two locations and the infant allowed to search for it. Once the infant has successfully searched in location A, the object is hidden in location B. Young infants tend to persevere in searching at location A. At around nine-months of age, infants develop the ability to instead look at the correct location B. This task has been conceptualised as a measure of spatial WM (Cuevas et al., 2012, Bell, 2002). Behaviourally, 8 month olds have been shown to possess rudimentary abilities to successfully search in A not B tasks. Cuevas et al. (2012) reported that only approximately 10% of their 8 month old participants were able to successfully search after a 2 second delay, while previous studies have demonstrated that at 12 months, infants can tolerate delay times of up to 10 seconds (Bell & Fox, 1992, Diamond et al., 1997).

In summary, infant WM has classically been assessed almost exclusively in terms of their visuospatial abilities, whereas Baddeley's model as a whole has not been thoroughly studied in the infant period. Both OP and A not B tasks provide evidence for infants' visuospatial WM development over the first 12 months of life. As reviewed, infants begin to show first signs of OP from as young as 4 months, which manifests in overt search from around 8-9 months. Around that time infants also become better able at remembering changes in an objects' location, and even when the hiding of the object and the search phase are separated by delays of up to a few seconds.

It has been shown that behavioural change in the infant period is accompanied by functional changes in brain areas that have been associated with WM processes in adults. In the following two sections, I will

first provide an overview of WM neural correlates in adults, before laying out developmental changes in these relevant areas.

5.1.2 Neural correlates of WM. Research into the neural correlates of WM have heavily implicated a network comprising aspects of the frontal cortex (FC), specifically the dorsolateral prefrontal cortex (dlPFC) and inferior frontal gyrus (IFG) as well as areas of the parietal cortex (PC) and the cingulum, particularly the anterior cingulate cortex (ACC, Nelson, 2000). In adults, it has been proposed that different nodes of this network are more heavily involved depending on task demands. Hereby, the dlPFC (including the superior and middle frontal gyrus) have been more heavily implicated in processing of spatial WM, whereas the ventral aspects of the frontal cortex, including the IFG have been shown to be more involved in the context of WM for objects and faces (Goldman- Rakic, 1996, Ungerleider et al., 1998). Some evidence for this stems from primate research, specifically from single neuron recordings in non-human primates. Primates with implantations in the dlPFC or the IFG showed activity in these two areas that was specific to task demands. Neurons in the dlPFC were most strongly activated in response to spatial tasks, whereas neurons in the IFG were more attuned to a pattern-based WM task (Wilson et al., 1993). Further, in a study comparing infants' and rhesus monkeys' performance on an A-not-B task, it was found that monkeys with lesions to the dlPFC performed similarly to the pre-permanent human infants, whereas those without lesions and those with parietal lesions were unimpaired (Diamond & Goldman-Rakic, 1989). Converging evidence from human research stems from an fMRI study by Courtney et al. (1998) who reported the superior frontal gyrus to be mostly involved in spatial memory tasks, whereas the left IFG was more active in a face recognition task.

In addition to the well-established link of FC activity and WM processes, a dose-response relationship has been demonstrated for activation of the

dlPFC and WM load. In an fNIRS study, Fishburn et al. (2014) assessed young adults on an n-back task, requiring participants to memorise a string of items and subsequently decide if a test item matched a target item in the previous sequence. The target item was either the one that immediately preceded the test stimulus (WM load 0) or an item that was presented a number of trials ago (WM load n). In this study, three load conditions were used (load 0, 1 and 2). Results showed that activation in the PFC and specifically the dlPFC increased linearly as a function of WM load. This study further demonstrated the sensitivity of fNIRS to measure load dependent activation modulations in adults.

Some evidence demonstrates the involvement of regions in the PC and of subcortical structures in WM processes. In line with the notion that regions in the posterior PC are part of the dorsal visual stream, or 'where-pathway', these regions have been shown to be active in the processing of spatial information during WM tasks (Ungerleider et al., 1998). Corroborating evidence stems from single neuron recordings in monkeys, which have shown involvement of the posterior PC in spatial tasks (Ungerleider & Haxby, 1994; Courtney, Ungerleider, Keil & Haxby, 1996). The ACC has been argued to be associated specifically with selective attention in WM tasks, as well as with language-based tasks (Cabeza & Nyberg, 1997). Carter et al., (1998) showed that competition between responses was strongly associated with ACC activity, indicating that WM tasks in which errors are likely to occur were most strongly associated with ACC activity. Some studies have investigated the functional neuroanatomy of WM in paediatric populations, as will be reviewed in the following.

5.1.3 Neurobehavioural development of WM

Neurobiological development.

While not much is known about the development of the parietal and cingulate cortices, an abundance of evidence suggests development of the FC starts in infancy and subsequently continue until well into young

adulthood (Nelson, 2000). Several forms of converging evidence suggest a protracted FC development. First, the number of synapses within the middle frontal gyrus has been reported to peak at around 12 month of age, at that time far exceeding adult numbers of connections. The subsequent reduction associated with pruning processes has been found to continue until mid to late adolescence (Huttenlocher, 1979, 1990, 1994, Huttenlocher & Dabholkar, 1997). Second, with regard to both metabolic efficiency and axonal myelination, the FC has been shown to lag behind other cortical areas, suggesting a slower developmental progression (Chugani, 1994; Chugani & Phelps, 1986, Deoni et al., 2011). Third, histochemical processes within the FC such as acetylcholinesterase reactivity associated with mature neural functioning is not fully developed until early adulthood (Kostovic, Skavic, & Strinovic, 1988; Mesulam & Geula, 1988). In summary, these findings strongly suggest that the FC continues to mature over the course of childhood and adolescence. From a functional perspective, changes in cerebral blood flow over frontal areas has been shown to linearly increase from birth until around 8-9 months of age when it plateaued. This increase was slower than that of other cortical areas responsible for sensory processes such as the occipital lobes, which plateaued at around 3-4 months of age (Fransceschini et al., 2007).

Neurobehavioural development in children and adolescents. Some paediatric research has examined brain- behaviour relationships of WM functioning. WM task performance has been linked to FC activity in both children and infants. Hereby, it first was shown in children that, reduced EEG power in frontal areas is associated with incorrect responses on a verbal WM task in 8-10 year olds (Fernández et al., 1998). Changes in FC dependent processes have also been demonstrated during adolescence. Using a range of WM dependent tasks such as the Tower of London paradigm, Luciana and Nelson (1998) demonstrated that performance gains from 4-8 years were

not larger than those from 8-12 years, indicating a certain continuity of the developmental change into older childhood and adolescence. Comparing children and young adults linear performance increases could be demonstrated with regard to visual- (Miles, Morgan, Milne & Morris, 1996) and spatial- and verbal WM (Isaacs & Vargha-Khadem, 1989).

Neurobehavioural development in infancy. A similar effect could be shown in infants, whereby reduced frontal EEG power was found to be linked to more errors on an A not B task (Bell, 2002). This was further corroborated by Cuevas et al. (2012) who showed that EEG coherence in frontal areas was associated with A not B task performance in infants between 8 and 12 months. Further, infants who passed a behavioural OP task, showed greater changes in the haemodynamic response in the prefrontal cortex (Baird et al., 2002). This latter study was the first to map out changes in the neuronal response relative to changes in behavioural performance. In this study, infants were presented with an OP task every month from the time they were 5 months old. They were tested using an OP paradigm with concurrent fNIRS recording. Data was then compared between object permanent and pre-permanent infants. Significantly greater increases in the haemodynamic response were measured in object permanent infants while they were performing the task. This task illustrates well the close brain-behaviour relationship between PFC development and behavioural performance on the task.

It has been suggested that WM capacity increases during infant development. It could be shown that even infants able to pass A-not B tasks fall prey to the A not B error when a delay in time between the hiding and subsequent search is too long (Diamond, 1985). This has been taken as evidence that infants are able to tolerate progressively more complex task demands. This is underlined by two studies related to this project, in which 12-14 month old infants were presented with a passive video version of an

OP paradigm. It could be shown that in both Gambian and British infants frontal activation was observed in response to an object being hidden over a 6 second delay but not a 3 second delay (Begus et al., 2016, Kischkel et al., 2016). In addition, in both these samples activation was observed over posterior temporal areas of the cortex, which was interpreted as indication of a wider network underlying the OP task.

Evidence for frontal – temporal network. While there is almost no evidence for more network based approaches of WM development, one study has investigated how neural networks develop on a dimensional card sorting task (DCT) during the preschool years (Buss & Spencer, 2018). In these tasks, children are typically asked to sort images according to a specific feature, which could be the images colour, before they are asked to switch rules and sort according to a different feature, for example the image's shape. While this task taps not only WM, but also a range of other executive functions, the discussion of developmental changes within the associated neural network help to extract some more general features of the orchestrating role of the FC, and is therefore informative in absence of research solely focused on WM development. Buss & Spencer (2018) administered a DCT in a sample of 3-5 year olds. Three year old children typically perform poorly on this task whereas at 4- 5 years of age children begin to be able to comply with the rule switch (Buss & Spencer, 2018). The authors hypothesised that this would be reflected in both frontal activity as well as in connectivity between frontal and posterior areas in the temporal and parietal cortices, indicating the increasing integrity of the neural network. Indeed, the study demonstrated frontal activity to be stronger in 4-5 than in 3 year olds, and that this activation in turn was related to task performance. In a next step, task difficulty was manipulated by administering both an easy and a hard version of the task. In the easy version, the two dimensions were not conflicting and thus sorting according to one or the other did not produce

different outcomes. In the second phase, the classical card sorting paradigm including the dimensional conflict was administered. In the hard version of the task dimensions were in conflict in both the first and the second phase of the assessment. A large proportion of three year old children was found to be able to perform the easy but not the hard version of the task and that further network integrity between frontal and posterior areas was associated with task performance. The authors argued that in the easy version, bottom up processes first provide input to the FC, guiding rule learning during the first phase which supported better performance on the hard version. In the hard version this initial bottom up process was pre-empted by the immediately conflicting task conditions, meaning immediate top down control needed to be exerted. This top down control as evidenced by increased activation of the FC – posterior network was observed in the able switchers but not in the children struggling in the task. The authors reiterate the importance of network-based approached and highlight how the study of broader networks could help elucidate the mechanisms on neural development of FC dependent tasks.

In summary, the presented studies strongly suggest FC involvement with WM processes in both children and adolescence, adults and infants. A dose-response relationship has been demonstrated for stronger activation in this area relating to higher memory load either by increased set sizes or by increased delays. Further, the brain behaviour link has been illustrated between FC and PFC activation and OP performance even in infants. As for the involvement of wider neural networks, Buss and Spencer (2018) have suggested some mechanistic pathways in which the FC might orchestrate WM dependent processes through a feedback loop of bottom up and top down control processes.

5.1.4 WM in clinical populations and resource poor settings. WM deficits are a commonly reported impairment in virtually all neurodevelopmental disorders and have been demonstrated to occur in children with autism spectrum disorder, attention deficit hyperactivity disorder and developmental coordination disorder (Alloway et al., 2009), William's syndrome (Vicari et al., 2003), Fragile X Syndrome (Munir et al., 2000) and Down's Syndrome (Lanfranchi et al., 2004). In adults, deficits have been reported in the context of many psychiatric conditions and neurodegenerative diseases, such as schizophrenia (Park et al., 1995), Parkinson's disease (Lewis et al., 2003) and Alzheimer's Dementia (Belleville et al., 1996). While WM deficits are a common feature of various clinical populations, specific profiles of impairment have been found to be associated with different clinical presentations. Children with language impairments for example have been shown to have impairments specific to the phonological loop and thus phonological processing (Pickering & Gathercole, 2004).

Very little is known about the nature and prevalence of WM deficits in infant at-risk populations. Some studies in infants born prematurely have indicated WM deficits. Woodward et al. (2005) examined infants at 24 months corrected age and recorded consistently lower scores on an A not B task when comparing the preterm infant to term born controls. This deficit was above that correlated with global measures of brain development, such as white matter reductions and increased volumes of cerebral spinal fluid. Similarly, Lowe et al. (2009) assessed infants with very low birth weight when they were 18-22 month old and found that they consistently scored lower than controls on an item sequence on the Bayley Scale of Infant Learning consisting of incrementally more complex OP dependent items. WM capacity has been investigated in children brought up under extreme deprivation in institutional care homes (Fox et al., 2011). When tested at 8

years of age, WM abilities of children who had spent part of their infant period in institutional care homes performed significantly worse than children who grew up within the community. The studies reviewed above give some indication that the common WM deficits observed in children and adults can also be found in a variety of infant at-risk populations. While there are currently some ongoing investigations into early WM memory development in infancy underway in other LMIC settings such as India (Spencer et al., personal communication), to my best knowledge there currently is only one published study that has investigated the neural correlates of WM in infancy in a LMIC, which formed part of a previous phase of the BRIGHT project (Begus et al., 2016).

In Phase 1 of the BRIGHT project a new paradigm was piloted in 12-14 month olds in both the Gambia and the UK. The paradigm consisted of a passive OP task, which required infants to watch an object being hidden from view and recovered after a delay of either 3- or 6 seconds. The activation patterns from both studies are displayed in Figure 5.2. The results obtained from the Gambian populations are published in Begus et al. (2016). In both cohorts, differential patterns of activation were observed in response to the 3- and the 6 second delay condition. While posterior temporal activation was obtained in both the 3 and the 6 second delay condition, more frontally localised activation was obtained exclusively for the 6 second delay. This pattern was observed both in the Gambian cohort, as well as in preliminary analysis of data collected in the UK (Kischkel et al., 2016). Overall, activation was found across a larger number of channels in the Gambian sample relative to the UK: this could be due to reasons of statistical power, as only n = 6 infants were tested in the UK while n = 24 infants were tested in The Gambia.

Alternatively, it can be speculated that a higher variance in brain development in the Gambian cohort might be associated with more broadly

spread neural generators for the observed activation, thus leading to more wide spread activation pattern.

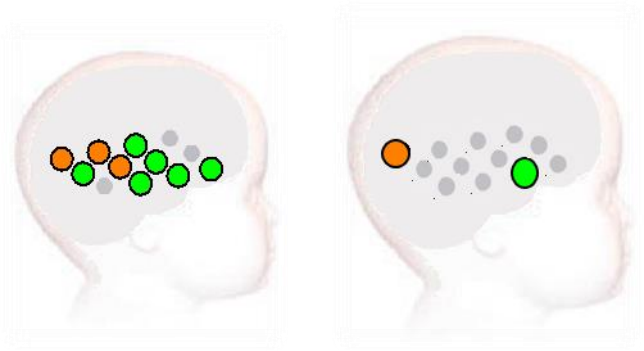


Figure 5.2. Activation patterns from pilot studies conducted in 12-14 month old infants in the Gambia (left) and the UK (right). Highlighted channels indicate activation in response to 3- and 6- second delay condition (orange) and 6 second delay condition only (green). More wide spread activation was observed in Gambian sample. Note that even though a control condition was presented in the UK sample, this did not elicit any significant activation against baseline. Note also that the right hemisphere is displayed as a unilateral array was used in these studies.

From the neural activation it became apparent that infants were employing regions in the medial and posterior temporal lobes known to be involved in adult memory functioning (Begus et al., 2016) as well as more frontal regions when the object was hidden for longer, possible reflecting the prolonged retention delay. Following on from these results, the paradigm was further refined in some key ways and administered longitudinally in the current study.

5.1.5 Rationale for use of the current study

. As reviewed above, the brain behaviour relationship associated with different types of WM is well-characterised in adults and older children. Further, there is some evidence on how early WM abilities emerge during infancy. A few studies have examined neural correlates of object permanence as an indicator of typical

WM development in infants (Bell, 2002, Baird, 2002) as well as in at-risk populations (Begus et al., 2016).

This study seeks to follow on from this work in four key ways. First, by extending knowledge on the localisation of a possible wider cortical network, relevant to OP and thus WM development in infancy. Secondly, the findings from Begus et al. (2016) will be expanded upon. The stimulus presentation used during the pilot phase is illustrated in Figure 5.3.

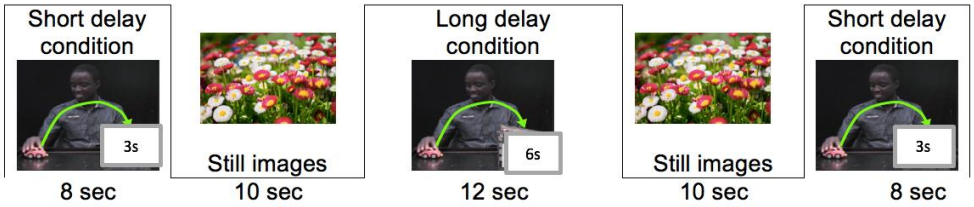


Figure 5.3. Schematic of stimulus presentation utilised in Begus et al. (2016). Trials varied in length between the 3 and the 6 second delay condition. No control condition was presented.

By implementing a control condition in this larger cohort it will be possible to disentangle effects associated with WM demands from effects due to other stimulus features. Third, the longitudinal implementation of this task during development in which a majority of infants have been shown to reliably represent a hidden object will elucidate associated neurodevelopmental processes. Fourth, by concurrently implementing this study in both the Keneba and the Cambridge cohort, we will be better able to parse out developmental trajectories for each site and identify in how far they differ or coincide.

The paradigm used in this study differs from previous studies in a few key ways. First, fNIRS will be recorded from a larger cortical area than was available in Baird et al. (2000), thus not only giving an indication about the PFC but also other potential areas of interest. Second, the paradigm used in Begus et al. (2016) will be modified through equalisation of trial length to

unify the duration of the visual input across trials. Third a control condition will be implemented in which the object is placed on top of the box, thus not creating any WM demands. While this condition was part of the previous UK pilot study (Kischkel et al., 2016), the small sample size of that study did not give the best possible indication of associated activation patterns and will thus be re-assessed in the current study.

5.1.6 Hypotheses. Based on the previous literature discussed above, the following hypotheses will be tested in the context of this study.

Experimental manipulation:

1. In line with the findings on FC activation in WM tasks, activation in frontal channels is expected for both experimental conditions but not the control condition.
2. It is further expected that this activation will be stronger in the long delay condition, compared to the short delay condition due to increased task demands.
3. The more posterior activation patterns are not expected to be related to WM demands but rather to be present in all three stimulus conditions.

Developmental change:

4. It is expected that activation in frontally localised channels will increase between the age time points in the experimental conditions.
5. It further is expected that this increase of frontal activation will be stronger in the 6 second delay condition due to higher WM demands in this condition.

Differences between sites:

6. It is expected that frontal activation will be reduced in the Keneba compared to the Cambridge cohort, and that the developmental increase will be larger in Cambridge.

5.2 Method

5.2.1 Participants. The fNIRS study was performed when infants were 8 and 12 months of age. Participant information can be found in Table 5.1.

Table 5.1. *Sample sizes and age in days for 8 and 12 month time points for participants completing the fNIRS assessment.*

	8m		12m	
	N (girls)	Age in days X ± SD	N (girls)	Age in days X ± SD
Keneba	42 (18)	246.54 ± 10.56	23 (13)	379.92 ± 17.47
Cambridge	24 (10)	249.45 ± 9.88	21 (13)	376.72 ± 11.874

5.2.2 Stimuli & Design. Infants were presented with 10s-long video stimuli of an actor picking up an object, attracting the infant's attention to it with a non- speech vocalisation (i.e. 'ooh!') and subsequently moving the object towards a box. There were three stimulus conditions. In the short delay condition, the object was hidden in the box out of sight for 3 seconds. In the 6 second delay condition, the object was hidden for 6 seconds and in the control condition the object was placed on top of the closed box for 6 seconds and thus remained in sight throughout the trial. After the

respective delays the object was taken out or picked up from the box, lifted up and placed next to the box in its original position again. Four stimuli of each condition were presented, which were interspersed with a range of still images for blocks of 10 seconds. The presentation block began and ended with a baseline of still images, which consisted of pictures of animals and landscapes. While a Gambian and a British actor were filmed for videos shown to the infants at the respective sites, still images were chosen that were relevant to infants from either cohort. The stimulus presentation is illustrated in Figure 5.4.

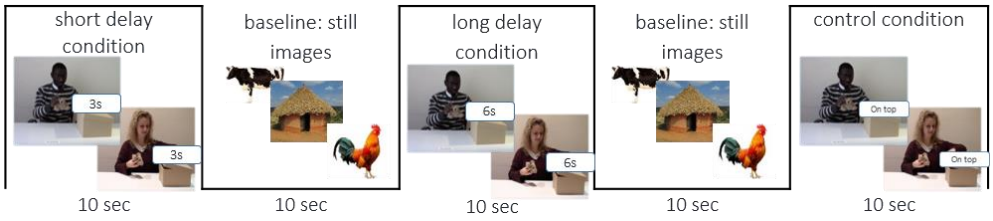


Figure 5.4. Schematic of fNIRS stimulus presentation. Video stimuli were presented showing an actor lifting an object and then moving it towards the box. The object was then either hidden in the box for 3 seconds, for 6 seconds or placed in sight on top of the box (control condition). Videos differed between sites to show a British and a Gambian actor. Video stimuli were interspersed with a baseline of alternating still images (3 images per 10s baseline).

5.2.3 Apparatus & Procedure. Infants sat on their parents' laps approximately 80cm from a screen on which the stimuli were presented. The study was presented as part of a larger fNIRS battery, and was presented second after a task consisting of social and non-social stimuli that was presented for 6 minutes. Sound levels were set so that the vocalisations were presented at a mean of 60db SPL. When infants looked away from the screen, additional sounds were played at the discretion of the experimenter to attract their attention back to the screen. Whenever infants became too fussy to redirect their attention, the study was briefly paused and one of the researchers spoke to the infant to calm them down. Depending on the parents' recommendation, a break was taken or a snack was offered, in some cases the study was stopped early. The study set up is illustrated in Figure 5.5.

Figure 5.5. Study set up for fNIRS WM study with 8 month old infant in the UK.

Data was recorded using the Gowerlabs Near Infrared Topography System (NTS, Everdell, 2005). A silicone headband holding two arrays with 12 source and 14 detector fibres was positioned on the infant's head. The optodes were positioned in the same layout across all of the fNIRS studies in this project, which is illustrated in Figure 5.6.

Figure 5.6. Reproduced from Lloyd-Fox et al. (in press). Optode placement over either of the temporal and the lateral frontal cortices.

Each fibre was clipped into a plastic washer within the arrays arranged at a source detector separation of 2cm. Headgear was aligned so that the same sources lined up with the preauricular point over each hemisphere across infants. To account for different head sizes, the optode positions could be adjusted in the headband with some flexibility, so that across infants and ages the same sources were aligned with the anatomical landmarks of the infants' head. Sources were set to 80% of the maximum light intensity in Cambridge and to 100% in Keneba to account for darker skin and hair. Fibres were bundled together and rested on the parent's shoulder in a loose loop to accommodate a degree of movement by the infant. To ensure that the fibres would not be pushed and displaced by the infant leaning back, a rolled towel was put on the parent's lap and the infant sat in front of it to provide some additional distance.

5.2.4 Pre-processing and analysis. Light attenuation was measured between each adjacent source-detector pair, giving an indication of the degree of absorption that occurred in the area between each source and detector, or channel. The degree of light attenuation was then converted into changes in the concentration of (HbO_2) and deoxy-haemoglobin (HHb) in µMol which served as a measure of the haemodynamic response associated with neural activation. Data was analysed using Matlab based programs (Matlab 2015b,

The Mathworks, Inc, 2015) generated by Drs. Anna Blasi and Sarah Lloyd-Fox which were customised specifically for analysis of data collected in this project. Pre-processing parameters were aligned with those of previous work by this group to be able to compare findings across studies. Further, parameters were as closely aligned with those used in two pilot studies using this task (Begus et al., 2016, Kischkel et al., 2016) to be able to compare across findings from this longitudinal study to the two previous cross-sectional ones. Data was first checked on the basis of signal quality of the light intensity measures of each individual participant. Data was excluded based on high variance in the signal as defined by an inflated coefficient of variation, excessive drifts in the data (indicating movement of the headgear relative to the head) and interference caused by the simultaneously used eye tracker in the frequency domain.

Data rejection. Prior to pre-processing, datasets were excluded based on the placement of the headgear relative to anatomical landmarks of the head to ensure channels covered similar areas of underlying cortex across infants. This was done according to the procedure described in Blasi et al., (2014), in which two lines are fit across the preauricular point of each hemisphere. Alignment of the relevant optode with this point is then checked, and the dataset is excluded whenever divergence exceeds a threshold of 10 mm. Data was further discarded based on infants attendance to the screen during each trial. Whereas for other studies, a criterion is typically set based on the entire trial (i.e. >60% attendance for inclusion), the sequential order of steps in the current study led to a modification, where infants had to attend >50% of each segment of the initial object presentation, the hiding and the subsequent uncovering process. To code infants' attention, video data synchronised to the stimulus presentation was coded by an automated program developed by Drs Luke Mason and Anna Blasi as part of the BRIGHT project. Trials were discarded if infants attended <50% on one of the

segments, datasets were discarded if less than three valid trials per condition remained. The remaining data was analysed based on signal quality. Attenuation measurements from each channel were assessed and channels that were outlying with regard to the light intensity detected were discarded. Data rejection and retention rates are displayed in Figure 5.7, additional detail on valid channels and trials can be found in Table 5.2.

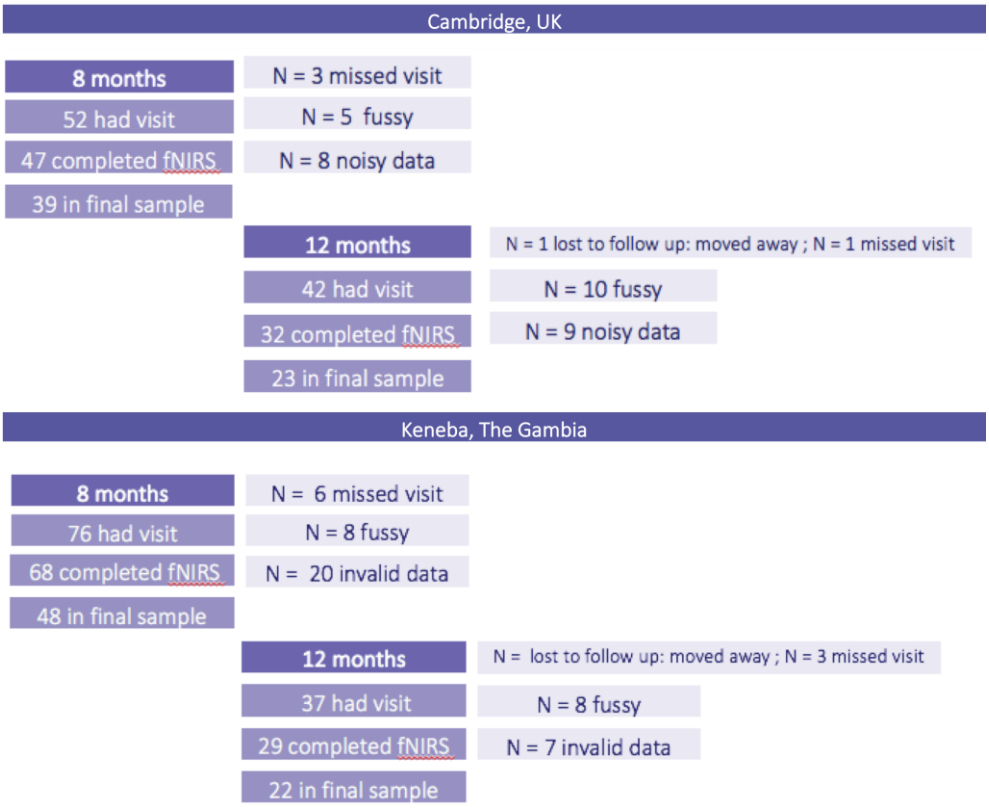


Figure 5.7. Numbers rejected and retained per site and age point.

Table 5.2. *Data quality measures. Proportion of datasets retained after checking for cap fit and looking time, as well as number of valid trials per group.*

Site	Age (months)	Valid channels	Mean number of valid trials per infant and		
			Control	3 sec delay	6 sec delay
		$\bar{X} \pm SD$	$\bar{X}$	$\bar{X}$	$\bar{X}$
Cambridge	8	30.24 ± 3.41	2.3	2.8	2.1
	12	28.26 ± 2.56	2.6	2.7	3.2
Keneba	8	29.43 ± 3.62	2.8	2.6	2.1
	12	28.79 ± 3.4	3.2	2.4	2.9

The intensity data were then low pass filtered at 1.8Hz and detrended by linear fit between the beginning and the end of the session. Following this pre-processing on the intensity data, it was converted into concentration changes of HbO_2 and HHb in µMol using the modified Beer-Lambert law (Delpy et al., 1988), with a differential pathlength factor of 5.13, which is conventionally used in infant research (Duncan et al., 1995). After conversion, trials that showed either a µMol change greater than 3.5 during the baseline phase or 8 during the task phase were discarded, as near-zero values are expected during the baseline period, and a change of 8 µM is extremely unlikely to occur as a result of a true physiological change. Trials were then averaged for each channel across conditions and participants,

yielding one averaged time course of HbO_2 and HHb per channel and condition.

5.2.5 Statistical analysis. Conventionally, both peak and mean amplitudes are used in fNIRS research (Wilcox & Biondi, 2015). Contrary to ERP analyses, peak amplitudes provide a good measure for the haemodynamic response for two reasons: 1) the haemodynamic response is less well temporally resolved and due to the limited literature available it is difficult to pre-determine time windows over which to average that are not purely data driven; 2) high frequency contamination is frequently a problem in ERP research but is less of an issue in the haemodynamic response, particularly after the frequency correction implemented as part of the pre-processing pipeline implemented in this project.

Peak amplitudes were analysed for each channel in a time window of 6-10 seconds after the object was either hidden or placed on top of the box. This was equivalent to a time window of 10-14 seconds post stimulus onset. By time locking responses to this event rather than the stimulus onset, activity was anticipated to be more specific to the experimental manipulation than the visual properties of the stimuli. The time window was chosen based on prior studies in infant populations evidencing haemodynamic response changes from around 6 second post stimulus onset. Results were corrected for multiple comparisons using the false discovery rate (FDR) correction (Benjamini & Hochberg, 1995).

Peak amplitudes were then analysed in three consecutive steps. First, a channel-wise analysis was conducted in which peak oxyhaemoglobin changes were compared against baseline activation within the 4 seconds prior to stimulus onset for each channel, correcting for multiple comparisons using the FDR method (Benjamini & Hochberg, 1995).

Secondly, condition contrasts were examined, to elucidate condition specific activation patterns. Thirdly, based on the results from the channel- wise and condition contrast analyses, regions of interest (ROI's) were defined, averages across which were entered into a repeated measured mixed effects model examining the effects of the within factors Cluster of ROI (anterior / posterior), Condition (Control / 3 seconds / 6 seconds) and the between factors Age (8 months / 12 months) and Site (Cambridge / Keneba). Note that despite the longitudinal nature of this data at the point of analysis for this thesis too few datasets had been collected at both age points, leading to the factor Age to be treated as a between factor. Future analyses should appropriately model Age as a repeated measure.

5.3 Results

5.3.1 Channel wise analysis. In a first instance, oxyhaemoglobin changes in a time window of 6-10 seconds post object hiding (thus 10-14 seconds post stimulus onset) were compared per channel against baseline via FDR corrected t-tests. Results are displayed in Figure 5.7.

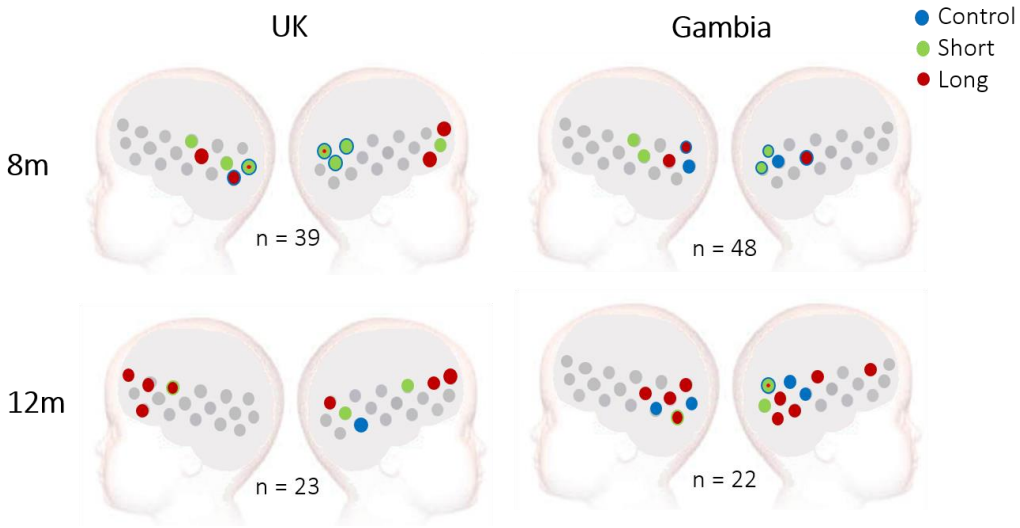


Figure 5.8.. Results of channel wise analysis. Highlighted channels showed significant oxyhaemoglobin changes within 6-10 seconds post object hiding or placement on the box.

Peak changes were compared to activation within the 4 second baseline prior to stimulus onset. Significant changes are displayed separately for the control- (blue), 3 second delay- (green) and 6 second delay- (red) conditions.

Results from the channel wise analysis indicate a few important trends. First, activation over the frontal portion of the array was only observed in the experimental conditions but not the control condition. Activation over areas covering the posterior temporal areas however occurred in both the control and experimental conditions and therefore appeared less specific to task demands. Secondly, there was a developmental change towards stronger activation at 12 compared to 8 months over frontal areas in both experimental conditions, particularly the 6 second delay condition. At the 8 month age point, some frontally located channels showed significant activation in the Cambridge cohort only, and this activation becomes much stronger at the 12 month age point. In the Keneba cohort, none of the frontally localised channels show significant response changes at the 8 month age point, and only one channel shows a significant change at the 12 month age point. Further, a larger number of channels over posterior regions showed activation in the Keneba cohort. All of these initial findings will be further explored in the following analyses.

5.3.2 Condition contrasts. To further understand differential patterns of activation between conditions, condition contrasts were examined. Hereby, all channels evidencing a significant change from baseline for the 6 second condition in the channel-wise analysis were examined further. The contrast with the control condition hereby provided evidence of activation related to an object being out of sight vs in sight for the same amount of time. The contrast with the 3 second delay provided evidence for activation related to an increased vs a shorter duration of occlusion. Oxyhaemoglobin changes

averaged over all trials of one condition were compared to one another per channel and infant via paired t-tests. As only channels found to be active in the above analysis were considered and these had been found to be significant after correction for multiple comparisons, no further correction was applied in the condition contrast analysis. Results are displayed in Figure 5.8.

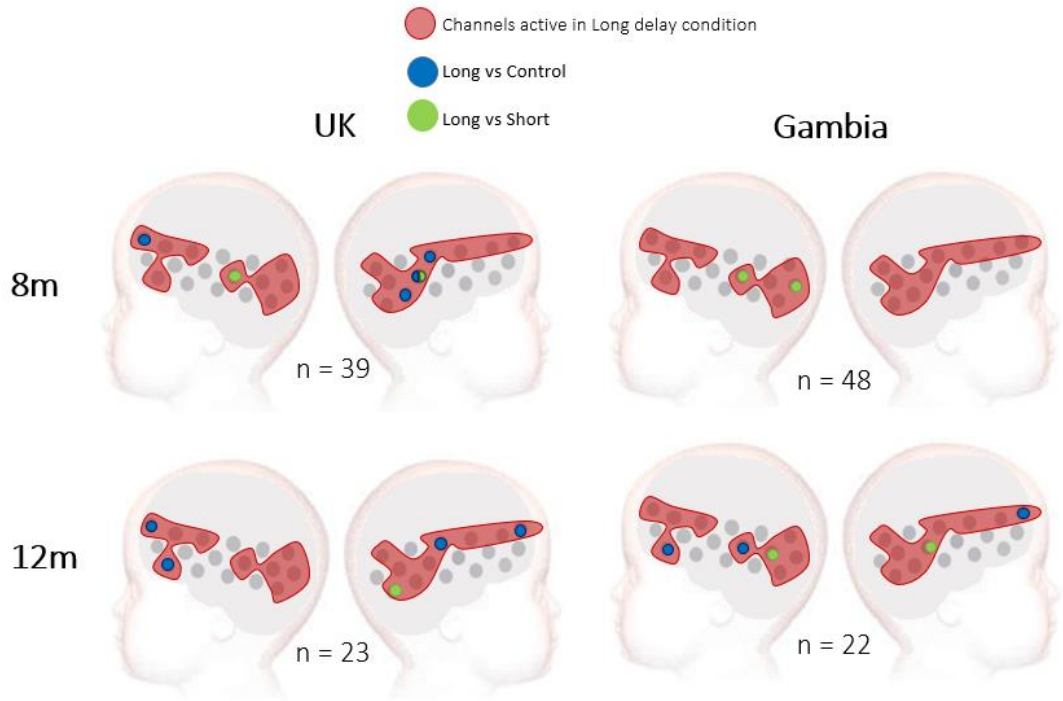


Figure 5.9. Results from condition contrast analysis. Highlighted are channels in which activity in the 6 second delay condition was greater than that in the control (blue) and 3 second delay condition (green). Only channels that showed significant changes in for the 6 second delay condition in the channel wise analysis were considered (highlighted in red).

The examination of condition contrasts adds to the channel-wise analysis in several key ways. First, it highlights thatdifferences regarding the magnitude of activation between the 6 second and the control condition can be

observed both over posterior temporal areas, where a greater number of channels is active in the 6 second delay than in the control condition. Secondly, activation over several of the frontal channels was found to be greater thanin the control condition. This further suggests activation in this area to be related to WM demands. With regard to the 6 second – 3 second delay contrast, differential activation patterns were only observed in posterior channels. In summary, results suggest recruitment of broader areas of cortex for the 6 second than for both other conditions, and suggest that activity over frontal areas is related to an increased retention delay.

As both the channel –wise and the condition contrast analysis suggested differential patterns of activation between posterior and anterior channel clusters, it was decided to examine this further in an ROI based analysis.

5.3.3 Region of interest (ROI) analysis. Two ROI's were defined, one covering a subset of channels over an anterior and the other over posterior parts of the array. These ROI's approximately mapped on to the lateral prefrontal and posterior temporal cortices, respectively. This mapping process was guided by prior work in which arrays were co-registered to MRI scans, in order to understand the cortical origins of activation observed in the channel space (Lloyd-Fox et al., 2014)

The approximate regions in which clusters were defined was guided by the channel-wise analysis. Channels were however also included if they had not shown a significant response change but were localised in the approximate anatomical regions that were to be assessed. Homologue regions were fit over each hemisphere, as no lateralisation effects were anticipated prior to analysis. ROI's are displayed in Figure 5.9. Descriptive statistics of the oxyhaemoglobin changes within these Clusters per Condition, Age point and Site can be found in Table 5.3.

Figure 5.10. Regions of interest (ROI's) fit to examine differences across an anterior (purple) and a posterior (yellow) located channel cluster.

Table 5.3 *Descriptive statics of peak oxyhaemoglobin changes in anterior and posterior ROI for Cambridge and Keneba at 8 and 12 months and.*

		8 month		12 month	
		anterior cluster	posterior cluster	anterior cluster	posterior cluster
		$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD
Cambridge	Control	.0891 ± .709	.0836 ± .986	.0607 ± .731	.0346 ± 1.09
	3 second delay	.249 ± .736	.2271 ± .906	.1323 ± .714	.0655 ± 1.08
	6 second delay	.1771 ± .788	.959 ± .113	.2528 ± .783	.0522 ± .72
Keneba	Control	.0168 ± .686	.2789 ± 1.06	-.2319 ± .719	.0612 ± 1.02
	3 second delay	-.0632 ± .664	.3409 ± 1.07	.0395 ± .735	.3097 ± .574
	6 second delay	-.1708 ± .699	.1334 ± .929	.0606 ± .705	.2914 ± .871

Activation was then averaged across these channels. Averaged peak changes were examined in a repeated measures mixed effects model looking at the effects of the within factors Cluster of ROI (anterior / posterior), Condition (Control / 3 seconds/ 6 seconds) and the between factors Age (8 months / 12 months) and Site (Cambridge / Keneba).

Prior to fitting the model, assumptions of normality, homoscedasticity and sphericity were tested for the oxyhaemoglobin changes. It was found that all subgroups were approximately normally distributed, and homoscedastic as indicated by non-significant Leven's tests ($.493 < p < .766$). The assumption of sphericity was met for both the repeated factor of Cluster (Mauchly's W = .971, $p = .398$), Condition (Mauchly's W = .99, $p = .78$) and their interaction Cluster * Condition (Mauchly's W = .99, $p = .80$).

Results of the ROI analysis are shown in Table 5.4. As can be seen, three effects were found to be statistically significant. There was a significant main effect for Cluster, which when followed up in pairwise comparison was found to indicate greater activation in the posterior over the anterior cluster ($t_{230} = -2.895$, $p = .004$). This was in line with the hypothesis that activation in the posterior cluster would be common to all conditions at both age points, whereas activation in frontal channels was thought to emerge with age and only be present for the experimental conditions. Further, a main effect for Site was found, which when followed up showed an overall greater activation in the Keneba compared to the Cambridge cohort ($t_{203} = 4.269$, $p < .001$). Both main effects were qualified by the significant interaction effect of Cluster * Site, follow up analyses of which showed higher activation in the posterior cluster in the Keneba cohort ($t_{203} = 3.945$, $p < .001$), but higher frontal activation in the Cambridge cohort ($t_{203} = 2.211$, $p = 0.04$).

Table 5.4. *Analysis of variance for peak oxyhaemoglobin change with Cluster, Age, Condition and Site as independent variables.*

IV	*F*	*df*	*p*	η_p^2
Cluster	28.973	1,203	<.001*	.125
Condition	.076	1,203	.927	<.001
Age	.003	1,203	.956	<.001
Site	5.358	1,203	.022*	.026
Cluster * Condition	.293	1,203	.746	.001
Cluster * Age	.052	1,203	.820	<.001
Cluster * Site	4.746	1,203	.031*	.023
Condition * Age	2.052	1,203	.130	.010
Condition * Site	2.262	1,203	.105	.011
Age * Site	.761	1,203	.384	.004
Cluster * Condition * Age	.106	1,203	.900	.001
Cluster * Condition * Site	.129	1,203	.878	<.001
Cluster * Age * Site	.739	1,203	.391	.004
Condition * Age *Site	1.228	1,203	.294	.006
Cluster * Condition * Age * Site	.636	1,203	.529	.003

Note. IV = Independent variable, df = degrees of freedom, η_p^2 *= partial eta squared.*

In order to integrate results from the channel wise, condition contrast and cluster ROI based analysis, they will in the following be discussed in the context of the hypotheses previously formulated.

5.3.4 Experimental manipulation.

Hypothesis 1. In line with work documenting a relationship between FC activation and performance in WM tasks, activation over frontal channels was expected for both experimental conditions but not the control condition. Overall data was consistent with this analysis as evidenced by both the channel- wise and the condition contrast analysis. The channel-wise analysis showed activation over areas covering the FC that only occurred in the experimental conditions, whereas the condition contrast analysis showed that this activation indeed was more pronounced in the 6 second delay compared to any sub-threshold activation present in the control condition. No Cluster * Condition ($F_{1,203}$ = .293, p = .746) interaction was found in the ROI analysis though, indicating that the differential pattern of activation between conditions did not hold when directly compared statistically.

Hypothesis 2. It was further expected that activation over frontal channels would be stronger in the 6 second, compared to the 3 second delay condition due to increased retention duration. Again, this hypothesis also was partially supported by the data. In the channel-wise analysis, a greater number of channels in the frontal region were found to show significant response changes for the long than for the short condition. Further, responses were of a higher magnitude in the 6 second delay condition, and thus a subset of channels that had shown a significant response clearly showed greater activity when contrasted against both other conditions. However, the ROI analysis showed no significant Cluster * Condition interaction, which might be due to a relative dilution of the effect with only few single channels being active in a relatively large ROI cluster.

Hypothesis 3. The more posterior activation patterns were not expected to be related to WM demands but rather to be present in all three stimulus conditions. Results from the channel wise analysis were in line with this hypothesis, as activation in the posterior cluster was evident for all three conditions. In follow-up pairwise comparisons between conditions for activation in the posterior cluster only (Table 5.5), no significant differences between the control and both experimental conditions were found, further corroborating this finding.

Table 5.5. *Pairwise comparisons for factor Condition on activation within posterior ROI cluster.*

	Mean Difference	*p*
3 second delay - Control	.0392	.636
6 second delay -Control	.0749	.396
3 second delay – 6 second delay	.0199	.797

Note: P-values bonferroni corrected for multiple comparisons.

5.3.5 Developmental change

Hypothesis 4. It was expected that activation in frontally localised channels would increase between age points in both experimental conditions. This was partially supported by the data. As for the channel wise analysis, a greater number of channels showed significant activation in frontal areas at the 12 month compared to the 8 month age point. However neither the related Cluster * Age ($F_{1,203} = .052$, $p = .820$) nor the Cluster * Condition * Age ($F_{1,203} = .293$, $p = .746$) interaction effects were found to be significant.

Hypothesis 5. It was further expected that the increase of frontal activation would be stronger in the 6 second delay condition due to the longer retention interval posing higher WM demands. Some evidence for this hypothesis was provided in the channel wise analysis. Overall, a greater number of channels was found to be active within the frontal cluster in response to the 6 second delay condition, but only very few in response to the short delay condition. For a subset of channels, this activation was robust enough to not only show when comparing it against baseline, but also when contrasting it against the control condition. However, as mentioned in the context of the above hypotheses, this effect was not evident in the ROI analysis in form of a Cluster * Condition effect.

5.3.6 Differences between sites

Hypothesis 6. It was expected that frontal activation would be reduced in the Keneba compared to the Cambridge cohort. Additionally, the hypothesised developmental increase between age points was expected to be larger in Cambridge. Evidence was in line with this hypothesis, as indicated by the channel wise analysis in which fewer channels of the frontal part of the array showed significant oxyhaemoglobin increases. When assessed formally in the ROI analysis, a significant Cluster * Site interaction effect ($F_{1,203}$ = 4.746, p = .031) was found. Post hoc comparisons revealed that stronger activation was evident in the frontal cluster in Cambridge compared to Keneba (t_{203} = 2.211 , p = 0.04).

5.4 Discussion

The current study is the first to provide longitudinal evidence regarding the neural correlates of early WM development in both a high and a low resource setting. The study design draws from previous work which has helped establish that a) infants' behavioural development towards mental

representation of an object is related to increased haemodynamic response changes over prefrontal cortex (Baird et al., 2002) and that b) similar activation patterns could be elicited in passive video paradigms in infants both in the UK and in The Gambia (Begus et al., 2016, Kischkel et al., 2016). This study now provides evidence regarding the developmental change across two critical age points, and longitudinally follows two infant cohorts in parallel. By implementing and refining a previously established paradigm it was possible to add to observations made during the pilot studies. Findings demonstrate not only that this paradigm can be used to elucidate early neural correlates of WM development, but also that this tool holds potential for implementation in low and high risk populations, as is indicated by differential patterns of cortical activation.

5.4.1 Experimental manipulation. Previous findings were partially supported, in that differential patterns of activation were found between all stimulus conditions. All three conditions were associated with activation over posterior temporal areas of the cortex, whereas only the experimental conditions were found to elicit any activation over FC. FC activation was found to be most pronounced in the 6 second delay condition, which is in line with the expectation of it requiring the highest WM demands. The newly introduced control condition allowed for an investigation of non-WM related cortical activation. While quantitative differences were observed regarding activation over posterior temporal region, with the 6 second delay condition being associated with a larger number of active channels, it seems likely that the activation at least in part can be attributed to features distinguishing the video stimuli from the still images presented during the baseline phase, rather than with WM demands. Associations have previously been demonstrated between activation in similar regions in the context of processing object features (Wilcox, 2005, inferior temporal cortex), as well as in the context of biological motion processing (superior

temporal sulcus, Lloyd-Fox et al., 2011, Pelphrey et al., 2005). Since both of these processes are manipulated between stimuli and still image baselines some of the activation measured over posterior temporal areas can likely be attributed to them.

5.4.2 Developmental change. The anticipated developmental change of increased FC activation, particularly for the 6 second delay condition was partially supported. The channel wise and condition contrast analysis showed that indeed a larger number of channels activated at the 12 compared to the 8 month age point, but this effect did not hold when averaging across the entire ROI cluster of channels.

5.4.3 Differences between sites. With regard to differences between both cohorts, it was expected that FC activation at each time point, as well as the developmental change would be more pronounced in Cambridge than in Keneba. This was confirmed both by the channel wise and the ROI analyses. The data thus provide evidence for differential patterns of activation and developmental trends in the two cohorts investigated. While from the present data it is not possible to draw strong conclusions on longer-term developmental trajectories of the two cohorts, it will be crucial to relate this data to the upcoming two study time points at 18 and 24 months.

5.4.4 Implications for early WM development across the two sites. The current study provides valuable additional insight into the emergence of WM in the two cohorts under investigation. From the activation patterns observed over frontal regions we can deduce that in both cohorts there is a developmental trend towards increased WM capacity at 12 compared to 8 months in both cohorts. This activation as well as the developmental change however is much reduced in the Keneba cohort. This is surprising, particularly in context of previous findings on related passive paradigms, in

which behavioural differences based on looking time could be observed even in much younger infants (Baillargeon, 1986). While only elucidating one WM subdomain from Baddeley's (2010) model, the present study shows clear differences between the two cohorts with regard to visuospatial WM. The way in which WM was assessed taps one of the most basic forms of abstract thought, and the ability to represent previously encountered sensory input is an essential building block of higher order cognition. However, as of now we do not know whether these differences are predictive of any future WM deficit within the Keneba cohort.

5.4.5 Limitations. The current findings must be regarded in light of several limiting factors. The three analyses presented here offer increasingly conservative insights into patterns observed in the data. Several hypotheses were supported by the channel wise analyses but only few effects held within more stringent examination within the ROI based analysis stream. This latter analysis can be deemed the most conservative of the three analysis streams, and it therefore is not surprising that only the strongest effects were picked up by it. Due to the theoretical underpinnings however, this approach will be pursued further when extending the analysis to the entire dataset and further age points, as it is likely to capture meaningful developmental trends. As no statistical models were implemented in either of the pilot phases, it might be considered to further refine the model fit. Especially hypotheses regarding activation within the anterior ROI were frequently not supported. This is not surprising given that fewer channels in were significant in this ROI and through averaging across a large cluster bilaterally. While it was initially planned to address this dilution of activation by considering one cluster per hemisphere, statistical power in this subset analysis was not high enough for the additional factor of Hemisphere (left/ right) to enter the model. While ROI based analyses can be useful in the context of fNIRS data to extract strong effects, they do reduce spatial

accuracy and therefore one of the main advantages of the method over other functional methods such as EEG. For future analyses, methods will be explored that allow for analysis on a channel by channel level of resolution, however taking into account the spatial alignment of adjacent channels and therefore being sensitive to changes within larger cortical regions.

Secondly, it needs to be acknowledged that the current paradigm taps processes dependent on early experience of infant-caregiver interaction. Whereas infants were not required to perform active object search in this study, their mental representation of the object is closely linked to an anticipatory response of the objects' reappearance. Although infants in the UK can be assumed to have frequently been exposed to games that involve this very process, less is known about prevalence of these games the in the Gambian cohort. Contextual information is being collected as part of this project: it could be investigated further whether games relevant to this study are being played during our parent-infant interaction studies or even whether differences in their frequency allows for a stratified analysis of the corresponding neuroimaging data.

Validity of Site and Age comparison. Some non-uniform changes between the two age groups and sites need to be considered when interpreting results presented in this chapter. One important issue with regard to spatially resolved measures such as fNIRS is how it can be generalised across different head sizes. In order to cover the same anatomical landmarks, arrays were positioned slightly differently at the two age points. The choice of where the headgear was placed was guided by the infants' head circumference. This allowed to counteract the effects of differential head growth trajectories across the two sites to a degree, however still may lead to subtle differences in the spatial precision of measurements, given greater variance in the Gambian compared to the UK cohort. Relatedly, an ongoing

issue within the field of fNIRS research is the optimisation of the separation between optical sources and detectors. While for infant populations the current approach is to use fixed distances for each pair, and thus a uniform penetration depth of tissue across the cortex, it needs to be considered how physiological changes (i.e. skull thickness) may unfold differently in different populations, both typically and atypically developing, as this may result in distorted estimates of cortical activation and maturation. One final issue to be mentioned here is that fNIRS relies on measurements of oxygen saturation of the haemoglobin compound in the blood. In cases where participants may differ with regard to their haemoglobin status, for example due to anaemia, it needs to be considered in how far cohort differences might be attributable to this issue.

5.4.6 Future directions. In the context of the limitations laid out above the following suggestions are being made for further analysis of this dataset. First, newly incoming data should independently be modelled as described here, in order to assess replicability of the current findings. Secondly, the model should be refined further once sample size and power are sufficient, and should model ROI's in more detail, by assessing interhemispheric differences, as well as further developmental changes through inclusion of follow ups from the 18 and 24 month time points. Lastly, it will be crucial for the current data to be interlinked with other datasets collected in the same infants. While this is recommended for all studies in this thesis, it is of particular importance to this study in particular, as comparison of several paradigms within the fNIRS modality can help identify general developmental trends.

Chapter 6. Deferred Imitation of Novel Action Sequences

Study 3: Behavioural Study at 8 and 12 months

6.1 Introduction

A significant proportion of human learning is facilitated through imitation. Facing a novel situation, for example while travelling in a foreign country, adults will observe others and model their actions accordingly in order to behave appropriately. In school, children are routinely presented with situations in which copying a particular behaviour leads them to learn novel things. As social animals, humans are excellently adapted for observational learning (Bandura, 1969). Of course, imitation is also well-documented in our closest relatives. Chimpanzees have been shown to use tools and obtain food through watching and imitating others (Goodall, 1986; Itani & Nishimura, 1973). By imitating an individual who is proficient in the task at hand, others can vastly reduce the time and effort than would be necessary to achieve the same result through trial and error learning.

While imitation plays a significant role throughout the entire life span, it bears particular importance during infancy. Presented with a novel action, young macaques have been shown to be much more likely to subsequently reproduce the behaviour than older ones (Kawai, 1965). Similarly, infant chimpanzees presented with a task involving the use of tiles representing words became proficient much more quickly when observing their mothers perform the task, than did older individuals (Rumbaugh & Savage-Rumbaugh, 1994).

Experimentally, the natural tendency to imitate behaviours can be utilised to draw conclusions not only about infants' abilities to imitate, but also their abilities to mentally represent an action without immediately copying it. In contrast to elicited imitation paradigms in which infants are modelled an action and then encouraged to immediately copy the behaviour, deferred imitation paradigms introduce a delay in between the two phases, making

the paradigm more reliant on retention over a time delay. The deferred imitation paradigm therefore provides a window into infants' early memory abilities as will be laid out below.

6.1.1. Imitation and memory.

One of the first researchers to attribute importance to imitation in light of memory development was Piaget (1952, 1962), who argued for deferred imitation to be an indicator of the development of symbolic thought. As for experimental paradigms, he argued that they relied on an infant observing an action, mentally representing it, and later enacting it, rather than on a memory trace of a prior attempt to actually perform the action. For this reason, the ecological validity of deferred imitation tasks is considered to be comparatively high, as infants frequently passively observe others perform actions, which are enacted at a later time. As deferred imitation is not reliant on motor practice and its retention, but rather on an abstract mental representation of said action, it has been argued to tap explicit (knowledge that can be brought to consciousness, such as experiences and facts), rather than implicit (memory for skills, motor actions) memory. Some evidence in support of this view stems from studies in patients suffering from amnesia as a consequence of damage to the MTL (McDonough et al., 1995). The studied patients, who were also strongly impaired in their explicit (but not implicit) memory functioning, were found to show a strongly reduced performance in deferred imitation tasks. A control group of patients with damage in regions not associated with explicit memory functioning and without behavioural impairments in this domain did not show deficits on the deferred imitation task they were presented with. Not only does this study illustrate co-occurrence of explicit memory capacity and deferred imitation skills, but also gives in an indication of the underlying neural structures, which will be discussed in the following.

6.1.2. Neural basis of deferred imitation behaviours. Deferred imitation paradigms have been argued to tap explicit memory, and as such have been associated with brain areas known to be involved in explicit memory functioning, such as MTL structures (de Haan et al., 2006). In their study of amnesic patients on the deferred imitation paradigm, McDonough et al. (1995) assessed healthy controls, patients with focal frontal damage and patients suffering from amnesia. Amnesia was either due to Korsakoff's syndrome, which is known to affect projections within the MTL, or due to specific hippocampal damage. It was found that while control subjects and frontal lobe damaged patients who were shown a sequence could imitate it after a 24 hour delay, this ability was much reduced in the amnesic patients. Both frontal cortex and MTL lesions were anticipated to negatively impact performance, in terms of either sequencing the required steps (hypothesised to be frontal lobe dependent), or remembering the actions (hypothesised to involve the MTL). It was however demonstrated that only lesions to the hippocampus and its projections resulted in impairments, highlighting these structure's importance on deferred imitation paradigms. Convergent evidence stems from a study of patients with developmental amnesia, associated with bilateral hippocampal volume reduction with an early developmental onset (Adlam et al., 2005). While these patients showed similar baseline performance compared to controls, their ability to imitate action sequences after a delay was reduced. These studies demonstrate a relationship between impairments in MTL structures and performance on deferred imitation tasks.

From a developmental viewpoint, maturation of structures such as the MTL has been argued to map onto performance on deferred imitation tasks during infancy. Initially, maturation of the MTL was assumed to progress in a step-like, rather than a gradual, manner with maturational change at around 9 months of age rendering infants much better able to perform MTL

dependent tasks (Nelson, 1995). However, a closer examination of the gradual behavioural development on imitation paradigms is not entirely consistent with such a view. Findings from both humans and primates are more in line with an ongoing change across infant and child development. As reviewed in Jones and Herbert (2006), the cytoarchitecture of the human hippocampus is mature from the 24-25th week of gestation (Seress, 2001). Structures mediating the input to the hippocampus such as the dentate gyrus however, develop in a more protracted manner and are not fully developed until 4-5 years of age (Eckenhoff & Rakic, 1991). In reviewing the neural basis of explicit memory during infancy Bauer (2004) concluded that the MTL areas involved in this function reached maturity between 2 and 6 months of infant age, with more refined development thereafter. Bachevalier and Vargha-Khadem (2005) come to a similar conclusion in light of primate development. They propose that even though structures involved in explicit memory functioning such as the hippocampus and its projections are present at birth, modifications in the connections from and to these structures mature gradually for a prolonged period of time. This more maturational viewpoint maps onto the developmental changes observed behaviourally in the deferred imitation paradigm during infancy.

Some research has suggested that the MTL structures implicated in deferred imitation might develop at different rates (Lavenex & Lavenex, 2013), and also possibly undergo a functional change between infancy and adulthood (de Haan et al., 2006). As reviewed in Chapter 1, it has been shown in animal models that MTL structures such as the perirhinal cortex support recognition based memory processes with developmental changes in the hippocampus being associated with the emergence of more complex recollection based memory (Tulving, 1985). While this has not formally been tested in infants, due to obvious constraints in structural assessment, these findings provide evidence for a potential functional shift with

parahippocampal tissue supporting early infant memory and gradually becoming more reliant on the maturing hippocampus.

6.1.3. Development of deferred imitation abilities during infancy. Due to the non-verbal nature of the task, deferred imitation paradigms lend themselves to the study of pre-verbal infants. As an indicator of early memory development, deferred imitation paradigms have been used extensively to elucidate the developmental trajectories of memory and observational learning over the first years of life. With the basis of their later behavioural repertoire vastly expanding during the first months and years of life and their concurrent development of a motor repertoire, infants are particularly attuned to imitate novel actions. In fact, infants have been estimated to acquire approximately 1-2 novel actions per day (Barr & Hayne, 1996). Piaget (1962) argued that infants start to show deferred imitation with a rather sudden onset at between 18-24 months. Other studies have however show that infants are able to imitate starting at a much earlier age (Meltzoff, 1985, 1988), as well as a gradual improvement rather than a sudden onset of the ability. Even 6 week old infants have been shown to imitate facial expressions (Meltzoff & Moore, 1994). While the very limited behavioural repertoire of 6 week old infants limits the assessment of object based actions, the use of facial expressions in imitation studies in older infants has been criticised based on their high baseline occurrence (Byrne & Russon, 1998). Further, it can be argued that the innateness of some facial expressions, such a smiles that are observed even in congenitally blind infants (Freedman, 1964), make them a suboptimal tool to study imitation behaviours. Further, base rates of infants' facial expressions can be influenced by caregiver responsiveness and therefore confounded when studying samples that differ in that regard. In infants between 6-18 months, the salience to imitate gestures has been reported to decrease in favour of actions in relations to objects (Abravanel et al., 1976).

Object based imitation has been demonstrated to occur in infants as young as 6 – 9 months, who were able to imitate an action after a 24h delay (Barr et al. 1996; Collie & Hayne, 1999). The notion that even the youngest infants show some forms of imitation behaviour rests on the assumption that actions demonstrated and imitated are much simplified in these young age groups. Increasingly sophisticated actions can be examined in older infants. Some of the parameters along which paradigms have been frequently manipulated in order to examine the extent of infants' imitation abilities is reviewed in the following.

Contextual and object feature changes. Two important factors shown to affect imitation behaviour are changes to the object used to demonstrate the target action (i.e. by using a differently coloured object in the test phase), as well as changes to the testing environment which are irrelevant to the task. Such contextual changes, even though not task related, have been shown to bear relevance when eliciting imitation behaviours both in chimpanzees and human infants (Bjorklund & Bering, 2003; Barr et al., 1996). At 6 months of age, human infants' imitation behaviour has been shown to be reduced when a change in the presentation context had occurred (Hayne, 2000). This rigidity is typical for younger infants and gradually is replaced by a change towards higher representational flexibility as infants develop. It has been demonstrated that 6 month old infants can still successfully imitate when presented within one room of their home and tested in another (Learmonth et al., 2004). The same infants were however shown to not be able to imitate an action in the lab, which they were shown in their home setting (Hayne, 2000). From around 12 months, infants become much better at transferring between contexts. Even with a long retention interval of one week, 12 month olds have been shown to be able to transfer an action from home to the lab setting (Klein & Meltzoff, 1999). It was further shown that 14 month old infants could generalise between

contexts, such as from the laboratory or their day-care setting their home setting (Meltzoff, 1993).

As for object feature changes, 6 month olds infants are able to imitate actions after a 24h delay when tested with the original object with no contextual changes in the testing environment (Barr & Hayne, 1996; Hayne et al., 1997). From around 12 months of age infants become much better at generalising across object feature changes and can still imitate if the colour of an object changed, but not if its form changed, whereas by 18 months they seem able to generalise across both of these dimensions (Hayne et al., 1997).

Enabling and arbitrary sequences. Another parameter of interest is the complexity of the action sequence, as well as the degree to which steps interlink towards an end state. A frequent distinction is hereby made between enabling and arbitrary sequences. In an enabling sequence, steps need to be performed in a specific order to reach a desired goal. Barr and Hayne (1996) provide the example of driving a car, whereby the door needs to be unlocked and the key turned before being able to drive. As for arbitrary sequences, they more resemble making a salad, as the outcome will not differ depending on the order in which ingredients are added. It has been demonstrated that both adults (Ratner, Smith & Dion, 1986) and children (Brown, 1975; Fivush, Kuebli & Clubb, 1992; Hudson & Nelson, 1983) make use of enabling relations between events to facilitate recall. In children, better performance on recall measures has been shown for events with enabling relations than with arbitrary ones (Fivush et al., 1992; Hudson & Nelson, 1983; Murachver et al., 1993). In studying 24 month old infants, Bauer et al. (1987, 1989, 1995) have shown that enabling sequences are more reliably imitated by participants both immediately after demonstration and following a delay. This effect was found to be

independent of number of steps in an action sequence, number of presentations, or length of delay. Barr and Hayne (1996) found that enabling sequences were much better retained within their sample of 18 month old infants than were arbitrary sequences. Sequence structure was found to be strongly linked with imitation after a delay, whereas introducing an immediate imitation condition in which infants could practice the behaviour right after demonstration, was not predictive of later outcome. Interestingly, even though several studies in infants find enabling sequences aid imitation after a delay, the same has not been found to be true for the developmental amnesia patients studied by Adlam et al. (2005). Regardless of whether action sequences were enabling or arbitrary, patients performed below the levels of control subject, indicating that logical sequential steps do not necessarily facilitate imitation in clinical, older populations.

Further studies have interpreted findings on enabling and arbitrary sequences as evidence for infants' abilities to infer an experimenter's intent. By adding components to a sequence that were irrelevant to a task outcome, it could be shown that even when participants performed these irrelevant actions, and thus over imitated, they tended to perform them out of sequence as they were not causally linked to an outcome. In an influential study of infants' imitation behaviour, Meltzoff (1988a) placed a light on a table in front of the experimenter. The light could be switched on by pushing down its surface. The experimenter demonstrated that it could be switched on by leaning over the table and pushing the light with one's forehead. A majority of 70% of 14 month olds infants was shown to copy this action after a 24h delay. Rather than emulate the desired outcome of switching on the light in any possible way, they imitated the exact action. In a follow up study, Gergely et al. (2002) presented infants with a similar set up. In one condition the experimenter had their hands within sight (hands-free condition), in the other, she signalled that she was cold and needed a

blanket, which covered her hands (hands occupied condition). A majority of infants would copy the unusual behaviour in the hands free condition (69%) but fewer would do so in the hands occupied condition (21%), indicating that their imitation was related to their evaluation of the experimenter's intent. This can be taken as evidence that enabling sequences do not only support infant memory, but that infants to some degree shape their imitation based on the sequential steps leading towards a desired outcome.

Sequential performance of actions. Participants' responses can further be regarded in terms of whether target actions are performed in sequence, or whether steps within a sequence are performed in any possible order. This concept is distinct to the notion of enabling and arbitrary sequences as in both type of sequences there can be instances where steps contributing towards the target action can be performed in an order different to the order that was demonstrated. This has for example been assessed in the aforementioned work on adult amnesic patients, where performance was scored according to the number of correct action pairs as well as overall sequential performance on the target action (McDonough et al., 1995). Performance of actions in order of the original sequence can be regarded as a more mature form of memory than recall of any action pair in isolation (de Haan et al., 2006). While this concept is an important consideration in studies with older infants, children and adults, it does not so much apply to many studies with young infants who only just show first signs of imitation behaviour, as many studies rely on single action items. In the present study, only few items were composed of more than a single step and sequential or non-sequential performance was therefore not evaluated.

Immediate imitation. As discussed, the majority of studies have found that immediate practice after demonstration did not aid infants' deferred imitation. What an immediate imitation condition does provide though, is a

measure of imitation behaviour with reduced memory demands, as the action needs to only be retained very briefly. In the current study we therefore introduced an immediate imitation condition for half of the presented items. Hereby, it was assessed whether imitation after only a minimal delay (immediate imitation) would be better than after a longer delay (deferred imitation).

Of course deferred imitation studies can be varied along other parameters, such as the delay time between the presentation and test phase (Barr & Hayne, 2000), number and kind of cues provided by the administrator (Simcock, Garrit & Barr, 2011), number of presentations of the action (Barr et al., 1996), familiarity of the demonstrator (Teiser et al., 2014), reward expectancy (Bandura, 1977) and complexity of the action sequence (Barr & Hayne, 1996). In summary, the presented studies suggest that some crucial parameters have to be considered when designing imitation paradigms for use in young infants. However, if manipulated correctly they also show that imitation can be elicited in infants in different ages and across different populations.

6.1.4. Deferred imitation in resource poor settings. Imitation behaviour is a universal means of learning and many studies have demonstrated commonalities across cultures. Similar developmental levels of spontaneous imitation have been observed between toddlers from the United States and those from Papua New Guinea (Eckerman & Whitehead, 1999), even though the imitative play they engaged in was found to be centred on themes relevant to their social environments. Imitation of task irrelevant features or overimitation has been shown to occur to similar degrees in both urban Australian and Kalahari bushman children (Nielsen & Tomaselli, 2010).

There is some controversy as to which cultural and sociodemographic factors foster or hinder imitation behaviours. While in western, industrialised countries infants frequently are surrounded by a smaller number of siblings and a distinction is frequently made between the infant-appropriate and the caregiver's environments (Keller, 2007a), infants in many non-western settings live in larger family structures and are surrounded by other children that can serve as role-models in acquiring the ability to perform novel actions (Rogoff, Mistry, Goncu & Mosier, 1993). On the other hand, great emphasis is placed in western settings on structured, dedicated learning opportunities such as play groups and nurseries, as well as dedicated, frequently dyadic face to face interaction. In non-western countries on the other hand, there often is less emphasis placed on specific instruction, however role models performing relevant actions are present for the majority of the time (Nsamenang & Lamb, 1993). Results from previous studies reflect these contradictory trends. Goertz et al. (2011) examined both middle-class German and Cameroonian infants from the Nso ethinc group, who were tested when they were 6 months old. They did not measure any differences in either baseline performance of the target action or imitation between the two ethnic groups at this age point. In some regards, it has been suggested that with infants growing older, differences begin to emerge. In a follow up study, Graf et al. (2014) demonstrated that the lack of difference in performance could be partially attributed to the young age of the infants and their relative lack of experience with objects. In their study, both 6 and 9 month old German and Cameroonian Nso infants were assessed. They found that baseline performance at 9 months was shown to differ significantly between the two cultures, with the German infants with relatively higher exposure to toy play performing more target actions in both the baseline and the imitation phase. Following on from this work, Teiser et al. (2014) examined whether age of the demonstrator (child

or adult) differed in the two cultures, the rationale being that in Nso culture more child- infant interaction is prevalent, whereas in German culture adult-infant interaction is more common. In their sample of 9 month old infants, they showed that all infants, regardless of cultural group imitated more with an adult than with a child model. German infants also performed the target action more frequently overall than the Nso infants. They further found differences between the two groups in the change between baseline and test phase which further interacted with age. German infants performed significantly higher numbers of target actions in the test compared to the baseline phase at both time points, while this was only true for the Nso infants at 9 months.

Several conclusions can be drawn from these studies. First, imitation can be elicited across cultures, regardless of whether toy play is the norm or the exception. Secondly, imitation abilities increase with age, and increase more between the baseline and test phase in older infants. We therefore predict that in our studies, (a) infants from Keneba and Cambridge will both show imitation behaviours, and (b) that more imitation behaviours will occur at the later age point.

The described studies in the German and Cameroonian populations put a great emphasis on imitation behaviour and how it is influenced by cultural factors, such as cultural appropriateness of the model's age, and do demonstrate interesting differences between age groups. Some open questions however remain as to the driving factors of the differences seen between the two cultural groups. In a related study, Graf et al. (2014) attribute the difference seen between the German and the Nso infants at 9 months primarily to familiarity to object play, which is highly likely to be one of the driving factors. This study will assess in how far imitation might be affected by differences such as the engagement with the objects infants are

presented with. Due to the differences in exposure to toy play between groups, differences in object engagement are expected to be observed. Further, in this study both an immediate imitation condition and a deferred imitation condition will be used in order to elucidate whether expected differences between assessment sites can in part be attributed to reduced imitation behaviour or reduced retention of the demonstrated action over the delay. As a consequence of higher exposure to toys, and possibly imitative play, in the Cambridge cohort, as well as possible neurodevelopmental differences between cohorts, it is predicted that infants in this group will perform the target actions more frequently, both in baseline and in the imitation conditions. It is further predicted that baseline corrected imitation scores will differ, with infants in Cambridge imitating more than infants in Keneba. Based on studies in Cameroon and Germany (Goertz et al., 2011, Graf et al., 2014, Teiser et al., 2014), we expect that the difference between Cambridge and Keneba will be larger for the deferred imitation than the immediate imitation condition. Lastly, as laid out in Chapter 1, neurodevelopment is hypothesised to be more variable in Keneba, due to the more diverse impact of environmental influences. This is anticipated to be reflected in all tasks presented in this thesis, and it expected to lead to a greater variance of scores within the Keneba compared to the Cambridge cohort.

6.1.5 Hypotheses. As will be described in more detail below, two different kinds of outcome measures will be considered: non-baseline corrected raw scores to assess performance of the target action in general, as well as baseline corrected scores, giving an indication of how much infants imitate, rather than spontaneously perform the target action. The following hypotheses will be tested in this study.

Experimental manipulation

1. More frequent performance of the target action (non-baseline corrected) will be observed in the imitation conditions, compared to the baseline condition.
2. Higher imitation scores (baseline corrected) will be expected in the immediate compared to the deferred condition, reflecting a shorter retention interval.
3. No difference will be found on deferred imitation (baseline corrected) scores on items which were preceded by immediate imitation and those that were not.

Developmental change

4. Baseline scores will not change between time points, as an indicator that difficulty of the items was age appropriate.
5. Imitation scores (baseline corrected) will be higher at 12 than at 8 months, with a larger developmental change in the Deferred than in the Immediate condition.

Differences between sites

6. Engagement (here defined as touching the object) with the novel items will be higher in Cambridge at both age points.
7. Performance of the target actions (non-baseline corrected) will be higher in the Cambridge.
8. Imitation scores (baseline corrected) overall will be higher in the Cambridge cohort as evidenced by higher immediate and deferred imitation scores at both age points.

9. The discrepancy between Cambridge and Keneba will be largest for scores in the deferred imitation condition, indicating an effect of memory for the action over the retention delay.
10. Higher variances are expected in the Keneba cohort compared to the Cambridge cohort regarding infants' baseline and imitation scores at both age points.

6.2 Method

6.2.1 Participants. This study was performed when infants were 8 and 12 months of age. Participant information can be found in Table 6.1.

Table 6.1. *Sample sizes and age in days for 8 and 12 month time points for participants completing the behavioural deferred imitation assessment.*

	8 months		12 months	
	N (girls)	Age in days X ± SD	N (girls)	Age in days X ± SD
Keneba	64 (34)	244.46 ± 9.87	54 (28)	382.60 ± 15.45
Cambridge	30 (18)	248.93 ± 10.57	32 (14)	369.71 ± 12.13

6.2.2 Stimuli & Design. While deferred imitation paradigms have been widely used in infant research, items used in this project needed to meet some specific criteria. First, previous studies have frequently examined infants aged 18 months and over, meaning that items were too motorically demanding for 8-12m old infants and had to be simplified. Secondly, studies using similar tasks in younger infants frequently used items requiring infant to push buttons or switch levers (Collie and Hayne, 1999). As infants in Cambridge were expected to be much more familiar with these types of actions than infants in Keneba, a different set of items was generated which were intended to be novel to infants in both settings. This was especially important because infants in Keneba do not usually play with toys.

As we were interested in memory functioning at 8 and 12 months, rather than retention of items from the first to the second time point, two sets of items were used in this study. They required similar actions to be performed, however items used at the 12 month time points were slightly more difficult compared to those used at 8 months to avoid floor or ceiling effects at either age point. The difficulty of items used at 8 and 12 months was expected to be adequate for each age point, and it was expected that baseline scores would not change between time points, reflecting that infants could not just easily infer the target from the object at either age. Lastly, items were designed so that they would require mostly fine and little gross motor movement, due to the fact that fNIRS data was recorded during this task at the Cambridge site. For this thesis however, only the behavioural data will be considered.

The criteria along which the items were chosen were therefore:

1. Items need to be in line with 8-12 month old infants' motor development.
2. Items need to be versatile enough so they could not be used for only one specific action.
3. Items should consist of novel objects not yet familiar to infants of these ages to make them as novel as possible for infants in both groups.
4. Item difficulty should be slightly different between age points to avoid ceiling and floor effects at either time point.
5. Gross motor movement should be minimised due to parallel recording of fNIRS data.

A range of items including all items in the final set were piloted with seven 8 month old and five 12 month old infants at CBCD Birkbeck. Items entered the final set if at least one of the infants could perform the associated action. Further, parents were asked whether their infants had previously been exposed to any of the items. One item (Xylophone) was replaced as a consequence of parents reporting previous use of this object. It was replaced by a wooden frog, which is used to elicit a similar action but was deemed to be visually distinct enough to appear novel. All items as well as the phrase used to narrate the action are summarised in Table 6.2. Scoresheets used at both age points can be found in Appendix 6.1 and 6.2.

Table 6.2. *Overview of items used at 8 (left) and 12 (right) months, including actions and phrases used to narrate the action during demonstration.*

	8m		12m
	Action: Push down light Narration: 'Look, I push it!'		Action: Push the stamp down and lift up. Narration: 'Look, I push it down and I lift it!'
	Action: Lift up ball to extend and pick up small ball that falls out Narration: 'Look, I lift it up, and I take the ball!'		Action: Stroke frog with stick Narration: 'Look, I stroke it!'
	Action: Turn around rain maker Narration: 'Look, I turn it around!'		Action: Turn around timer Narration: 'Look, I turn it around!'
	Action : Twist cabasa Narration: 'Look, I twist it!'		Action: Throw ball in cup Narration: 'Look, I throw it in!'
	Action: Lift up with both hands and pull apart Narration: 'Look, I pick it up, and I pull it!'		Action: Lift up with both hands and push together Narration: 'Look, I pick it up, and I push it!'
	Action: Lift bell using the magnet and shake Narrative: 'Look, I pick it up, and I shake it!'		Action: Put ball in box, close lid and shake Narrative: 'Look, I put the ball in, I put the lid on and I shake it!'

6.2.3 Procedure. In Cambridge, the deferred imitation task was administered as part of the fNIRS protocol. Infants sat on their parents' laps wearing the fNIRS headgear and a small table was placed in between the infant and the experimenter. In Keneba, the study was performed independently of the fNIRS study, with infants sat on their parents lap at a table opposite the experimenter. Initially, the experimenter played with the infant for a few seconds using a small ball to establish a back and forth between infant and experimenter. Infants were then presented with the first item which was placed in front of them by the experimenter, who encouraged them to explore it. This free play phase lasted for approximately 30 seconds, depending on the infant's temperament, with shy infants being given a little longer than very engaged ones. After this phase, the experimenter took the item back and placed it in front of themselves, out of the infant's reach. They then performed the action while narrating the steps (i.e. 'Look- I turn it around'). Each action was presented 3 times. The item was then either presented to the infant again for immediate imitation or put out of sight for delayed imitation. The same procedure was then performed for all further items. During each session, three of the six items were presented both immediately after presentation as well as after a delay, while the other three were presented only after the delay. Which half of the items was in the immediate or delay- only condition was counterbalanced across infants. The groups of items that were in the same condition were kept constant as items differed slightly with regard to difficulty and it was tried to avoid that all more difficult or all easier items were in the same condition. Subsequently, there was a 20 min delay. The delay was chosen for two main reasons. First, the initial aim was to concurrently record fNIRS data at both sites to in the future assess haemodynamic changes associated with the imitative response. For this reason the study needed to fit within the time constraints posed by the fNIRS protocol, as it was anticipated that beginning

a separate fNIRS session after a longer delay might have led to data loss due to infants' non-compliance to have the headgear placed twice. Similarly due to the design of the study it was not routinely possible to call back participants the following day therefore only enabling a delay within the assessment day. In previous literature, a wide range of delay time has been implemented, ranging between as short as 10 minutes (Goertz et al., 2011, Graf et al., 2014, Teiser et al., 2014) to several days (Barr et al., 2001) weeks (Meltzoff 1995) or even months (Herbert & Hayne 2000). Developmentally, delays over a few weeks or moths can be considered problematic, as results can be confounded by developmental changes occurring over the delay. While a delay of one or several days might have been more in line with previous literature, it was not possible to reliably arrange call backs for all participants. Theoretically, the shorter delay allowed us to control what infants were exposed to during the delay, which in context of the importance of contextual information of infant memory was useful in controlling their exposure to external stimuli.

In the UK, infants completed the remainder of the fNIRS protocol during this delay, in which they first listened to a habituation study with a repeating auditory stimulus and then watched an audio-visual stimulus of spoken nursery rhymes and moving toys. In Keneba, for the aforementioned reasons, the task was performed as part of the behavioural protocol and the demonstration and delayed study phases were separated by the parent child interaction study, during which infants played with their parent using a range of toys.

After the 20 min delay, infants were placed at the table again and in turn presented with each item. Similar to the free play phase, infants were presented with the item and then given approximately 30 seconds to perform the target action. A little longer time was given for items with

several steps (i.e. rattle). During the initial immediate imitation phase, it was avoided to excessively praise the infant, to avoid a change in performance due to increased motivation. While experimenter never helped the infant to initiate an action, they could help with items with increased motor demands (i.e. closing the lid of the rattle) if the infant's intent to perform the action was observed and they had unsuccessfully tried to close the lid. The procedure of this study is illustrated in Figure 6.1. The full standard operation procedure as provided to the testing team can be found in Appendix 6.3.

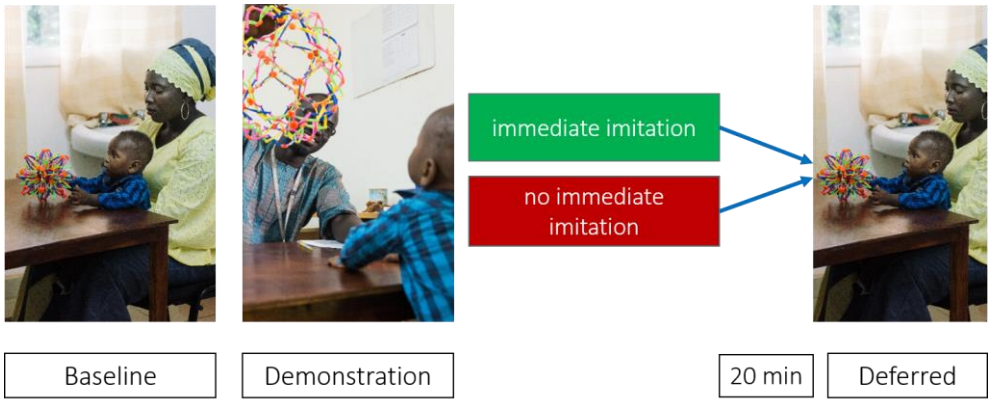


Figure 6.1. Procedure of deferred imitation paradigm.

6.2.4 Outcome variables and scoring. Scores were given based on number of steps of an action completed. Due to the implementation of this task in a relatively young age group, most actions had only one step and were scored as 0 (not completed) or 1 (action completed). For some items, two steps were scored (i.e. picked up object with one hand either side – 1, pulled object apart -2). As other studies have been able to use a more continuous measure of performance, for example by employing multi step sequences, it was initially tried to use a different scoring scheme. In this scheme, administrators had the option to also score an attempt. For items that were ultimately scored in a dichotomous fashion, this produced an additional

scoring option (0 – no interaction, 1- attempts, 2- completes). In reliability meetings however it was decided that this introduced too much inter-rater variability, which led to the change in scoring. Even though this scoring method places less emphasis on subtleties in infants' behaviour, inter-rater reliability was found to be high, which in a study with multiple sites and administrators was of crucial importance.

Inter-rater reliability was discussed during the initial phase of data collection for each age group to ensure accurate scoring criteria were developed. The free play phase served as a baseline. Immediate and Deferred imitation scores were analysed both as raw scores as well as baseline corrected difference scores. Scores in each condition were obtained by taking the sum of scores on all items. For the baseline corrected scores, the score on the baseline items was subtracted from the score on either imitation condition. All outcome variables can be found in Table 6.3. The different outcome variables were scored to address the aforementioned hypotheses. Analyses aimed at exploring occurrence of the target behaviour across conditions, the raw count data was used. As different conditions have different maximum numbers of scores that could be reached (i.e. immediate imitation has a lower maximum score than the total deferred imitation score, as only half the items entered the immediate imitation condition), these analyses were all offset corrected within the appropriate model for count data. For other analyses, proportions scores were calculated, by dividing each raw score by the maximum number possible in the corresponding condition. These proportions will be used in all hypotheses interested in baseline corrected, normally distributed scores.

Table 6.3. *Outcome variables of deferred imitation study.*

Variable label	Calculated as	Purpose	Maximum score	
			8m	12m
Engagement with object	Scored as 1 if infant touched the object, otherwise 0. Sum was obtained from Baseline, Immediate and deferred phase	To assess in how far infants engaged with the novel objects, as a control for object exploration that did not involve the target action.	15	15
Baseline	Sum of target actions performed in baseline phases prior to demonstration of target actions	To obtain an estimate of infants' spontaneous performance of target actions	9	11
Immediate imitation$_{RAW}$	Sum of immediate imitation scores across all items	To assess how often target actions were performed right after demonstration	5	5
Immediate imitation$_{COR}$	Sum of immediate imitation scores across all items, subtracting baseline scores	To assess how often target actions were performed right after demonstration controlling for baseline performance	5	5
Deferred imitation$_{RAW}$	Sum of deferred imitation scores across all items	To assess how often target actions were performed after a delay	9	11
Deferred imitation$_{COR}$	Sum of deferred imitation scores across all items, subtracting baseline scores	To assess how often target actions were performed after a delay controlling for baseline performance	9	11
Deferred without Immediate$_{RAW}$	Sum of deferred imitation scores of those items that were not tested in the immediate condition	To assess performance in deferred condition when there was no immediate practice after demonstration	5	5

Deferred without Immediate$_{COR}$	Sum of deferred imitation scores of those items that were not tested in the immediate condition, subtracting baseline scores	To assess performance in deferred condition when there was no immediate practice after demonstration, controlling for baseline performance	5	5
Deferred with Immediate$_{RAW}$	Sum of deferred imitation scores of those items that were tested in the immediate condition	To assess performance in deferred condition when there was immediate practice after demonstration	5	5
Deferred with Immediate$_{COR}$	Sum of deferred imitation scores of those items that were tested in the immediate condition, subtracting baseline scores	To assess performance in deferred condition when there was immediate practice after demonstration, controlling for baseline performance	5	5

Sessions were scored during administration by the experimenter. During the first sessions, an observer scored in addition to the administrating researcher and scores were discussed to ensure inter-rater reliability. To formally assess inter-rater reliability prior to data analysis, 20% of sessions were double scored from video. The inter class correlation coefficients (ICC) for each group can be found in Table 6.4.

Table 6.4. *Interclass correlation coefficients for inter-rater reliability between session administrator and second score from video.*

	8 months	12 months	total
Keneba	0.827	0.809	0.823
Cambridge	0.913	0.964	0.936
total	0.868	0.919	0.885

6.2.5 Data pre-processing and retention. Data were cleaned according to adherence to the delay time of 20 minutes, and with regard to a minimum number of items having been administered. The Criteria for exclusion thus were:

1. Infant became fussy early in the session, leading to < 3 items to be administered.
2. Delay time was either <15min or >45min.

Based on these exclusion criteria, high retention rates were achieved, as summarised in Figure 6.2.

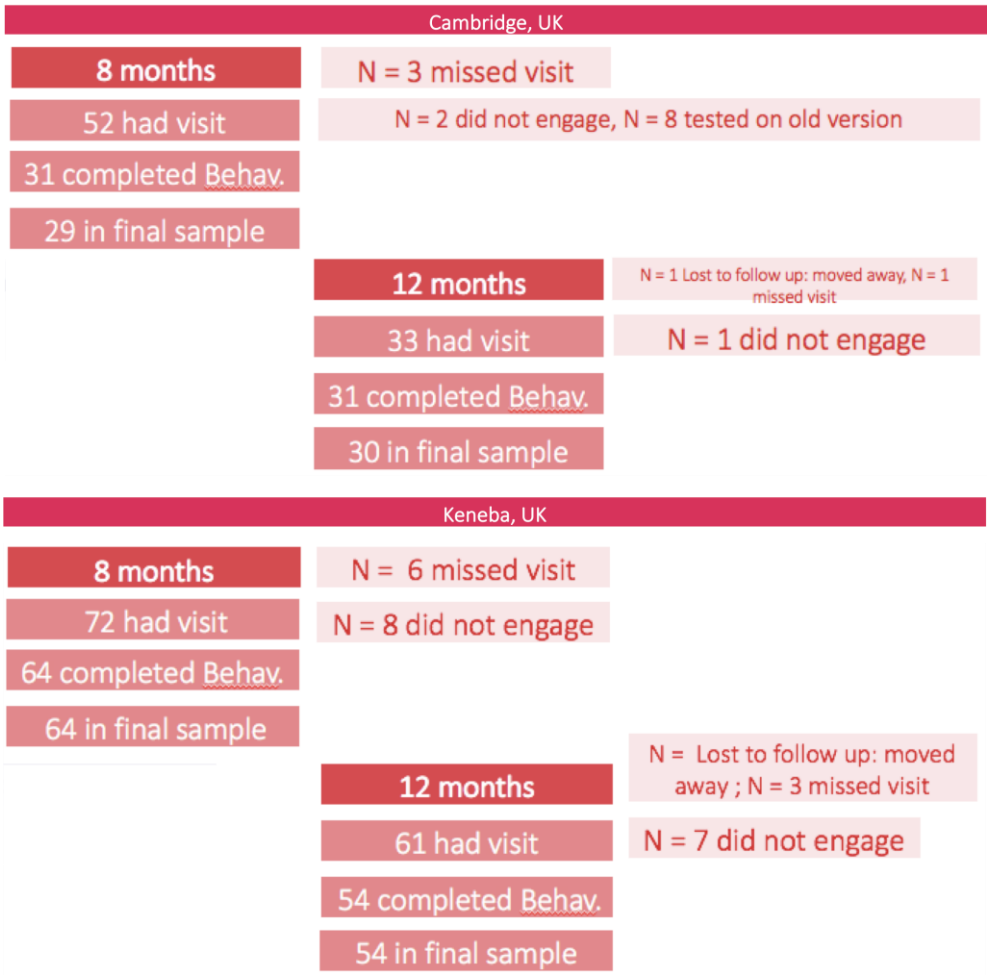


Figure 6.2. Numbers retained/rejected from samples per site and age point.

6.2.6. Repeated measures design. Even though this study was performed at 8 and then again at 12 months and therefore will ultimately yield repeated measures data, at the time of analysis for this thesis only 22% of infants were tested at both age points. This was due to protocol changes during the first implementation of the study, yielding early collected 8 month data sets to not enter this analysis. As at this time point primarily early 12 month datasets are available, only few participants who completed two time points are available. For this reason, all analyses will treat the two age points as cross-sectional samples. Ultimately, it is recommended to repeat the

described analyses on the final sample, using Age as a within subject rather than a between subject variable.

6.3 Results

Some descriptive statistics on the outcome variables per Site and Age point are displayed in Table 6.6. As will be laid out in the following section, raw scores were anticipated and found not to be normally distributed, and thus described in terms of their median and inter-quartile range (IQR). The baseline corrected proportion scores exclusively followed normal distributions and are therefore described in terms of their means and standard deviations.

Table 6.6. *Descriptive statistics of raw and corrected imitation scores per Site and Age point.*

		8 month			12 month		
		possible maximum score	raw data	baseline corrected proportions	possible maximum score	raw data	baseline corrected proportions
			$\mu_{1/2} \pm$ IQR	$\bar{X} \pm$ SD		$\mu_{1/2} \pm$ IQR	$\bar{X} \pm$ SD
Cambridge	Baseline score	9	2 ± 2.5	*N/A*	11	2 ± 3	*N/A*
	Immediate imitation score	5	1 ± 1.5	.0276 ± .274	5	2 ± 2	.2070 ± .156
	Deferred imitation score total	9	4 ± 3	.0641 ± .244	11	5 ± 4.25	.2091 ± .191
	Deferred imitation score with immediate imitation	5	2 ± 2	.0923 ± .242	5	3 ± 2.5	.2414 ± .309
	Deferred imitation without immediate imitation	5	1 ± 3	.0769 ± .325	5	2 ± 3	.2483 ± .254

Keneba	Baseline score	9	2 ± 2	*N/A*	11	2 ± 2	*N/A*
	Immediate imitation score	5	1 ± 1.75	-.0278 ± .219	5	1 ± 2	.0838 ± .192
	Deferred imitation score	9	1 ± 2	.0291 ± .269	11	2 ± 2	.0472± .147
	Deferred imitation score with immediate imitation	5	0 ± 1	-.0167 ± .228	5	2 ± .75	.0462 ± .212
	Deferred imitation without immediate imitation	5	1 ± 2	-.007 ± .249	5	1 ± 2	.0615 ± .222

Note. $\mu_{1/2 = \text{median value}}$, IQR = inter quartile range, $\bar{X}$ = mean, SD = standard deviation

6.3.1 Assumptions check. As count data are frequently positively skewed, it was anticipated that raw data would not follow a normal distribution. Rather, it was expected that the non-baseline corrected variables would follow a Poisson or a negative binomial distribution, as is typical for count data (Gardner at al., 1995). It was first assessed whether outcome variables were indeed positively skewed. This was found to be the case for all non-baseline corrected scores. The degree of skewness was subsequently formally assessed. As proposed by Kim (2013), skewness values were standardised (Z_{skew} = skew/SE $_{skew}$). For medium sample sizes (i.e. 50 < N < 300) like the one in this study, Kim (2013) proposes that those variables with a $Z_{skew} > 3.29$ should be regarded as significantly diverging from normality. Table 6.7 shows skewness for all variables. As can be seen, all variables based on raw scores, with exception of baseline scores, violate the assumption of normality, whereas all baseline corrected values comply with it. Thus models more appropriate for positively skewed count data were explored to more adequately represent the raw scores. Both Poisson- and negative binomial regression models were considered. Negative binomial regression models are similar to a Poisson regression models, in that they usually provide an adequate fit for positively skewed count data, however they are more lenient in terms of the variance present in the data (Gardner at al., 1995). Poisson regression models are affected by overdispersion of data points, frequently brought about by a zero inflation in the data. As zero inflation was found to be an issue in this dataset, it was decided to use negative binomial regression models whenever raw data outcomes were modelled. As for the baseline corrected proportions data, variance across subgroups was found to be homoscedastic with results form Levene's test for each subgroup ranging between $.093 < p < .984$.

Table 6.7. *Summary of skewness of imitation outcome scores.*

	Skewness	SE_{skew}	Z_{skew}
Total	.732	.183	4.00*
Baseline	.455	.183	2.432
Immediate Raw	.768	.183	4.197*
Immediate Corrected	-.402	.183	-2.197
Deferred Raw	.921	.187	4.925*
Deferred Corrected	.424	.187	2.267
Deferred with immediate Raw	.866	.188	4.606*
Deferred with immediate Corrected	.357	.188	1.898
Deferred without immediate Raw	.991	.188	5.271*
Deferred without immediate Corrected	.077	.188	.409

Note. * indicate values above criterion proposed by Kim (2013) for significant divergence from normality.

6.3.2 Full model. In an initial exploration of the data, two models were fit to explore both the raw scores and baseline corrected scores in relation the relevant factors. Both models included Age (8 month / 12 month) and Site (Cambridge / Keneba) as between subject factors. Condition was entered as a within subject factor with either three (Baseline / Immediate / Deferred) or, in case of the baseline corrected model, two levels (Immediate / Deferred).

Raw scores. A Negative Binomial model was fit to predict raw scores from the factors Condition (within), Age and Site (between). An offset variable was created and used to control each stimulus category for the maximum possible score. Results from the model can be found in Table 6.9. Main effects were found factors Condition, Age and Site. Interaction effects were found for Condition * Age and Condition by Site. The main effects were further followed up (Table 6.10). It was shown that Baseline scores were lower than both Immediate and Deferred imitation scores. Immediate and Deferred imitation scores, including both the score on items with prior practice during the immediate phase and those without, did not significantly differ from one another. Imitation was further found to be higher at 12 compared to 8 months and to be higher in Cambridge compared to Keneba. These results will be revisited in context of specific hypotheses in the following.

Table 6.9. *Results from negative binomial regression of factors Condition, Age and Site on raw scores.*

Independent variable	*Wald chi-square*	*df*	*p*
Condition	10320.125	2	<.001*
Age	123.786	1	<.001*
Site	53.966	1	<.001*
Condition * Age	441.611	2	<.001*
Age * Site	.246	1	.620
Condition * Site	6.650	2	.036*
Condition * Age * Site	.174	2	.917

Table 6.10. *Post hoc tests for main effects of Condition, Age and Site.*

	Wilcoxon Signed Rank Z statistic	*p*
Condition (Baseline – Immediate)	3.096	.002
Condition (Baseline – Deferred)	4.017	<.001
Condition (Immediate – Deferred)	.425	.671
	Mann-Whitney U Z statistic	*p*
Age (8 month – 12 month)	2.962	.003
Site (Keneba - Cambridge)	-7.209	<.001

Note: significance level of α = .05 Bonferroni corrected.

Corrected scores. Results are summarised in Table 6.11. Main effects were found for the factors Age and Site, but not for Condition. None of the interaction terms reached statistical significance, however there was a trend towards an Age*Site interaction, which when followed up revealed a stronger increase in scores for within the Cambridge cohort. These results will be expanded upon in the following to address the previously formulated hypotheses.

Table 6.11. *Results of RMANOVA with Condition, Age and Site as independent variables.*

Independent Variable	*F*	*df*	*p*	η_p^2
Condition	.596	1,165	.441	.004
Age	17.240	1,165	<.001*	.095
Site	11.085	1,165	.001*	.063
Condition * Age	2.162	1,165	.143	.013
Condition * Site	.052	1,165	.820	<.001
Age * Site	2.967	1,165	.087	.018
Condition * Age * Site	.424	1,165	.516	.003

Table 6.12. *Post hoc tests for main effects of Age and Site.*

	Mean Difference	*t*	*p*	*Cohen's d*
Age (8 month – 12 month)	-.103	-.425	<.001	.459
Site (Keneba - Cambridge)	-.099	-3.832	<.001	.428

Note: significance level of α = .05 Bonferroni corrected.

6.3.3 Experimental manipulation. *Hypothesis 1.* More frequent performance of the target action was expected in both imitation conditions, compared to the baseline condition. The data were found to be in line with this hypothesis, as shown by a significant main effect for Condition in the full model examining raw scores (Table 6.9) as well as significant results in the post-hoc comparisons comparing Baseline scores against Immediate and Deferred imitation scores (Table 6.10). Thus, infants showed higher performance of the target action after having been shown the action, indicating that the experimental manipulation of eliciting an action was successful. This effect is illustrated in Figure 6.3.

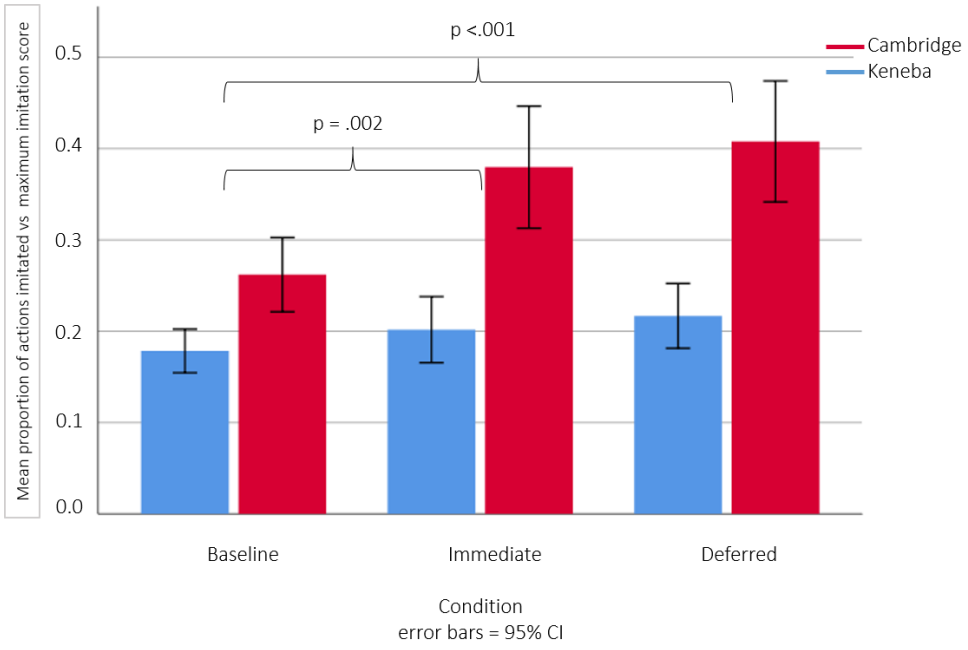


Figure 6.3. Proportion of raw scores relative to maximum score by Condition and Site. Error bars indicate 95% confidence interval.

Hypothesis 2. Higher imitation scores were expected in the immediate compared to the deferred condition, as task requirements were identical in both conditions and only assumed to differ in length of the retention interval. The full model on baseline corrected proportion scores did not

show a main effect for Condition (Table 6.11). Data did thus not show significantly higher levels of imitation in the immediate compared to the deferred condition that could solely be attributed to imitation rather than spontaneous behaviour. The result also was unlikely to have been a result of baseline correction, as no difference was found between performance of the target action between the immediate and the deferred condition in the raw score analysis.

Hypothesis 3. Items that were tested in the immediate imitation condition will not elicit better performance in the deferred imitation condition than those that were not in the immediate condition. To address this, baseline corrected deferred imitation scores with and without a prior immediate imitation phase were compared. Deferred imitation scores were baseline corrected, using only those items that were in the immediate or delayed imitation only condition. A paired samples t-test revealed that there were indeed no significant differences between items of the two conditions (t_{166} = -.268, p = .789). The reliability of this null effect was further assessed. As frequentist statistics do not allow for accepting the null-hypothesis in light of a non-significant result, Bayesian statistics were applied to further examine the reliability of this predicted null- effect. In contrast to conventional significance tests, Bayesian statistics can provide evidence not only for the presence but equally the absence of a difference, and can thus be used to quantify evidence for a genuine null effect (Rouder et al., 2017). The Bayes Factor (BF) denotes how likely it is for one hypothesis (null or alternative) to be true compared to the other. The BF_{01} (Bayes factor denoting how likely it is for the null hypothesis to be true compared to the alternative) was calculated. Reliable evidence for the null- hypothesis is accepted to be indicated by a $BF_{01} > 3$ (Jeffreys, 1961), which would indicate that the observed data is at least 3 times more likely under the null- than under the alternative hypothesis. The BF for this model was calculated using

the applet provided by Rouder et al. (2009). It was found to be BF_{01} = 11.183, and thus in support of a genuine null-effect.

6.3.4 Developmental change

Hypothesis 4. Baseline scores were not expected to change between time points. The full model based on raw scores showed significant main effects for Age and Condition as well as a Condition * Age interaction, indicating differences in scores between conditions that changed non-uniformly between age points. Baseline score changes between Age points were thus assessed further. As can be seen in Table 6.7, even though Baseline scores were count based, they were found to be approximately normally distributed and therefore assessed using parametric methods. A univariate analysis of variance model was fit, using Age and Site as independent variables and Baseline score as an outcome. Results of the model can be seen in Table 6.13.

Table 6.13. *Results from univariate analysis examining Age and Site differences in Baseline scores.*

Independent variable	*F*	*df*	*p*	η_p^2
Age	.101	1,173	.751	.001
Site	15.693	1,173	<.001*	.083
Age * Site	.685	1,173	.409	.004

As predicted, the main effect for Age did not reach statistical significance. The BF_{01} was calculated based on the result of a post hoc t-test of the factor

Age (t = -.227, p = .820) in the above model. For the current effect, the test revealed a BF_{01} = 5.988, thus supporting the presence of a reliable predicted null- effect.

Hypothesis 5. It was expected that infants would be better able to imitate the novel actions at 12 months compared to 8 months. As shown in Table 6.11, the full model for baseline corrected proportions scores showed a significant main effect for Age. The post hoc comparison showed that indeed imitation scores were at 12 compared to 8 months (Table 6.12). It was further expected that the developmental change would be more pronounced in the Deferred compared to the Immediate condition. However, the full model did not show a significant interaction effect for Condition * Age, and did thus not provide evidence for differences in change between Age points in one condition over the other.

6.3.5 Differences between sites

Hypothesis 6. As object play is less common in Keneba than it is in Cambridge, and parents' attitudes towards exploration of novel objects is much more liberal in western countries, it was expected that engagement scores would be lower in Keneba compared to Cambridge. Engagement scores were entered into a two-way ANOVA with Age (8 months / 12 months) and Site (Gambia / UK) as between subject factors. Results showed that contrary to the expectation, there were no Site differences ($F_{1,117}$ = .536, p = .466). No main effects were found for Age ($F_{1,117}$ = .922, p = .339) or Age * Site ($F_{1,117}$ = .063, p = .802) either, indicating that engagement with the novel object did not differ by Site or Age.

Hypothesis 7. Infants tested in Cambridge were expected to show higher rates of performance of the target action compared to infants in Keneba in the Baseline, Immediate and Deferred imitation condition. As can be seen in

Table 6.9, significant effects were found for the factor Site, as well as a Condition * Site interaction. This interaction was followed up by post hoc tests, results of which are displayed in Table 6.14. The effect is also illustrated in Figure 6.2. As can be seen from Figure 6.2 and the effect sizes in Table 6.11, scores were found to be consistently higher in the Cambridge than in the Keneba cohort.

Importantly, the higher baseline scores in Cambridge might affect the pattern observed in the baseline corrected scores, which will be explored in the following.

Table 6.14. *Post-hoc comparisons for Site and Site * Condition effects.*

	Condition	*Mann-Whitney U Z statistic*	*p*	n_p^2
Site (Keneba-Cambridge)	Baseline	-3.271	.001*	.061
	Immediate	-4.436	<.001*	.112
	Deferred	-6.318	<.001*	.236

Hypothesis 8. It was expected that higher imitation scores would be observed in Cambridge, when comparing both cohorts. From the full model of baseline corrected proportion scores, (Table 6.11) it is evident that there was a significant main effect for Site. The post hoc tests demonstrated that imitation scores were indeed higher in Cambridge compared to Keneba (Table 6.12). There further was a trending effect towards a greater developmental change in Cambridge compared to Keneba, as can be seen in Figure 6.3.

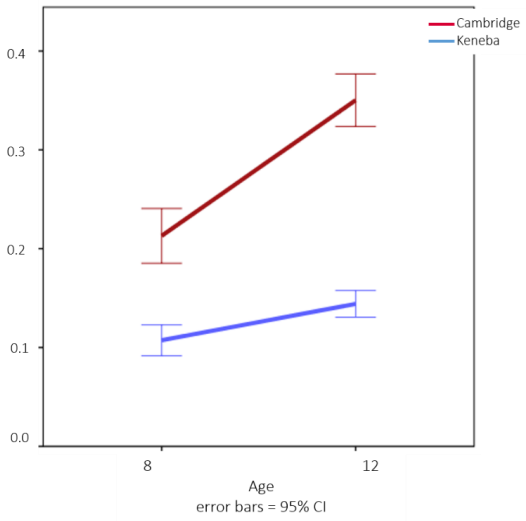


Figure 6.4. Trending interaction effect of Age * Site, showing a trend towards lower scores in Keneba at both age points as well as a reduced developmental change.

Hypothesis 9. The discrepancy between Cambridge and Keneba will be largest for scores in the deferred imitation condition, indicating differences in memory rather than imitation ability. As can be seen from the full model in Table 6.11 no interaction effect of Condition * Site was evident. As the analysis of baseline scores had shown higher performance of infants in Cambridge compared to Keneba however, the interaction effect was also examined on the basis of raw scores. The full model in Table 6.9 showed a significant interaction effect of Condition * Site and the follow up analyses in Table 6.10 showed this effect to hold for each of the conditions. The effect sizes for both imitation conditions indicated that Site differences indeed were more pronounced for the deferred compared to the immediate imitation condition. These findings provide partial evidence in favour of the hypothesis.

Hypothesis 10. Performance was expected to be more variable in the Keneba cohort at both time points. The baseline corrected proportion

scores were entered into univariate ANOVA models and the Levene's test of equality of variance were examined. One model examining differences in Score between Sites was fit separately for each combination of Age point and Condition. Results are displayed in Table 6.12. As can be seen, no significant differences in variance were evident from the data in any of the subgroups. Our current dataset therefore does not provide evidence for higher variance in the Keneba cohort. While none of the effects reach statistical significance, a slight trend can be observed for greater differences in variance between Sites at the 12 month time points in both the Immediate and the Deferred conditions, which could be indicative of growing disparity between groups.

Table 6.12. *Results of Levene's test of equality of variance for univariate analysis models examining differences in proportion score by Site.*

	Age	*Levene's Statistic*	*df*	*p*
Immediate	8 month	1.021	1,91	.315
	12 month	3.055	1,82	.084
Deferred	8 month	<.001	1,85	.984
	12 month	2.512	1,80	.117

6.4 Discussion

This study illustrates the development of imitation behaviours in infants the UK and The Gambia between 8 and 12 months of life. It extends knowledge gained from previous studies comparing imitation behaviour and its developmental change in a western and non-western setting (Goertz et al., 2011, Graf et al., 2014, Teiser et al., 2014). The questions asked in this study varied somewhat from these previous studies, necessitating some specific adaptations to the otherwise well-established deferred imitation paradigm.

6.4.1 Adaptation of established paradigm. Items used in this task were newly developed, taking into consideration differences between sites with regard to prior exposure to particular objects. Further, this study used items that were adequate with regard to motor demands at these young age points, as well as being versatile enough to not only offer one possible action, thus preventing infants from spontaneously performing, rather than imitating, the target action. Items were aimed at being slightly more demanding at the 12 month age point, to prevent ceiling effects. As baseline scores reflecting spontaneous performance of the target action remained similar across age points, the item difficulty appeared to be appropriate. Administrators of this paradigm had previous experience with standardised behavioural infant testing from other studies on the BRIGHT project (i.e. the Mullen Scales of Early Learning). Training on this task thus took the form of a detailed protocol description, one in-person training and several regular check-up meetings to discuss questions and provide feedback on specific items. As part of these discussions, scoring criteria were simplified and made more objective, leading to high inter-rater reliability rates. Having implemented these adaptations, the following key findings were obtained.

6.4.2 Experimental manipulation. Item difficulty seemed to be adequate as infants performed target actions more frequently after, than before demonstration, indicating imitation. Similar levels of baseline performance were observed between age points, indicating that item difficulty was adequately gauged from one age point to the next.

6.4.3 Developmental change. Data were in line with the prediction that infants would become better at imitation with age, as evidenced by higher imitation scores at 12 compared to 8 months. Contrary to the hypothesis, no differences were found between performance in the immediate vs deferred imitation condition. This indicates that, in contrast to the

expectation, the 20 minute delay did not lead infants to imitate less than they did immediately after demonstration. While some previous studies have used similar delay times of 10-20 minutes (Teiser et al. 2014, Graf et al. 2014, Goertz et al., 2011), most studies using both an immediate and a deferred imitation phase used substantially longer delay times. As in this study families come in for a one-day visit, a much longer delay time could not be implemented, it might however be the case that 20min were not long enough to elicit differences between the immediate and the deferred condition. This was not so much driven by a lack of response and resulting floor effects in the imitation conditions, but rather a similar level of imitation in both imitation phases. As can be seen in Figure 6.2, there appears to be a possible trend towards more imitation in the Deferred compared to both other conditions, accompanied by a drop of imitation in the immediate imitation condition. While this is speculative, from personal observation this could be explained by infants reduced interest in the item during the immediate imitation phase, after having explored it during the baseline phase, then observing the demonstrator perform the target action and subsequently being again presented with the item.

Further, as predicted, no differences were found between the deferred condition with or without previous immediate imitation. This underlines the point made by Barr and Hayne (1996) that motor practice, and therefore implicit memory is not the defining feature driving the ability to imitate a novel action.

6.4.4 Differences between Sites. As for the differences between the two cohorts studied, it was found that infants in Cambridge performed the target action more frequently during all three conditions, and imitated more than infants in Keneba. This latter result is of particular importance, as the baseline corrected scores can be regarded as a more conservative measure

of imitation scores in Cambridge, due to the elevated baseline scores. An analysis of the overall engagement with the objects showed no differences between Sites, indicating that the effects are not purely driven by infants in Keneba being more careful in exploring the novel object.

Partial evidence was found for a larger discrepancy between Sites in the deferred compared to the immediate imitation condition. While the relevant interaction effect did not hold in the baseline corrected model, it did hold on the raw score model. It can therefore be concluded that there is a trend for differences to be higher in the deferred than the immediate condition. Lastly, no evidence was found for there to be more variance between individuals in one cohort over the other. While the more variable exposure to different environmental factors in the cohort tested in Keneba was expected to result in more variable responses, the current data did not provide any evidence for this.

6.4.5 Implications for memory development across the cohorts. The findings of the current study bear relevance in context of infant memory development via imitative learning in the two cohorts under investigation. The study clearly illustrates a developmental change regarding the imitation behaviour between the two age points. This change in itself is widely documented and in context of the present study underlines its utility as an indicator of early explicit memory development. The reduced imitative behaviour observed in Keneba gives an indication that modelled actions are recreated less readily in this cohort. Particularly the vastly reduced developmental change and reduced scores at the 12 month age point are indicative of a persistent trend, as reliable imitation responses are usually assumed to be present from 9-10 months of age (de Haan et al., 2006). While significant as an indicator of early memory abilities, these findings further suggest that a crucial avenue of early learning relevant behaviours

through observation of others might not be easily accessible to infants in Keneba. This is further supported by the large discrepancy between cohorts in the deferred imitation condition. As prior research has suggested that immediate imitation is not the most significant predictor of successful longer term learning (Barr & Hayne, 1996), the reduced imitation behaviour after a delay further corroborates the notion that learning through imitation might be impeded in the Keneba cohort.

6.4.6 Strengths and limitations. *Ecological validity of task.* Particularly for the behavioural studies conducted in the Gambian cohort, ecological validity is an important consideration. While objects in these studies were chosen in such a way that the actual objects and their use was novel to all infants, the concept of toy and exploratory play is strongly encouraged in one but not the other cohort. While the protocol included time for infants to opening up towards the experimenter and to engage in some form of play with them, this still only partly eliminates the situational novelty.

Confounds with motor development. As the current study involves manipulation of a range of objects, motor development should ultimately be controlled for. Even though experimenters were able to assist the infant whenever they were struggling with an aspect of the task that was clearly motor dependent, a cleaner comparison could be obtained by controlling for this factor in future analyses. Data on gross and fine motor development is currently being collected and curated as part of the Mullen Scales of Early Learning.

Consistency of administration. As demonstrated by Teiser et al. (2014), experimenter characteristics can make a difference in eliciting imitation behaviour. Even though administration was regularly monitored and reliability was high, some differences between sites remain. One

confounding factor in this study is that all infants in Keneba were assessed by male project staff, as no female staff are conducting study visits, whereas in Cambridge all project staff are female. This might affect results in that infants in Keneba are almost exclusively taken care of by female family members and as such interacting with a male experimenter could have further added to the novelty of the testing situation.

Validity of Age and Site comparison. For the current study, it is important to consider the vast differences in infants' exposure to object play between the two sites, and how this might be closely linked with their developmental improvements on the deferred imitation task between the two age points. While some of this bias was attempted to be controlled for (i.e. by taking into account infants' spontaneous behaviour, and by contrasting immediate and deferred imitation), the underlying cohort difference in object play cannot be fully accounted for. Further, it needs to be taken into consideration that differences in child care practices between the two sites may have brought about some of the observed outcome (i.e. infants being frequently carried on their mum's back and being more restricted in their ability to explore freely). One way to enable a cleaner comparison might lie in the assessment of imitation to more universal behaviours, for example by measuring tongue protrusion, contagious yawing or scratching. While this would likely yield important insights into mechanisms of imitation, such designs do however not lend themselves to a deferred imitation paradigm, and thus would address a different set of research questions than were set out to answer in this thesis. Another possible way to address this site difference might lie in measuring infants' overall motor abilities and to adjust analyses accordingly.

6.4.7 Future directions.

For analysis of this sample once data collection is complete, there are a few issues that should be considered. First, while the

analyses presented in this chapter tease apart whether we observe imitation or spontaneous behaviour by baseline correcting scores, overall engagement with the objects was only scored very broadly in terms of touching each object that is presented. From communication with the local testing team and from own observations, infants in Keneba are on average less engaged in play-based sessions. As it is uncommon especially for young infants to either play with toys or engage in regular face-to- face interaction with their carers, they engage more slowly with assessments centered on interactive play. Infants tested in Keneba have further been found to more frequently be overwhelmed by situations in which a lot of external stimulation was offered (i.e. fast paced audio-visual stimuli in eye tracking task, exposure to a range of objects in current study). In coding the object engagement it was discussed that an approach coding either the time it took until the first contact with the object or the total time of handling the object could provide better insight into the issue. Due to time constraints this approach could not be pursued in the current data set, but is planned to be applied with a subset of the final sample. Thereby, we can add another layer of understanding on where the observed differences originate from.

While the data presented in this chapter is purely behavioural, it was attempted to collect fNIRS data at the time of administration and time lock it to object presentation. In relation to aforementioned issues with infants in Keneba becoming overwhelmed more easily, it was decided that no valid data could be collected during the fNIRS session as infants were not engaging with the object play while also wearing the fNIRS hat. The study was therefore administrated purely behaviourally. For the Cambridge cohort however fNIRS data was collected at both time points for the majority of infants. In a future analysis, the behavioural response should be examined concurrently with the time-locked haemodynamic changes to better understand the underlying neural response.

Lastly, as discussed in Chapter 1, one goal of this deferred imitation study was to examine whether imaging measures obtained early could be shown to bear relation to a later behavioural outcome. An investigation of whether early measures held any predictive utility in relation to scores in this study could help explore pathways of early identification of at risk infants. This is especially relevant in this current study as imitation is such a significant mechanism for infant learning.

Chapter 7. General Discussion

Concluding remarks and directions for future research

The neurocognitive development of infants in low and middle income countries is a largely unexplored area of research. This thesis aimed to contribute to this emerging field in two main ways; firstly to prove the feasibility of selected objective, easy-to-use neuroimaging measures. This was achieved through (i) the implementation of electrophysiological markers during the first 5 months of life, (ii) the further refinement and implementation of an fNIRS paradigm and (iii) a specifically adapted behavioural task. Secondly, this thesis had a particular cognitive focus on early memory development and was aimed at understanding developmental trajectories in this domain in two cohorts, with high and low risk of adverse developmental outcomes. This was achieved across all three studies with developmental changes differing in some key aspects between the two study cohorts. In this chapter, I will first summarise results across all studies in the context of the research aims initially set out, before providing a critical review of the presented research and a contextualisation within practical, clinical and theoretical frameworks.

7.1 Results in the context of the research aims

7.1.1 To develop and further refine paradigms to assess early memory development for adaptation and use in wider, global settings.

Due to the novelty of this line of research, one important aspect of this thesis was to further the development of tools that could potentially provide objective, mechanistic insights into early neurocognitive development in a range of contexts. All three of the presented studies were successfully implemented in both cohorts and were thus able to inform our understanding of early development across a range of memory subtypes. The ERP auditory novelty detection study has been widely implemented in a range of infant populations (Burden et al., 2007, Cheour-Luhtanen et al., 1996, Siddappa et al., 2004), and has now been shown to also be successful in measuring early

neurocognitive development inrural Gambia. This provides a proof-of-principle for the utility of electrophysiological markers in young infants in Africa. Being reliant on relatively neutral stimuli, this paradigm in particular holds potential for more wide-spread use across different cultures. Similarly, the fNIRS working memory paradigm offered some mechanistic insights into the development of abstract object representation during the latter half of infants' first year of life. This study was the first to demonstrate developmental changes in frontal cortex activation in the absence of an active behavioural task, providing a useful tool for contexts in which overt behaviours are notreadily elicited. Lastly, the behavioural imitation task produced valuable insights in both cohorts but did prove to be more difficult to implement in Keneba compared to Cambridge. This might be partly accounted for by the increased novelty of the overall assessment situation experienced by the infants in Keneba, who from qualitative observation of the testing team were more easily overwhelmed and unable to be engaged in some of the behavioural measures. Future studies should take into account differences in infants' temperament in the context of similar tasks to avoid skewed results due to infants' ability to engage with the task.

Overall the successful implementation of all three of these paradigms enabled the assessment of similarities and differences with previously reported literature, which will be discussed in the following.

7.1.2 To examine the replicability of established paradigms assessing early memory capacity in both rural Gambia and the UK.

While some adaptations were made to successfully implement the presented studies in both cohorts, the underlying paradigms have all previously been implemented in infant research, allowing for an examination of their replicability in the current project. As a widely used tool in infant research, there is a well-defined developmental change from an initial intensity based response

towards a response based on stimulus novelty that has been described in previous literature (Kushnerenko, 2013). Indeed, a replication of this effect could be shown in this thesis within the Cambridge cohort. In relation to the different developmental changes in the Keneba cohort, this provides an indication for the paradigm's potential use in understanding developmental progressions in different cohorts, and its potential sensitivity to altered developmental trajectories in the context of environmental adversity.

The fNIRS paradigm, having been developed more recently and not applied to many populations and age groups, provided additional insight into early working memory development, by enabling us to apply a passive paradigm, using the resulting neural response to better understand early WM development. Being based on a wealth of behavioural data as well as some prior fNIRS data collected in the US (Baird et al., 2002), the UK (Kischkel et al., 2016) and The Gambia (Begus et al., 2016), the current study extended this line of research in several key ways. First, compared to pilot studies collected in the context of this project in the UK and the Gambia, the current study added a developmental dimension by implementing the task around an age range critical for early development of working memory and abstract thought (Baird et al., 2002, Cuevas et al., 2012). Secondly, compared to previous fNIRS studies examining brain-behaviour relationships (Baird et al., 2002), this study demonstrated activity in key areas such as the frontal cortex within a passive paradigm, i.e.in the absence of an overt behavioural response. Third, it demonstrated a specifically localised response by examining a broader cortical network than was assessed in previous studies. As for the behavioural imitation paradigm, the well-documented change towards more reliable imitation behaviour with increasing infant age (Jones & Herbert, 2006) was also evident in the current study. Also some more subtle effects previously reported, such as the irrelevance of an immediate practice attempt (Barr & Hayne, 1996)

could be replicated in both cohorts. In summary, findings do show a substantial overlap with previous literature regarding documented developmental effects. They therefore contribute to our understanding of early developmental trajectories of memory development across a range of domains as will be reviewed in the following.

7.1.3 To establish normative longitudinal data on early memory development on a set of tasks in the two cohorts. Results presented in this thesis have all contributed to a more thorough understanding of early memory development in the two investigated cohorts. The presented tasks have each highlighted the developmental change occurring between the relevant age points, and thus added to our understanding of early memory development across a range of domains, including habituation, novelty detection, working memory and imitation behaviours, during a critical developmental period. The main developmental changes observed in each study and cohort are summarised in Table 7.1.

Table 7.1. *Main developmental changes observed in each study in the two cohorts.*

	Cambridge	Keneba
ERP novelty detection	Initial strong response to stimulus intensity at 1 month changed to preferential response to trial unique sounds, and therefore novelty at 5 months	Strongest response to intense infrequent stimuli at both the 1 and the 5 month age point.
fNIRS working memory	Minimal activation over frontal cortex observed at 8 month increased in intensity towards the 12 month age point	Some activation over frontal cortex observed at 12, but not at 8 months of age.
Behavioural deferred imitation	Ability to perform novel action sequences increased between 8 and 12 month age point.	Ability to perform novel action sequences increased between 8 and 12 month, however less so than in the Cambridge cohort.

In their entirety, the findings can be regarded as describing developmental trajectories in memory development over the first year of life. Hereby, it

was possible to extract certain features indicative of the developmental changes that were anticipated based on the literature. Regarding the ERP novelty detection study, the described developmental change away from reaction to stimulus intensity towards reaction to stimulus novelty was replicated in the Cambridge cohort but not the Keneba cohort, suggesting utility of this feature in assessing development of novelty detection over the first months of life, as well as in understanding differential trajectories across populations. The fNIRS working memory study revealed an increase of activity within the frontal cortex (FC) with development, which in the context of previous literature highlights the importance of this area in abstract object representation. Finally, the deferred imitation task provided insight into the behavioural abilities of infants to learn novel actions they observed. It was found that both groups were able to imitate some novel actions and that this ability sharply increased between time points. This interlinks with previous research showing the ubiquity of imitation behaviours in different settings and their potential for use in infant research.

While an interrelation of the different studies ultimately was beyond the scope of this thesis, recurring developmental patterns that are seen across different assessment modalities and areas of neurocognitive development give an indication towards trends unique to each of the studied cohorts, as will be discussed in the following.

7.1.4 To identify potential differences and commonalities between the two cohorts regarding performance at each age point and subsequent developmental changes.

The most significant aspect of this thesis was to examine whether the proposed tools showed sensitivity to any potential differences between the cohorts and in how far developmental profiles where overlapping or diverging from one another. In the ERP novelty

detection study, it was shown that differences between the two tested cohorts were evident from as early as 1 month of age, both with regard to habituating to a repetitive stimulus as well as in the dishabituation/novelty response to a change in the sequence. The ERP amplitudes overall were reduced in the Keneba cohort and no distinction was evident between frequent and trial unique sounds. As laid out in the context of the spectral analyses, amplitude reduction could partly be attributed to lower phase coherence of the oscillatory response in the Keneba cohort. While at this point it can only be speculated what this finding indicates about brain development, in light of the notion that phase synchrony can be used to quantify the robustness of a response to repeated input, a lack thereof might be indicative of a less reliable neural response patterns in light of continuous stimulation. The anticipated developmental change between the 1 and the 5 month age points was not evident within the Keneba cohort, who again showed a large response to the infrequent sounds and similar amplitudes to the frequent and trial unique sounds. With regard to the preferential response to the frequent stimuli, no developmental change was apparent in the Keneba cohort, indicating a less mature processing style than would be expected from studies in other cohorts at the 5 month age point. With regard to the similar amplitudes to the frequent and trial unique stimuli, the hypothesis that this might be due to some deficit with auditory discrimination between the two sound categories was not supported by the spectral analyses which showed distinct spectral response patterns for the frequent and infrequent stimuli. Rather, it seems that discrimination between both types of stimuli does occur, but that on the whole session level responses to the trial unique stimuli appear to be of similar magnitude as those to the frequent sounds.

The fNIRS study showed increases in FC activation in both cohorts, however the increase in the Keneba cohort was much reduced, with no FC activation

being evident at 8 months and only one channel reaching significance at 12 months. This study further revealed a more wide spread pattern of activation in the Keneba cohort over posterior temporal regions, which could be indicative of increased structural and functional variance. While purely speculative, in terms of structural variance, both a recruitment of different neural structures as well as variance as to the location of relevant structures could have resulted in the observed response. In functional terms, recruitment of a wider but less localised neural network could have accounted for the observed findings.

The imitation study similarly showed reduced performance in the Keneba cohort at both age points, as well as a trend towards a reduced developmental change. Regardless of assessment modality, all findings revealed systematic differences in the developmental profiles of the two tested cohorts. In cases where previous developmental trends could be replicated, these tended to be more pronounced in the Cambridge cohort (i.e. latency decrease between time points, frontal lobe activation to object hiding, increase of overall imitation scores). While the current thesis only provides data from two age points for each study which prevents firm conclusions about developmental trajectories, the repeated occurrence of the developmental trends across assessment modalities shows a common trend across paradigms.

The following sections will discuss the practical, clinical and theoretical implications of the presented findings before moving on to a critical methodological review of the research presented in this thesis.

7.2 Implications of findings

7.2.1 Practical and clinical implications. This thesis provides evidence for the utility of three tools used to tap different subdomains of memory development over the first year of life to assess infants in a low resource

setting. Two neuroimaging measures were implemented, offering potential tools for objective assessment across a range of cultural contexts. The measures presented here include some of the first EEG studies performed with young infants in Africa, as well as an fNIRS paradigm assessing mental object representation without reliance on overt behavioural responses from the infant. Further, the utility of a behavioural imitation paradigm was shown. All three studies add to our understanding of early memory development in a unique way and offer potential for future use. Having demonstrated the feasibility of these tools to be implemented and to capture cohort specific developmental profiles supports their utility for wider use across different settings world-wide. The practical applicability of these measures should increase further, in the context of current technological advances, making it possible to employ both EEG and fNIRS measures in a decentralised manner in the field. While due to the scope of the BRIGHT project participants were required to come into the lab, there now are first advances implementing fNIRS measures in the field, in the absence of any research facilities which enables access to even the most remote and rural areas (Jasinska & Guei, 2018). Through reliance on battery powered, portable equipment, this approach could also already be pursued relying on the EEG system used in this thesis. Similarly, a new generation of portable, wireless and battery powered fNIRS devices has very recently become available, which will hugely benefit future studies of this kind.

Results further show that the proposed methods offer insight into assessment of infants from birth onwards, underlining their potential for use in early identification of at risk individuals, as well as continuous monitoring across critical developmental time points. Hereby, the presented findings provide converging evidence for early developmental changes within the Cambridge cohort, as well as first insights into age appropriate responses within the Gambian cohort which will be extended in future

follow up assessments. Similarly, the potential for continuous assessment and emerging normative data also provide the basis for tests of efficacy of early interventions, creating a window of opportunity to buffer against some otherwise detrimental impacts of environmental risk even before the emergence of overt behavioural differences.

7.2.2 Theoretical implications.

Models of memory functioning and development. The question in how far processes in infant memory development can be conceptualised in terms of a maturational process towards adult memory functioning has long been an issue of debate. As reviewed in Chapter 1, Nelson (1995) proposed a framework of the infant memory system, which to a degree mapped onto models of adult memory functioning. Hereby, three domains of memory functioning were proposed, each associated with a different set of brain structures and taxed by a number of conceptually distinct paradigms. He distinguished between early emerging explicit, or 'pre-explicit' memory reliant on temporal lobe structures such as the hippocampus, implicit memory more reliant on the striatum and cerebellum and working memory, primarily recruiting regions in the prefrontal cortex. Since the initial proposal of this framework, much research has focused on understanding developmental processes driving neurobehavioural changes across these different domains (Jabès & Nelson, 2005). In this thesis, infant memory development was assessed in different ways, taxing both (pre-)explicit (ERP novelty task, imitation task) as well as early working memory (fNIRS object permanence study). As reviewed above, each task was able to reveal some of the developmental effects proposed by Nelson (1995). With regard to preferential response to novel stimuli, we found a developmental change towards reaction to preferential response of truly novel sounds. While in the current paradigm at least partly driven by the physical properties of the stimulus (i.e. its intensity), a related process has been described whereby infants develop a genuine novelty

preference over the first months of life, after reacting equally strongly to both infrequency of a stimulus and its novelty (Nelson & Collins, 1991). Regarding infants' imitation ability significant improvements in their ability to perform novel action sequences after a delay were proposed to occur between 6-12 months (Barr, Dowden & Hayne, 1996), which was reflected in the current study. With regard to object representation a developmental change could be demonstrated in areas implicated as relevant initially through the study of non-human primates (Wilson et al., 1993).

While these findings provide additional neurodevelopmental evidence as previously discussed, the precise mechanism by which the described changes unfold still is debatable. Hereby, neither the question of when a structure can be regarded as having reached an adult-like level of functioning nor the question of when a structure can be said to be sufficiently mature to fully support a given process can definitively be answered. While the former is more of theoretical interest but not a focus of the current investigation, the latter bears relevance when trying to understand neurodevelopmental trajectories. As noted by Nelson (1995), observations from developmental changes observed on tasks of early memory cannot easily be used to make inferences back to maturational changes within the relevant neural structures for two main reason; first tasks used to assess infant memory do not represent the end state of mature memory functioning and as such cannot provide ultimate insight into any one structure's maturational status. Secondly, even in instances where a certain aspect of adult memory functioning maps onto activation in a highly localised structure, broader networks could be involved in the developing brain. Further, due to its focus on subtypes of memory which to varying degrees depend on the hippocampus, it needs to be noted that while we can infer a lot about the hippocampus and the MTL network from

cortical measures of brain functioning, infant research mostly cannot be reliant on direct measures of this subcortical structure.

Along these lines, it has been argued that while in addition to the hippocampus and MTL the dorsolateral prefrontal cortex (dlPFC) crucially supports early WM tasks during infancy, its maturation continues until well after infancy, when it takes over much broader functioning in terms of orchestrating processes within the PFC (Nelson, 1995). Therefore, viewing proficiency on WM tasks in infants around 12 months of age could therefore lead to the misleading assumption of maturity within the dlPFC by this point. Further, some evidence suggests that during early development the as of yet immature dlPCF is supported by subcortical striatal structures such as the caudate (Goldman & Rovold, 1972) while more sophisticated WM processes during child- and adulthood might more fully rely on the more mature dlPFC. Due to the methodological differences in imaging subcortical structure involvement in infants, this however remains a speculation based on lesion evidence based on non-human primates. While the fNIRS study presented in this thesis is one of the first to provide localised functional data on an early WM task across a critical developmental period, an interpretation based on a one to one correspondence between structure and function cannot entirely address this issue. In addition to illustrating the importance to consider subsidiary structures supporting certain processes during development, this example also provides an indication of the importance to consider the involvement of wider cortical and subcortical networks during development. While structures such as the dlPFC or the hippocampus obviously play a key role in many forms of memory functioning, they also act as hubs receiving and providing input to other structures. These interactive relationships between, as well as changes within neural structures, represent the two core features of frameworks aimed at conceptualising the dynamic nature of neural development.

In the following, the concepts relevant in this context will be reviewed and discussed in relation to early adversity and the findings of the thesis.

Early exposure to risk: interactive specialisation and developmental systems theory. The notion that neural circuitry does not develop in isolation is at the heart of the interactive specialisation (IS) framework proposed by Johnson (2000, 2001, 2011). The IS account proposes a multilevel interaction between an individual's genetics, brain structure, function and behaviour across development (Johnson, 2011) whereby these different developmental processes interlink and moderate one another. This principle of *circular causality* governing the interplay between genes, brain structure and function in the emergence of cognitive functioning is one of the core assumptions of the IS account and in stark contrast to accounts implying a more deterministic view whereby genes drive the development of brain structures which in turn result in cognition and behaviour. It further assumes an *interactivity within brain networks*, whereby a structure does not develop in isolation but is highly dependent on input from neighbouring regions. Thirdly, the developmental process is said to be *self-organising and activity dependent*, as for example illustrated by a developmental bias to orient towards novel input as discussed in Chapter 3 of this thesis. Lastly, the IS framework proposes *dynamic mapping between brain structure and cognitive function*, rather than a more modular one to one correspondence. This latter principle also holds that as specialisation on a functional level is accompanied by localisation on a structural level and links in with Nelson's notion of subsidiary structure involvement during development.

While the IS account only touches on the impact of wider environmental context and adversity on the developing brain, it can be extrapolated that if assuming a circular interaction of genetics, brain structure, function and behaviour it becomes apparent that exposure to environmental adversity as

is often the case for individuals living in poverty (Jensen et al., 2017) can negatively impact development and result in knock-on effects for further neurodevelopment.

One other framework to provide a more global approach in including levels of explanation that include the immediate as well as the wider societal environment is Ford and Lerner's (1992) Developmental Systems Theory (DST). In this framework the authors provide an account that at its very centre has the interaction between an individual and their environment. DST states that development is jointly affected by genetic and epigenetic, environmental influences. Six principles are proposed which govern developmental processes. Development is said to be *jointly determined by multiple causes* as well as *context sensitive and contingent*, thus to be influenced by multiple interacting factors, and responsive to the experienced environment. This latter point is also formulated in the principle of *development as a process of construction*, which holds that an organism actively partakes in creating its own environment. Further, DST proposes for there to be an *extended inheritance* of not only genes, but also the resources present in the environment, if not directly than at least in shape of epigenetic factors. The principle of *distributed control* holds that no single factors takes central control over an organism's development, but rather that multiple factors interact and result in an outcome. Lastly, *evolution is viewed as a constructive process*, whereby larger-scale developmental dynamics are considered which provides perspective of the trajectories of not only individuals but whole ecological or societal systems.

These developmental principles link in with the multilevel exposure to adversity often associated with growing up in LMIC's. Even though results in the current thesis have not been associated with any measures quantifying the exposure to environmental risk, previous work conducted in The

Gambia allow for some speculation as to the potential mechanisms by which infants in the Gambian population might be at risk.

In this thesis, evidence regarding brain function as well as behaviour is presented, which has demonstrated divergent developmental profiles in the Gambian cohort. Findings from the ERP novelty detection study showed reduced responses to novel environmental stimuli, a bias highly important in obtaining novel sensory input and therefore fundamental to learning. Results from the fNIRS WM paradigm indicate reduced activity of brain areas implicated in mentally representing an object, which can be regarded as a building block for higher order abstract thought. The imitation study lastly indicated reduced acquisition of novel actions, which if generalised to everyday functioning impedes learning. Thus, there is direct evidence on suboptimal functioning from a brain functioning and a behavioural level, which in both an IS and a DST framework would be hypothesised to result in a knock-on effect for further development.

From an IS point of view, the functional differences observed give an indication on possible differences at a structural level. While the current project does not collect data on structural brain development, prior research as reviewed in Chapter 1 has linked a range of factors to differences in brain structure, one central factor being nutrition. Hereby, both micro and macro nutritional deficiencies have been linked to global volume reductions as well as to changes in more specific region such as the hippocampus or the PFC (Fuglestad et al., 2008). Potential differences in brain structure in an IS framework would then be expected to have an impact on both the further structural as well as functional brain development, leading to a permanently altered developmental trajectory. From a DST angle, it further needs to be considered that these early observed differences are not only partly brought about by multifaceted

environmental factors but will also shape infants' further development through altered behaviours during the infant period.

While the reasons for the observed findings need to be elucidated in further research, DST provides a framework to better understand potential pathways in which factors previously found to be significant in the Gambian cohort might affect the developing brain. DST strongly emphasises the impact of genes, and gene expression through environmental factors. One relatively new and ongoing line of research in rural Gambia is seeking to explain the effects of the aforementioned critical factors these epigenetic processes. Hereby, it has successfully been demonstrated that both nutritional factors (Khulan et al., 2012) and exposure to infectious disease (McDermid et al., 2009) play a vital part in influencing gene selection and therefore the very core of all other downstream processes. While collected as part of different projects, these findings from individuals in the same community as out participants might ultimately lead to a better understanding in how far environmental influences effect development not only directly, but also cross-generationally.

With regard to individual developmental trajectories, it further needs to be noted that the early infant period is critical with regard to development across several sensory and cognitive domains, which will be discussed in the following.

Special relevance of sensitive periods. As is implied in the IS principle of development progressing in a self-organising and activity dependent manner, another important consideration is that of the sensitive and critical periods occurring at different stages of development. A distinction is hereby made between critical periods, characterised by a sudden on and offset, during which the organism actively seek out (or is experience expectant)

input relevant for the function under development (i.e. being exposed to patterned light as an infant in order to develop normal vision) and sensitive periods during which relevant input is met by a heightened sensitivity to stimulation, during which optimal and most efficient development can occur (i.e. language acquisition in young children). While distinct, both concepts at the core imply that in order for optimal development to occur, relevant input needs to be available to the developing brain during a temporally limited period of time. Both sensitive and critical periods occur across infant and child development, and to a degree are hierarchically dependent on one another (Hensch & Bilimoria, 2012). For this reason, early adversity is particularly detrimental, as it implies a fundamental disruption of subsequent developmental processes. The concept of critical periods also holds importance for the implementation of early interventions, are bound to be most effective when implemented before an early window of opportunity has closed (Nelson, 2009).

These concepts further bear relevance when considering whether a developmental difference between cohorts at any age point is indicative of a developmental delay or rather a more permanent divergence in trajectory. Whilst an interpretation of the findings presented here in those terms would merely imply a different rate of developmental progression, which could eventually be overcome through some kind of compensatory catch up effect, this might not be the most adequate representation of the ongoing process. Studies conducted in individuals who experienced both physical but also severe socio-emotional early deprivation for the majority of their infant life suggest that some effects cannot be remediated and continue to prevail in the long-term (Nelson, 2009). This thesis is limited in that it does not provide sufficient longitudinal data to draw out developmental trajectories, and therefore cannot provide a definitive answer as to whether the present results indicate a temporary

developmental delay in the Keneba cohort, or whether trajectories progress in a different manner across sites and/or individuals. Based on the work reviewed addressing neurocognitive development at early school age in many LMIC's including The Gambia (McCoy et al., 2015), it however needs to be noted, that performance below developmental age norms is still prevalent at school age, meaning that if a catch up effect were to occur, it might not occur until after the start of formal education. With regard to results presented in this thesis, particularly the differences in the working memory response warrant continuous follow up, due to their known association with school performance (Alloway et al., 2004)

Framing the findings: Deficit or Adaptation. Another consideration besides the interrelated nature of brain development is whether certain developmental processes should be understood as indicative of a deficit from a certain typical trajectory or rather constitute an adaptive process leaving an organism best fit to their respective environment. As proposed by Johnson, Jones & Gliga (2015), early brain development is commonly regarded in terms of typical and atypical development. This view however does not encompass the brain's fundamental property to react to its environment in ways allowing for an adaptive developmental outcome, thus resulting in a cortical structure and functioning most fit for their experienced circumstances. As is reviewed in Johnson et al. (2015), development in context of early adversity can be viewed in different ways. The first frame of reference is hereby provided by the concept of 'resilience', which in a neurodevelopmental context describes the brain's ability to develop typically in the face of adversity. This model implies that one typical trajectory of neural development exists that either can be achieved, or, in the presence of adversity, needs to employ additional resources to not divert from the set out course. The notion of 'ontogenic adaptation' on the other hand holds that individual brains develop in such a

way that allow an optimal fit between environmental demands and brain functioning. Rather than making the assumption that one trajectory is favourable over the other, this notion allows for adaptive advantages to be experienced in different contexts. While some developmental disorders such as ASD might be more appropriately viewed as an adaptation to particular characteristics of the affected person, this view is more problematic in the context of early adversity. An illustration stems from an influential study by Barker (1994), who, noted that fetal undernutrition resulted in metabolic processes designed to retain energy in responding to insufficient resources in utero. However, these metabolic changes in the context of a postnatal environment characterised by sufficient nutritional availability, have been shown to be associated with elevated risk for disease. This process has also been described in the context of nutritional status in The Gambia (Moore, 2016). Hereby it is noted that early specialisation to a given environment can only be regarded as truly adaptive if the early environment prevails throughout the lifespan. In light of the nutritional environment in particular, it can easily be appreciated that the seasonality in The Gambia (as described in Chapter 1) alone creates contrasting environmental demands during the two seasons, with consequence for optimal functioning of the developing infant.

With regard to neurocognitive development, one obvious issue lies in the reduced discriminatory response observed in infants over the first months of life. The discrimination between different kinds of auditory input as laid out in Chapter 3 serves many crucial functions, one of which is to lay the foundation of language acquisition through attuning to phonemes of one's native language (Bishop, 2007). In the Gambia however, one common believe held by many parents is that infants over the first months of life are not able to see or hear, affecting their interaction and the exposure to sounds and sights they offer to the infants (Bartram-Torrance, unpublished

data), creating suboptimal conditions for early language acquisition. Along the same lines, the reduction in FC activation in the Gambian cohort in relation to object representation can be speculated to impede further development. Furthermore, the reduced responses in the imitation study that were observed can be understood as indicative of an adaptive behaviour, due to the higher prevalence of physically harmful objects in the Gambian infant's environments. However, this behaviour still impedes a useful learning mechanism, therefore holding potential for a negative downstream effect in terms of imitative learning.

In summary, the IS provides a framework to think about early brain organisation, as well as about possible pathways in which poverty related environmental influences can affects the developing system as a whole. This is a particularly crucial consideration in the context of temporally constrained, critical developmental periods occurring during infancy and early childhood. The adaptation vs deficit framework adds a perspective to better understand some of the cognitive outcomes in terms of infant's experienced environments. While only partially applicable to data collected for this thesis the discussion of how to view any results from this project at large remains an important consideration, as it provides a starting point to fully consider infants' experienced environments and their relation to what is observed on a neurodevelopmental level.

7.3 Critical review

7.3.1 Participation, representativeness, Attrition. All presented findings have to be considered in the context of some specific characteristics of the study sites, the participant populations and infant behaviour and its effects on data quality. The following section will discuss the sample representativeness at both study sites, as well as any systematic bias that might have skewed the sample either between age points or between study

sites. At both sites, participants were sampled from within the community, at a local antenatal clinic. In Keneba all pregnant women within the surrounding villages undergo antenatal care at the local clinic in Keneba, and further it is very common for the local community to participate in ongoing research, with more families than not being enrolled in at least one study (Moore, personal communication). Because pregnant women are frequently approached about participation in ongoing trials, it also is more likely that our sample represents a cross section of the local community, as less self-selection occurs at the point of enrolment. In Cambridge, participants also were enrolled from an antenatal clinic open to all women receiving antenatal care at Addenbrookes Hospital. However, with its close links to the University of Cambridge and the elevated base rates of parents with higher education degrees, the sample obtained at this site still cannot be regarded as truly representative cross-section of the UK population. Further, the clinic at which recruitment took place was disproportionately frequented by first time parents, and first time parents were also more likely to enrol, whereas second or third time parents frequently could not commit to the time intensive, longitudinal study visits. While this mode of recruitment might be considered a disadvantage, many other projects rely on self-selection of families by having parents indicate interest and subsequently being invited for studies. By recruiting from the hospital we could ensure that we 1) were notified of the birth early on, bearing in mind that babies were first seen at 7-14 days of age and 2) that we could discuss any concerns parents might hold there and then, therefore ensuring that some families signed up who would not have considered it otherwise.

While an in depth analysis of variance in SES within each sample was beyond the scope of this thesis, recommendations are made in Chapter 2 as to how to best utilise the collected SES information in the future. While the vast difference in relevance of the collected SES indicators between the

study sites prevent a direct site comparison, these data can be used as a first indicator of variance within each cohort.

Once enrolled no differences were noted between age points or sites as to the parents' willingness for their infants to engage with all aspects of the study protocol. However, infant behaviours differed both between age points as to their tolerance to wear the necessary headgear and to attend for a prolonged period to the presented stimuli on the screen, with older infants being more reluctant to have the headgear placed and younger infants being more easily overwhelmed and fussy during stimulus presentation. In Keneba, it was further noted that infants of all ages were more likely to fall asleep during both the fNIRS and the EEG session. While no differences were observed at least in the ERP study as to whether infants were asleep or awake, these observations might suggest that data presently does not include a proportion of infants with a tendency to become overstimulated by the assessments.

Some differences were observed between sites in terms of the general structure of the visits, with visits in Keneba taking much longer at all age points. In communication with the local team this was partly due to infant's tendency to 1) require more breaks to feed and sleep throughout the day 2) behavioural assessments taking much longer to engage the infant and therefore taking a long time. To address this, the local testing team has primarily operated on a 7 day testing schedule, to allow for all visits to be completed within the appropriate age range, even in cases where infants had to be called back the next day for the remainder of assessments. In Cambridge, the same schedule was implemented, as many parents were only able to come in on weekends. This participant led approach enabled excellent retention at both sites. To date, only two families dropped out in Cambridge, and only four in Keneba. Excluding data loss due to equipment

malfunction, which was more attributable to the remoteness of the study site rather than participant characteristics, success rates in both neuroimaging and the behavioural studies were comparable across sites.

In addition to these participant and cohort characteristics, some methodological consideration have to be taken into account, as will be laid out in the following.

7.3.2 Methodological considerations. As touched upon in Chapters 2, 5 and 6 all data presented in this thesis need to be considered in the context of some methodological limitations. Hereby, any differences between testing protocols of different age points as well as between the two studied cohorts are of crucial importance in the context of the comparative analyses made throughout this thesis. As reviewed in Chapter 1, much of what we know about infant development stems from assessments designed for, and optimised in infant cohorts in high-income countries. In this thesis but also in the BRIGHT project one ongoing consideration was the appropriateness of the protocol in terms of culture and age of infants. As pointed out in Chapter 1, the main objective hereby was to implement culturally fair neuroimaging measures, to study infant development as objectively as possible. The two neuroimaging studies presented in this thesis have provided important mechanistic insight into neurodevelopmental trajectories within our cohorts without being reliant on infants' overt responses or interaction with an experimenter. Still, even cultural differences in familiarity with the presented stimuli (i.e. object hiding games) might have contributed to the observed results. In case of the imitation study, overt play-based behaviour was investigated, which is of course highly reliant on motor development and prior exposure to object based play. A site difference was anticipated for engagement with the behavioural task, which however did not occur in the current study. Infants

at both sites initiated contact with the presented items during the behavioural imitation task. As noted in Chapter 6 however, the way in which engagement was coded here was merely reliant on any instance of object touch, and more refined coding options could be applied in the future to further tease apart this issue. In contrast to the imaging studies this study offers an indication of infants' active behavioural ability to learn via imitation. While the neuroimaging outcomes hold an inherent scientific value, their ultimate clinical utility lies in their predictive relationship with infants' neurodevelopmental and behavioural outcomes.

In terms of the overall testing situation, it was ensured that only Gambian staff were involved in direct interaction with the infant, which helped control for negative impact of potential race effects, which were observed with our Caucasian researchers in between sessions. Similarly, all stimuli relying on either language or videos of human actors were adapted for use at each study site. Even in light of these adaptations, it must be considered though that prior exposure to language will have an effect on the associated neural response pattern.

While the neuroimaging studies can be argued to add objectivity in terms of standardisation of assessment, they can be prone to some seemingly trivial issues, such as an age dependent increase of the number of infants with dark hair impeding optical imaging, as well as braids, preventing good scalp contact of the EEG electrodes. While boys in our cohort for the most part continued to have shaved heads until 24 months of age, this was not true for girls, leading to a potential gender skew in the older age groups.

The comprehensive approach of the BRIGHT project, including its focus on devising protocols optimised per age point across the two sites, was necessary to document feasibility for each assessment modality and

paradigm. This will allow future projects to streamline protocols in relation to the feasibility of assessments demonstrated. Even with all these consideration accounted for, it needs to be clear that while the presented data does allow for a comparison between a high resource and a low resource setting, and therefore a low vs high risk cohort, there are many other accompanying factors that are vastly different between the sites. While this prevents a strong attribution of any of the findings to any one factor for the current results, this issue will be among the most significant ones to be addressed in future research.

Statistical power. Analyses throughout this thesis were conducted on subsets of the full sample, posing some constraints on the statistical power. Whereas sample sizes were sufficient to reliably detect strong effects, such as condition differences, accuracy for estimates of more subtle effects may have been limited. In context of modelling data with many outcome variables, such as presented in Chapter 6, the partial samples may have not been sufficient depict more subtle interactions, as is indicated some trending hypothesised interactions.

Limitations of imaging methods in context of cross-cultural research.

While neuroimaging holds many benefits when working with infants across a range of study sites, some limitations of these methods need to be considered. As laid out in context of the general methodology of this thesis, neuroimaging measures require a greater degree of infant-tolerance as they are more restrictive of participants' movement and require the placement of the respective headgear for sometimes extended periods of time. Further, while most neurodevelopmental studies pose a novel situation to an infant, behavioural assessments are usually freer in the choice of objects they employ in order to elicit the response of interest, whereas imaging will

always depend on equipment that is likely very different to what infants are usually exposed to. Together, these issues might bias imaging findings towards favouring those infants that are, for instance, a) less fearful of novel situations, b) less motorically active/developed. The former point is of importance when seeking to compare cohorts across settings that differ with regard to infants' exposure to unfamiliar environments, while the latter is crucial when studying infants that may be more or less active due to more advanced/delayed motor development or illness.

There are some further biases specific to certain imaging modalities. fNIRS being an optical imaging method is reliant on light transfer through tissue. While it has been shown that dark skin does not negatively affect the NIRS signal when adjusting laser intensity appropriately (Lloyd-Fox, 2014), it needs to be considered that when comparing populations that differ in skin/hair colour pilot studies should be used to determine optimal settings so as to not systematically include certain participants due to excessive light attenuation.

With regard to study design, neuroimaging studies are just as prone to cultural bias as behavioural assessments when it come to the choice of appropriate stimuli. A large body of infant EEG/fNIRS literature relates to neural responses related to language or face processing. If seeking to design tasks that can be used more universally, differences between groups regarding infant carer interaction or prevalence of infant directed speech need to be considered. Concerning the studies in this thesis, particularly the EEG studies hold potential for translation to other contexts, given that they are reliant on relatively basic sensory processes, which can be argued to be more easily to be used across settings than stimuli relying on images of faces or that involve language.

Another notable issue in this context is that neuroimaging evidence, can be attributed an objectivity that may not be fully justified when considering developmental brain-behaviour relationships. This is illustrated by the fNIRS task described in this thesis. While it is not reliant on infants' overt behavioural response to engage in object search, this paradigm is still closely linked to infants' prior experiences with objects' permanence, and therefore to cultural factors. For this reason, only the method by which object permanence is assessed can be deemed more objective, as it is not reliant on overt behaviour, whereas the capacity that is measured is culturally dependent.

Overall, it needs to be noted that the implementation of neuroimaging methodology in itself does not prevent assessments from cultural bias. However, in experimental designs that are taking into consideration how culturally dependent factors could affect the outcome, direct measures of brain function are a way to avoid having to wait until a cognitive process manifests in overt behaviour, thus allowing their early implementation, and eliminating some bias when used across cultures that differ with regard to their norms of whether infants are expected to engage with novel objects and people.

Insight of cohort comparisons as presented in this thesis. Counter to what analyses presented throughout this thesis may suggest, the goal of the current research is not to attribute observed differences between cohorts solely to experiences of environmental adversity in one but not the other cohort. The main rationale for the inclusion of the Cambridge cohort lay in the novelty of some of the assessments in terms of the: a) hardware used, b) experimental design, or c) feasibility of the scope of the protocol at the different age points. As the implementation of such a protocol can pose a range of challenges, studying an infant cohort under well-controlled

conditions had the advantage of being able to detect and trouble shoot these issues immediately, and to then translate any fixes to the Gambian site.

The Cambridge cohort however cannot be viewed as a control group as they do not differ only with regard to the dimension of interest (i.e. exposure to early adversity). Rather, the two groups should be viewed as parallel cohorts, which makes it easier to appreciate advantages of including both groups into analyses at this stage, as has been done in this thesis. While some of the assessments used in this thesis have been used before (i.e. ERP task, DI paradigm), others do not have much literature to back up what developmental changes might be observed using this task (fNIRS paradigm). Even where paradigms have been used repeatedly in different cohorts and age groups, differences in hardware/analysis streams makes it difficult to draw out longitudinal developmental trajectories. Data collection at the Cambridge site thus enabled the confirmation that taking into account the necessary adaptations we made to paradigms, previously reported developmental changes could indeed be replicated. This enhanced our level of confidence that any observed effects are likely due to developmental processes, rather than a function of the specific design employed. Secondly, the examination of the two cohorts in parallel allows an investigation of the universality of certain responses. Again, given the outlook of this project to define early markers, one issue lies in understanding processes that may indeed be affected by infants' environments. In instances where cohorts were found not to differ, this is of academic interest but might also disqualify some assessments as suitable to identify at-risk infants. Thirdly, even though the cohort comparison as presented here is relatively unspecific and does not allow an understanding of mechanisms between early life adversity and later outcome, it does provide differential neurodevelopmental trajectories in both a low and a high risk setting, thus

providing a first indication of which processes might be found to differ between the two settings. Lastly, it has to mentioned in this context that it was considered to adopt a design within the BRIGHT project to include an infant cohort growing up in the coastal region of The Gambia, which compared to Keneba is a higher resource setting. This approach could have yielded an easier group comparison, however, would have come with its own caveats; tourism in the coastal regions has vastly increased over the past years, which has led to significant, and ongoing, changes to infants' and children's home environment. Development in this cohort would thus have been confounded by rapid developments of the overall living situation, which again would have made it difficult to understand genuine neurodevelopmental processes. In conclusion it has to be said that the analyses presented here provide an intermediary step in understanding the brain development of a previously understudied cohort. Ultimately, the aim is to move entirely into within group comparisons, which will be far superior in terms of controlling to a range of extraneous factors.

7.3.3 Recommendations for future research.

As laid out above, the most significant limitation underlying all presented findings are the vast number of ways in which the two infant cohorts differ in terms of their environment, culture and socialisation.

The findings presented in this thesis constitute one of the first steps in examining what is an unprecedented, rich dataset. The presented findings bear relevance in terms of proving the feasibility of the methods used, demonstrating many of the anticipated developmental effects but equally providing a first indication of ways in which development in the two cohorts might be converging. The cohort differences described here are only the very first step and should not be interpreted as being directly, causally linked to any one factor that differs between sites. Assessment of the

Cambridge cohort was designed not as a control group, as this would necessitate the infants' similarity across all non-measured dimensions. Rather, it was designed as a reference sample, in which feasibility of certain newly introduced studies could be assessed. As many paradigms had not been used in the same way before this project, having a sample in which staff highly experienced with infant assessment could have continuous oversight of assessment and immediate control of the data much increased confidence in our findings from both sites. However as mentioned previously, the Gambian sample was powered with an analysis approach in mind more focused at delineating individual differences rather than performing a group wise comparison. Even though most findings in this thesis did not observe greater variance within the Keneba cohort, a closer examination of subgroups within this cohort will still be beneficial. Directions proposed to be taken for future analyses of data within this project will be laid out below.

Definition of developmental trajectories. Developmental trajectories are going to be examined further, by inclusion of additional age points at either study site. This will establish the basis of normative developmental trends within each cohort. It will further be crucial when considering whether differences observed between cohorts thus far fit are indicative of a differential or a temporarily delayed development, the latter would imply an eventual catch up effect at a later age point.

Examination of construct validity across formats of assessment. Further it should be assessed in how far the different modes of assessment interlink. Hereby, studies assessing the same construct (i.e. NIRS and EEG habituation data) should be considered together, examining relationships across measures per infant. As the BRIGHT project has a broad scope of assessments of various modalities collected on the same infants

longitudinally, this inter-relation will be one way to exploit this unique feature of the project. This will help increase confidence in the construct validity of all paradigms and help integrate functional and spatial information concurrently, which could not be achieved by any one assessment modality.

Predictive power of measures collected early. The predictive power of some measures should be closely investigated. As one of the long term goals of this research is to define early indicators of atypical development, one useful avenue to pursue is the assessment of predictions made based on measures taken soon after birth and their relationship to later outcomes, significant for everyday functioning. Based on this thesis specifically, it could be investigated in how far early novelty detection holds predictive value for imitative learning at 12 months of age. There is some indication that early novelty detection is a promising marker in predicting later life outcomes, such as IQ in adulthood (Fagan, Holland & Wheeler, 2007).

In the wider context of the BRIGHT project, it could also be investigated how any one early marker relates to general cognitive development at a later age point informing which marker or set of makers holds potential for future clinical use.

Long term follow up. While prediction can be made within the scope of the data collected as part of the BRIGHT project, it would be of interest to examine even longer-term outcomes of development within these same infants. Most studies examining neurocognitive development in LMIC's so far have considered the preschool to primary school period (McCoy et al., 2015), during which deficits are present in a large proportion of individuals in LMIC's. An association between markers from early infancy and outcomes such as those collected in previous, population based research would

provide further justification for the utility of taking a more global approach in the study of infant development.

Stratification of sample. While all these approaches focus on associations based on the entire infant cohort, there also is great potential in examining subsets within the full sample. Even though this has not been formally assessed to date, it is expected that a subset of infants in Keneba will be particularly prone to adverse developmental outcomes. Past research has shown, that for example with regard to nutritional status, a quarter of infants experience medium to long term undernutrition (Nabwera et al., 2017), meaning that this subset of infants might be at particular risk, thus enabling a comparison between these and more optimally developing infants. This would provide a more powerful comparison than the cohort comparison presented in this thesis, as it enables greater control over between group differences.

Examination of individual differences. The stratification based approach should be extended through a specific examination of individual differences. This approach holds a lot of potential, as it allows for a delineation of individual developmental trajectories within the context of a range of risk factors experienced around the time of each assessment time point. Therefore, it does not only enable specific associations between potential risk factors and outcomes, but also allows for an investigation of both the immediate and more long-term sequelae of infants environment during critical developmental periods.

Definition of mechanistic associations of risk- and resilience building factors. While the definition of early markers of atypical development needs to be pursued, the same argument is true for the definition of early resilience building factors. A thorough understanding of both of these influences on early development will enable to identify possible mediating and

moderating influences of relevant factors, which will be crucial in understanding preceding factors of any of our collected outcome measures. Together, risk and resilience building factors could feed into the identification of at risk individuals, and the implementation of interventions drawing upon mechanisms known to be associated with a more-optimal outcome.

Generalisation across settings. While this work focuses on an infant population in only one West-African country, a thorough mechanistic understanding of specific environmental factors and neurodevelopmental outcome also bears potential for wider application world-wide. Some research is currently ongoing, focusing on infant and child development in other African countries (i.e. Guinea-Bisseau, Roberts et al., 2017, Ivory Coast, Jasinska & Guei, 2018) as well as other LMIC's (i.e. Bangladesh, Storrs, 2017, India). The consideration of commonalities between findings obtained from these lines of research will be crucial to delineate processes specific to any one context, and to help identify those found to be more universal.

Sensitivity of markers in the context of interventions. Some of the proposed findings lend themselves more readily to the definition of early markers than others. One example from this thesis stems from the ERP study, in which the difference in reaction to the infrequent and trial unique sounds could be utilised as a developmental index. Differences in the timing of the response switch could be utilised as a marker of developmental maturity, implemented around critical developmental ages. Indeed, an implementation of this paradigm in the context of an early nutritional intervention study in infants in Bangladesh is under discussion, which could yield valuable insights into the role of this marker's sensitivity in response to external, specific intervention within a randomised controlled trial. Since the

ultimate goal of this research is to put in place interventions, the beginning of examining the feasibility of some of the outcomes described in this project will be useful and lead toward this goal.

7.4 Final conclusions

This thesis was aimed at providing an understanding of memory development during early infancy in the context of exposure to environmental risk. Results showed developmental changes of different magnitude across the two cohorts under investigation, with an overall reduced developmental change in the Gambian cohort. This was true for responses based on electrophysiological, haemodynamic and behavioural markers. This thesis extends the understanding of early memory development by taking a multi-modal approach implemented across a critical developmental period. Through the implementation of the implemented methods in a novel context, it could further be shown that the current protocol holds potential for future application across different settings. The presented findings form part of a newly emerging field of research, which will in the future yield valuable insights into global neurocognitive development and play a crucial role in enabling a greater number of infants and children world-wide to fulfil their cognitive potential.

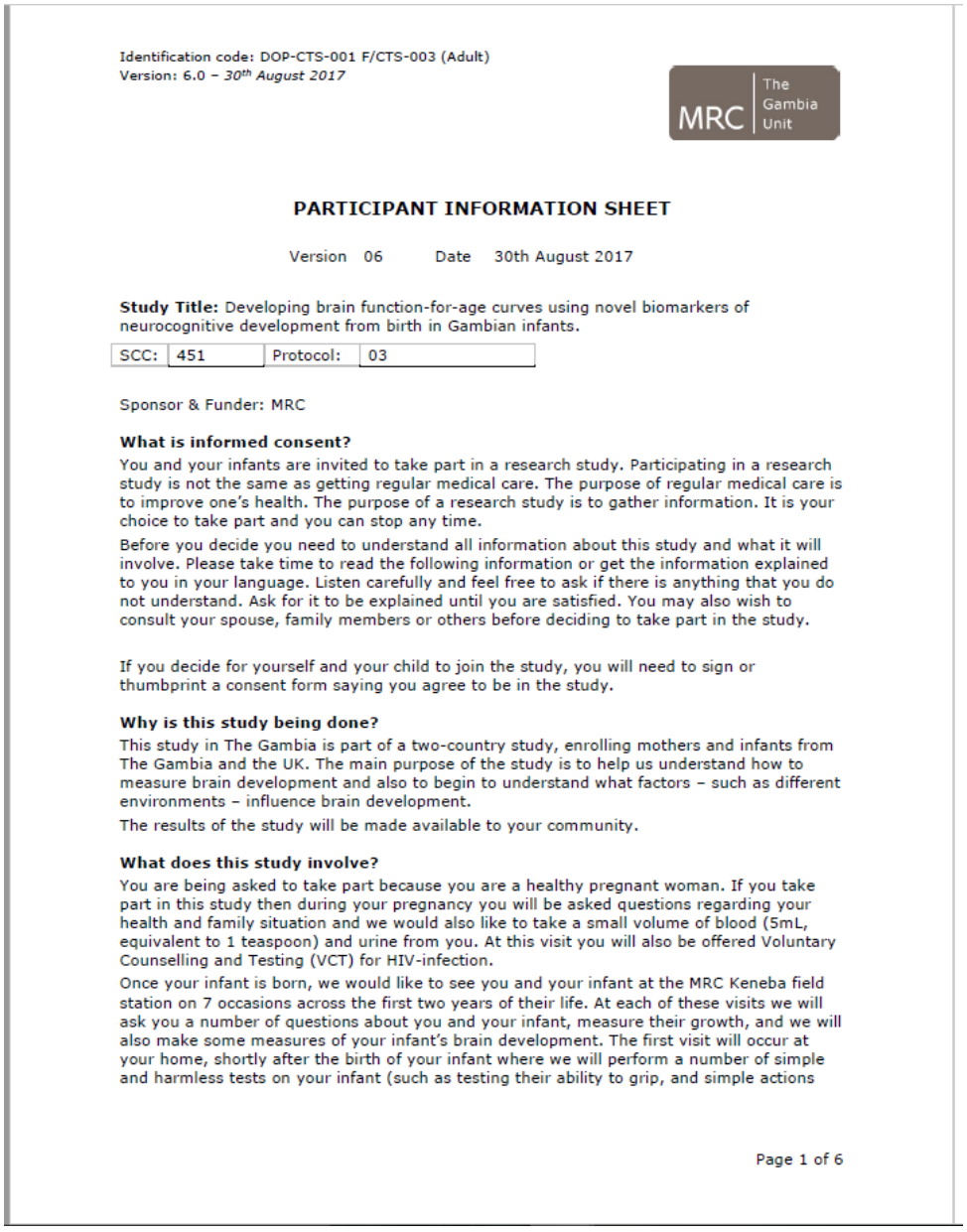
Appendix

Appendix

Appendix

Chapter 2. General methodology.

2.1 Parent Information sheet – Keneba



Identification code: DOP-CTS-001 F/CTS-003 (Adult)
Version: 6.0 – *30th August 2017*

MRC The Gambia Unit

PARTICIPANT INFORMATION SHEET

Version 06 Date 30th August 2017

Study Title: Developing brain function-for-age curves using novel biomarkers of neurocognitive development from birth in Gambian infants.

SCC:	451	Protocol:	03

Sponsor & Funder: MRC

What is informed consent?

You and your infants are invited to take part in a research study. Participating in a research study is not the same as getting regular medical care. The purpose of regular medical care is to improve one's health. The purpose of a research study is to gather information. It is your choice to take part and you can stop any time.

Before you decide you need to understand all information about this study and what it will involve. Please take time to read the following information or get the information explained to you in your language. Listen carefully and feel free to ask if there is anything that you do not understand. Ask for it to be explained until you are satisfied. You may also wish to consult your spouse, family members or others before deciding to take part in the study.

If you decide for yourself and your child to join the study, you will need to sign or thumbprint a consent form saying you agree to be in the study.

Why is this study being done?

This study in The Gambia is part of a two-country study, enrolling mothers and infants from The Gambia and the UK. The main purpose of the study is to help us understand how to measure brain development and also to begin to understand what factors – such as different environments – influence brain development.

The results of the study will be made available to your community.

What does this study involve?

You are being asked to take part because you are a healthy pregnant woman. If you take part in this study then during your pregnancy you will be asked questions regarding your health and family situation and we would also like to take a small volume of blood (5mL, equivalent to 1 teaspoon) and urine from you. At this visit you will also be offered Voluntary Counselling and Testing (VCT) for HIV-infection.

Once your infant is born, we would like to see you and your infant at the MRC Keneba field station on 7 occasions across the first two years of their life. At each of these visits we will ask you a number of questions about you and your infant, measure their growth, and we will also make some measures of your infant's brain development. The first visit will occur at your home, shortly after the birth of your infant where we will perform a number of simple and harmless tests on your infant (such as testing their ability to grip, and simple actions

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directed at them). These tests are specifically designed to test behaviour in the early neonatal period. This session may be video-recorded.

When your infant is aged 1, 5, 8, 12, 18 and 24 months of age we will then ask to see you and your infant at the field station in Keneba. At each of these visits we will test your infant's brain development using a special hat that contains light sensors linked to a computer. These light sensors are like tiny torches and are completely harmless to your child. Putting the hat on will only take a few minutes, after which your child will be shown a collection of pictures and hear a range of sounds. The light sensors will record how he/she responds to the pictures and sounds. The session will be videotaped and recorded using a small camera (called an eye-tracker) so that staff can record your child's behavioural responses, as well as the brain signals we measure from the light sensors.

When your infant is aged 1, 5 and 18 months of age we will also test their brain using another method (called electroencephalogram). Our brain communicates using faint electric signals. We can test this communication by placing an array of sensors of the head that can pick up the natural activity of the person's brain. The equipment we use is known as the Enobio, specifically designed for babies. This technique is completely safe and has been used for studying how the brain works for many years without using expensive equipment.

At each visit we will also ask your infant to perform some simple tasks (such as responding to toys) in order to assess their development. These will also be videoed. Finally, we would also like to make a short recording of you talking to your infant, which will be recorded. This helps us to understand how you are both communicating with each other. We may also ask if we can conduct these assessments in your home environment, so we can record you interacting with your infant at home. If the infant's father is available, we may also request to record him interacting with your infant also. We would also like to ask the infant's father some of the same questions we have asked you throughout the course of the study.

At each visit we will collect a finger prick blood sample from your infant, to measure the amount of iron in your child's blood. When they are aged 5, 12 and 24 months, instead of the finger prick, we would like to collect a small quantity of blood from their vein (3mL, < 1 teaspoon) and also a small amount of their urine. These samples will be used to measure the amount of different markers (such as nutrients) in your infant's body that may be associated to neurocognitive development.

In between these clinic visits to MRC Keneba, we will make regular visits to you in your home to ask simple questions regarding your infant feeding (fortnightly) and to measure your infant's growth (monthly).

When your child is aged 12, 18 and 24 months we will also visit your home to conduct some additional assessments. The first assessment involves a 1 – 2 day recording of language and environmental sounds of the child. Your child will wear an audio recording device within a t-shirt provided by the study team. We will not be assessing specific words used by you or the child's other relatives but rather the type of interactions the child has with others and the words that they can say. In addition to the recording device we will also conduct a home observation and/or interview to record who interacts with your child during this period. The second assessment involves your child wearing a monitor to record sleep quantity and quality. This monitor will be worn on the leg or wrist for 3 – 5 days to record daytime and night-time sleep during this period. We would also like to ask you some questions about the words that your child knows and can say, and what they play with while at home.

If you need to leave the clinic visits early before everything can be completed, we are also happy to arrange a further visit to the clinic, or to you at home to complete questionnaires at a time that is convenient.

In case the investigator discovers you or your child is sick and decides that you or he/she cannot participate in the study because of that, you or he/she will receive immediate care at the MRC Keneba clinic. If the research study needs to be stopped, you will be informed and your child will have the normal medical care.

What will happen to the samples taken in this study?

The samples collected in this study will firstly be processed and stored at the MRC laboratories, and then shipped overseas for analysis by the research team implementing the study.

What harm or discomfort can you expect in the study?

Collecting blood samples will cause a minor, temporary discomfort to yourself and your infant. However, we do not anticipate any other harm or discomfort from this study.

What benefits can you expect in the study?

The close contact your child and the family will have with our field staff will provide you with an immediate opportunity to address any health care concerns you have during the contact period.

Will you be compensated for participating in the study?

You will not get paid for participation of you or your child in the study. We will either visit you in your home, or bring you to the field station in Keneba for measurements, so there will be no transportation costs.

What happens if you refuse to participate in the study or change your mind later?

You are free to participate or not in the study and you have the right to stop participating at anytime without giving a reason. This will not affect the medical care that you would normally receive.

In case you decide to withdraw your participation during the study, any information already generated from the samples until the time of withdrawal will be used and samples already collected, for which you have given consent, will also be analysed and data used. The study doctor may also ask for tests for your safety.

Should any new information become available during the study that may affect your participation, you will be informed as soon as possible.

If you are injured in the study what compensation will be available?

We will be responsible to provide for treatment caused by procedures of the research study.

If medical treatment is required as an emergency, please refer to your health centre or clinic and contact the field worker who gave his/her telephone number to you or contact the Keneba research nurse, Mr Edrissa Sinjanka, on 7160857.

How will personal records remain confidential and who will have access to it?

All information that is collected about you or your child in the course of the study will be kept strictly confidential. Your personal information will only be available to the study team members and might be seen by some rightful persons from the Ethics Committee, Government authorities and sponsor.

Who should you contact if you have questions?

If you have any queries or concerns you can contact Dr Dr Momodou Darboe on 9904248 and you can always call the personal numbers of the study staff given to you.

Please feel free to ask any question you might have about the research study.

Who has reviewed this study?

This study has been reviewed and approved by a panel of scientists at the Medical Research Council and the Gambia Government/MRC Joint Ethics Committee, which consists of scientists and lay persons to protect your rights and wellbeing.

2.2 Consent Form – Keneba

Identification code: DOP-CTS-001 F/CTS-003 (Adult)
Version: 6.0 – *30 August 2017*

MRC Unit, The Gambia

SCC:	1451	Protocol 3

CONSENT FORM

Participant Identification Number: |__|__|__|__|__|__|__|__|__|__|__|__|__|__|

_____
(Printed name of participant)

☐ I have read the written information **OR**

☐ I have had the information explained to me by study personnel in a language that I understand,

and I

- confirm that my choice to participate is entirely voluntarily,
- confirm that I have had the opportunity to ask questions about this study and I am satisfied with the answers and explanations that have been provided,
- understand that I grant access to data about me, my infant and my infant's father to authorised persons described in the information sheet,
- understand that parts of the study will be recorded/videoed for research purposes,
- have received sufficient time to consider to take part in this study,
- agree to allow myself and my infant to take part in this study.

Tick as appropriate

I agree for my samples and those from my infant to be shipped outside of The Gambia — Yes ☐ No ☐

I agree to further research on my samples and those of my infant as described in the information sheet — Yes ☐ No ☐

Participant's signature/ thumbprint* _____

_____ _____
Date (dd/mmm/yyyy) Time (24hr)

Printed name of witness* _____

Printed name of person obtaining consent _____

I attest that I have explained the study information accurately in _____ to, and was understood to the best of my knowledge by, the participant. He/she has freely given consent to participate *in the presence of the above named witness (where applicable).

Signature of person obtaining consent _____

_____ _____
Date (dd/mmm/yyyy) Time (24hr)

** Only required if the participant is unable to read or write.*

Version	1451	Date	30th August 2017

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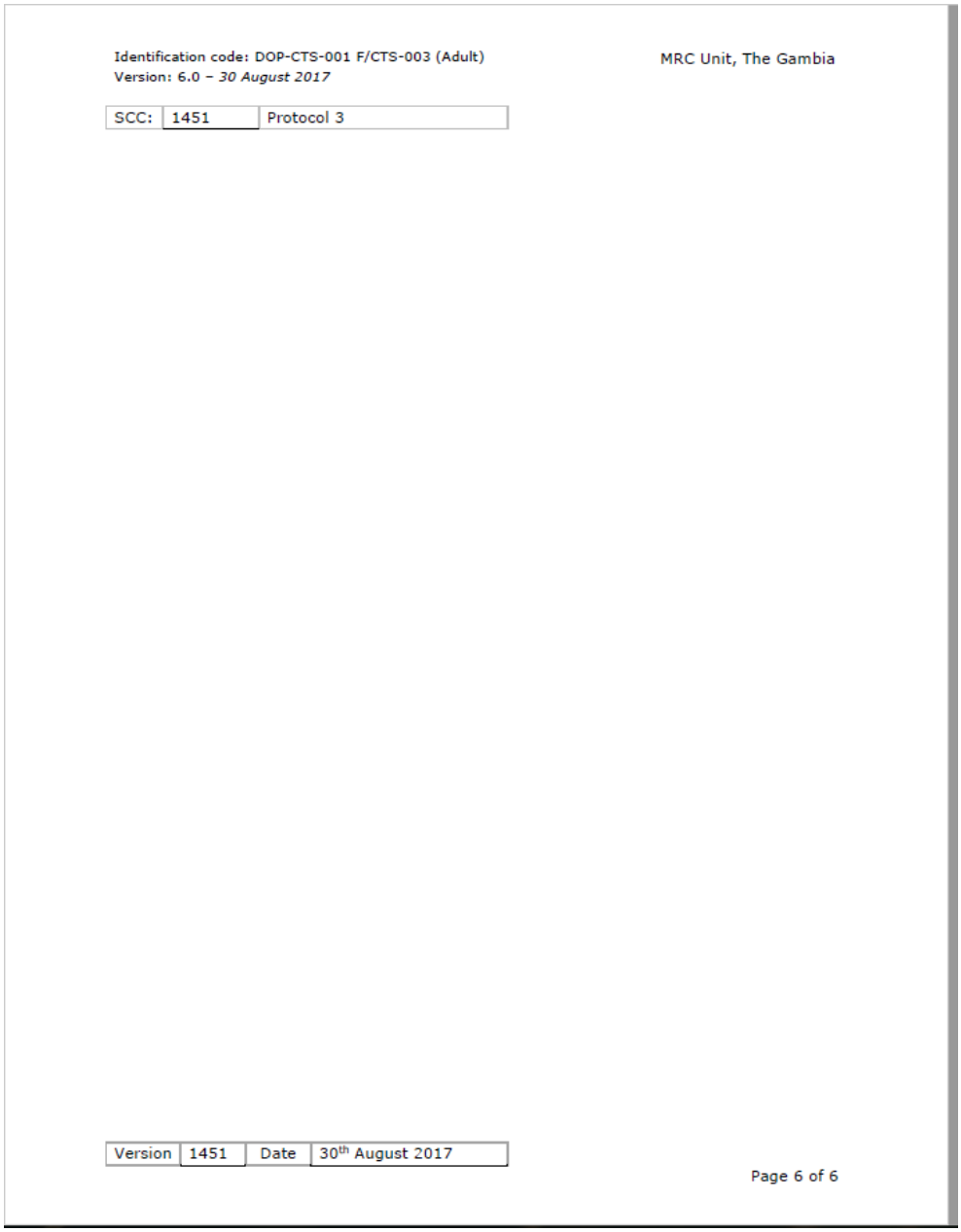

Identification code: DOP-CTS-001 F/CTS-003 (Adult)
Version: 6.0 – *30 August 2017*

MRC Unit, The Gambia

SCC:	1451	Protocol 3

Version	1451	Date	30th August 2017

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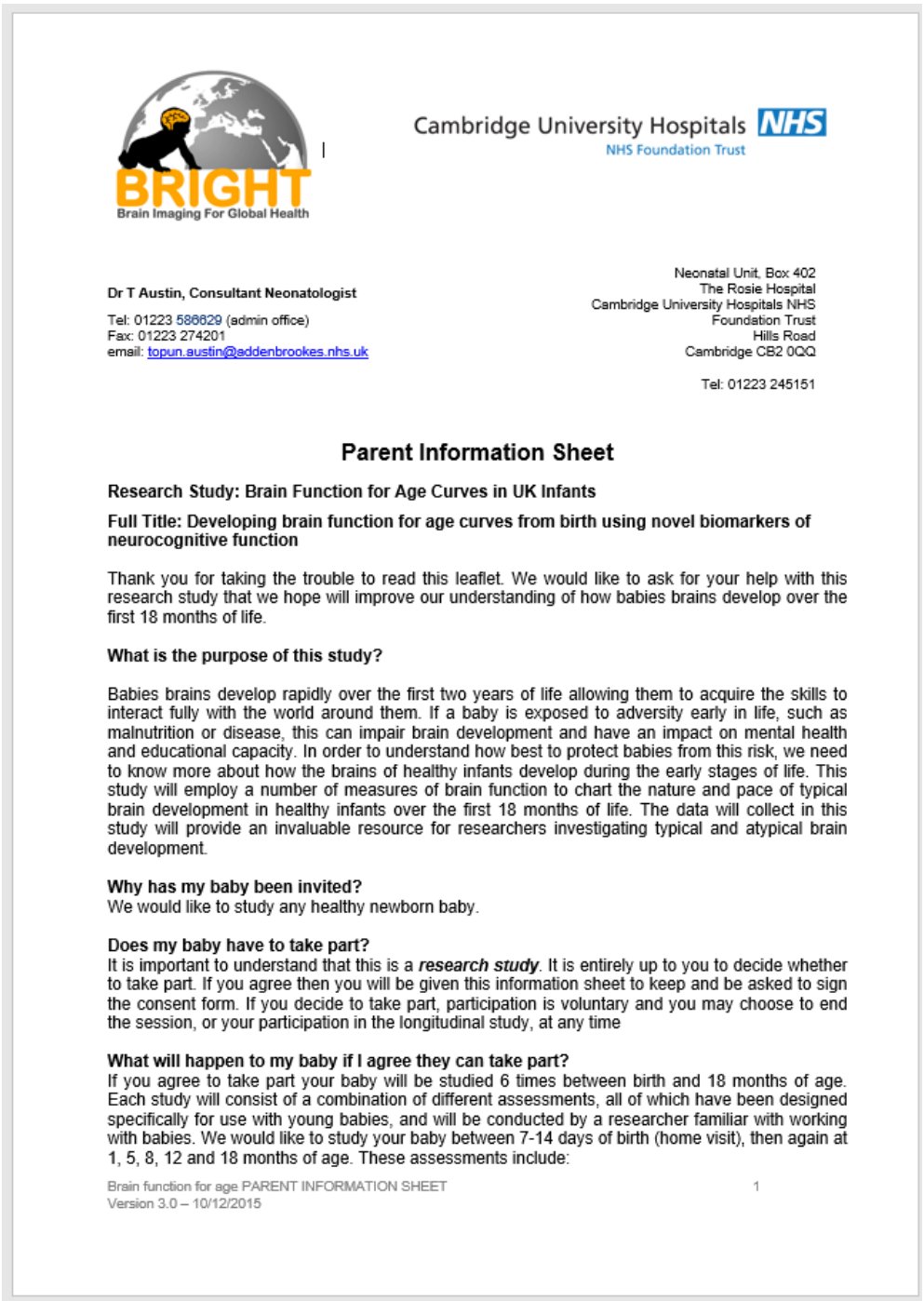
2.3 Parent Information Sheet – Cambridge



BRIGHT
Brain Imaging For Global Health

Cambridge University Hospitals NHS
NHS Foundation Trust

Dr T Austin, Consultant Neonatologist

Tel: 01223 586629 (admin office)
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email: topun.austin@addenbrookes.nhs.uk

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The Rosie Hospital
Cambridge University Hospitals NHS
Foundation Trust
Hills Road
Cambridge CB2 0QQ

Tel: 01223 245151

Parent Information Sheet

Research Study: Brain Function for Age Curves in UK Infants

Full Title: Developing brain function for age curves from birth using novel biomarkers of neurocognitive function

Thank you for taking the trouble to read this leaflet. We would like to ask for your help with this research study that we hope will improve our understanding of how babies brains develop over the first 18 months of life.

What is the purpose of this study?

Babies brains develop rapidly over the first two years of life allowing them to acquire the skills to interact fully with the world around them. If a baby is exposed to adversity early in life, such as malnutrition or disease, this can impair brain development and have an impact on mental health and educational capacity. In order to understand how best to protect babies from this risk, we need to know more about how the brains of healthy infants develop during the early stages of life. This study will employ a number of measures of brain function to chart the nature and pace of typical brain development in healthy infants over the first 18 months of life. The data will collect in this study will provide an invaluable resource for researchers investigating typical and atypical brain development.

Why has my baby been invited?
We would like to study any healthy newborn baby.

Does my baby have to take part?
It is important to understand that this is a ***research study***. It is entirely up to you to decide whether to take part. If you agree then you will be given this information sheet to keep and be asked to sign the consent form. If you decide to take part, participation is voluntary and you may choose to end the session, or your participation in the longitudinal study, at any time

What will happen to my baby if I agree they can take part?
If you agree to take part your baby will be studied 6 times between birth and 18 months of age. Each study will consist of a combination of different assessments, all of which have been designed specifically for use with young babies, and will be conducted by a researcher familiar with working with babies. We would like to study your baby between 7-14 days of birth (home visit), then again at 1, 5, 8, 12 and 18 months of age. These assessments include:

- Assessments of the behaviour of your baby:
  - We will observe your baby's behaviour as he/she plays some short games and interacts with you and the researcher. Depending on the age of your baby, these activities will include looking at how your child sits, stands, crawls, walks, grasps onto objects, responds to sounds, and creates their own sounds.
  - We will record a video of yourself and your baby interacting together.
  - When your baby is 18 months old they will play a simple game on a tablet computer. The computer will present them with a range of interesting shapes and sounds which they can interact with and we will record the way they respond to the game.
  - We will record how your infant tracks information presented on a computer screen. Your baby will be presented with videos to watch and a computer will record their eye movement.

- Imaging your baby's brain function.

A typical setup for our study showing a baby sat on her mother's lap watching a video on the TV screen

  - Optical Imaging: Your baby will sit on your lap and will wear a special hat that contains light sensors. These light sensors are like tiny torches and are completely harmless to your child. The light is able to measure oxygen levels in different parts of the brain, which change depending on brain activity. The system is completely safe and has been designed for and used with infants in many other studies. Putting the hat on will only take a minute or two while we adjust the size to your child. We would like to look at which areas of the brain respond in babies when they watch videos and listen to sounds. The session will be videotaped so that staff can record your child's behavioural responses, as well as the brain signals we measure from the light sensors.

  - Evoked Response Potential Imaging: We will repeat the same study with a system that measures electrical impulses from your baby's brain. A similar cap will be placed on your baby's head containing sensors to measure these electrical impulses. The system is completely safe and has been designed for and used with infants in many other studies. We will look at which areas of your baby's brain respond when they watch videos and listen to certain sounds.

- Measurements of growth: We will measure your baby's growth at each visit. We will measure their weight, length, head circumference, mid-upper arm circumference, knee-heel length and skinfold thickness.

- Family and Health Questionnaires: We will ask you to complete questionnaires about your baby's health , your health and their family and home environment.

- In addition to the visits with your baby we will also arrange one home visit approximately four weeks before your baby's due date to ask some of these questionnaires about your health, family and home environment. We will also access your medical notes to record details of your health, pregnancy and later the birth details.

Your baby will not undergo all assessments at each visit. We estimate the average time for each visit will be between 2.5 and 3 hours. We will conduct the assessments to fit in around your baby's feeding and sleeping pattern. You will be with your baby at all times if you wish to be.

All the data, images and videos collected from these tests will be stored on an encrypted computer. We will keep information collected in confidence. This means we will only tell those who have a need or right to know. Published reports based on these studies will not mention individuals. Your child's file will be given a code number rather than a name for us to identify it. Only staff members working on the project will have access to the videotapes. Images or short segments of selected videotapes, about 5 minutes in length, may be retained and shown to other mothers and researchers as examples of child behaviour or published in scientific journals with your consent. These images will not contain any identifiable information about or name of your baby.

Where will the study be done?
The first study will be done during a visit to your home. All other studies will be done in the Evelyn Perinatal Imaging Centre, Rosie Hospital Cambridge. This is a dedicated research facility for the study of newborn and young infants.

What are the possible risks/side effects of taking part?
We do not anticipate any risks or side effects from taking part. This is an observational study and all methods are widely used to study newborn infants and children and are completely non-invasive.

What are the potential benefits of your child taking part?
This study will not directly benefit your baby. We hope though that the results of this study will help us understand how healthy babies brains develop over the first 18 months of life. This will help us to develop strategies for protecting infants whose brains may be a risk due to malnutrition or other adverse conditions early in life.

What if we identify a problem?
The tests being used are not clinical or diagnositic tests, however if it is apparent to the investigator that there may be a problem, for example with development, or weight gain, we will discuss this with you and write to your GP recommending a more formal assessment.

What happens at the end of the study?
At the beginning of the study your baby will be given a unique study number. This number will be used when analysing the data. The list of the babies studied and their study number will be recorded in a study book which is kept in a locked office in the Evelyn Perinatal Imaging Centre. All electronic data will use the study number only; no identifiable data will be stored electronically.

The results will be presented at medical conferences and published in scientific medical journals. Your baby's confidentiality will be maintained throughout. No identifying details about your baby will be presented or published.

Will my own doctor know?

We will routinely inform your GP that your baby is participating in the study; if any concerns are raised by either the investigator or parent we will also write to your GP, as outlined above.

Who will have access to the case/research records?
The researchers will have access to your baby's medical notes to collect any relevant clinical data. The data and video files collected during the study, anonymised clinical data and images will be sent securely to Centre for Brain and Cognitive Development, Birkbeck College, University of London, for further analysis. These files will be identifiable by the unique study number only. The key researchers will be Professor Clare Elwell and Drs Sarah Lloyd-Fox, Sophie Moore, Topun Austin and Michelle de Haan (contact details below). The data will be analysed both at the Evelyn Perinatal Imaging Centre in Cambridge, the Human Nutrition Unit in Cambridge, Birkbeck University of London and University College London.

The Research and Development Department (R&D) at Cambridge University Hospitals NHS Foundation Trust will have access to the data collected during the study. This will be for audit and monitoring purposes. Individuals from regulatory authorities may also need access to the data where this is relevant to your child's participation in the study.

As mentioned above your baby will be given a study number at the begninng of the study. All electronic information/data will contain only this number and no other identifiable information. All data is stored under password protection by the researchers only. Research data is normally storred for a minimum of 25 years afterwhich time it is destroyed according to the Trust guidelines.

The use of personal information is safeguarded by the Data Protection Act 1998 (DPA). The DPA places an obligation on those who record or use personal information, but also gives rights to the people about whom information is held. If you have any questions about data protection, please contact the Data Protection Officer via the hospital switchboard on 01223 245151 ext 3768.

Who has reviewed this study?
The scientific content has been reviewed and approved by both an external reviewer with expertise in this area as well as the Research and Development Department at Cambridge University Hospitals NHS Foundation Trust. The study has also been reviewed and approved by a local ethics committee: NRES Committee East of England – Cambridge South.

What if something goes wrong?
It is unlikely that anything will go wrong as this is an observational study, using a standard monitors (such as the optical imaging system). However, research can potentially carry unforeseen risks and we want you to be informed of your rights in the unlikely event that any harm occurs as a result of taking part in this study. In the event that something does go wrong and your baby is harmed during the research and this is due to someone's negligence then you may have grounds for legal action for compensation against the Cambridge University Hospitals NHS Foundation Trust but you may have to pay legal costs. The normal National Health Service complaints mechanisms will still be available to you.

What if I withdraw from the study?
If you chose to withdraw from the study, we will ask if we can use the data already collected. If you do not want this, all electronic data will be deleted by the Chief Investigator.

Who is organising the study?
This is a multidisciplinary collaborative study between Cambridge and London.
The investigators and methods of contacting them are:

Cambridge University Hospitals NHS
NHS Foundation Trust

Chief Investigators:

Dr Topun Austin
Consultant Neonatologist
Evelyn Perinatal Imaging Centre, Box 402
The Rosie Hospital
Cambridge University Hospitals NHS Foundation Trust
Hills Road
Cambridge CB2 0QQ
Phone: 01223 256947
e-mail: topun.austin@addenbrookes.nhs.uk

Professor Clare Elwell
Professor of Medical Physics
Department of Medical Physics and Biomedical Engineering
Malet Place Engineering Building
University College London
London WC1E 6BT
Phone: 020 7679 0270
e-mail: c.elwell@ucl.ac.uk

Dr. Sarah Lloyd-Fox
Research Fellow
Centre for Brain and Cognitive Development
Malet Street
London WC1E 7HX
Phone: 0207 631 6323
e-mail: s.fox@bbk.ac.uk

Dr. Sophie Moore
Senior Investigator Scientist
MRC Human Nutrition Research
Elsie Widdowson Laboratory
120 Fulbourn Road
Cambridge CB1 9NL
Phone: 01223 426356
e-mail: sophie.moore@mrc-hnr.cam.ac.uk

Dr. Michelle de Haan
Reader in Developmental Cognitive Neuroscience
UCL Institute of Child Health
30 Guilford Street
London WC1N 1EH
Phone: 020 7905 2755
e-mail: m.de-haan@ucl.ac.uk

Who do I speak to if any problems arise and who can I speak to for independent advice about involvement in medical research?

If you have any complaints about the way in which this research project has been, or is being conducted, please, in the first instance, discuss them with the researcher.

If the problems are not resolved, or you wish to comment in any other way, please contact:

Cambridge University Hospitals NHS
NHS Foundation Trust

Patient Advice and Liaison Service (PALS)

PALS can provide objective help. They are located in the information centre near to the main entrance of Addenbrooke's Hospital. They are open all day during the week and in the afternoon on weekends. The mailing address is:

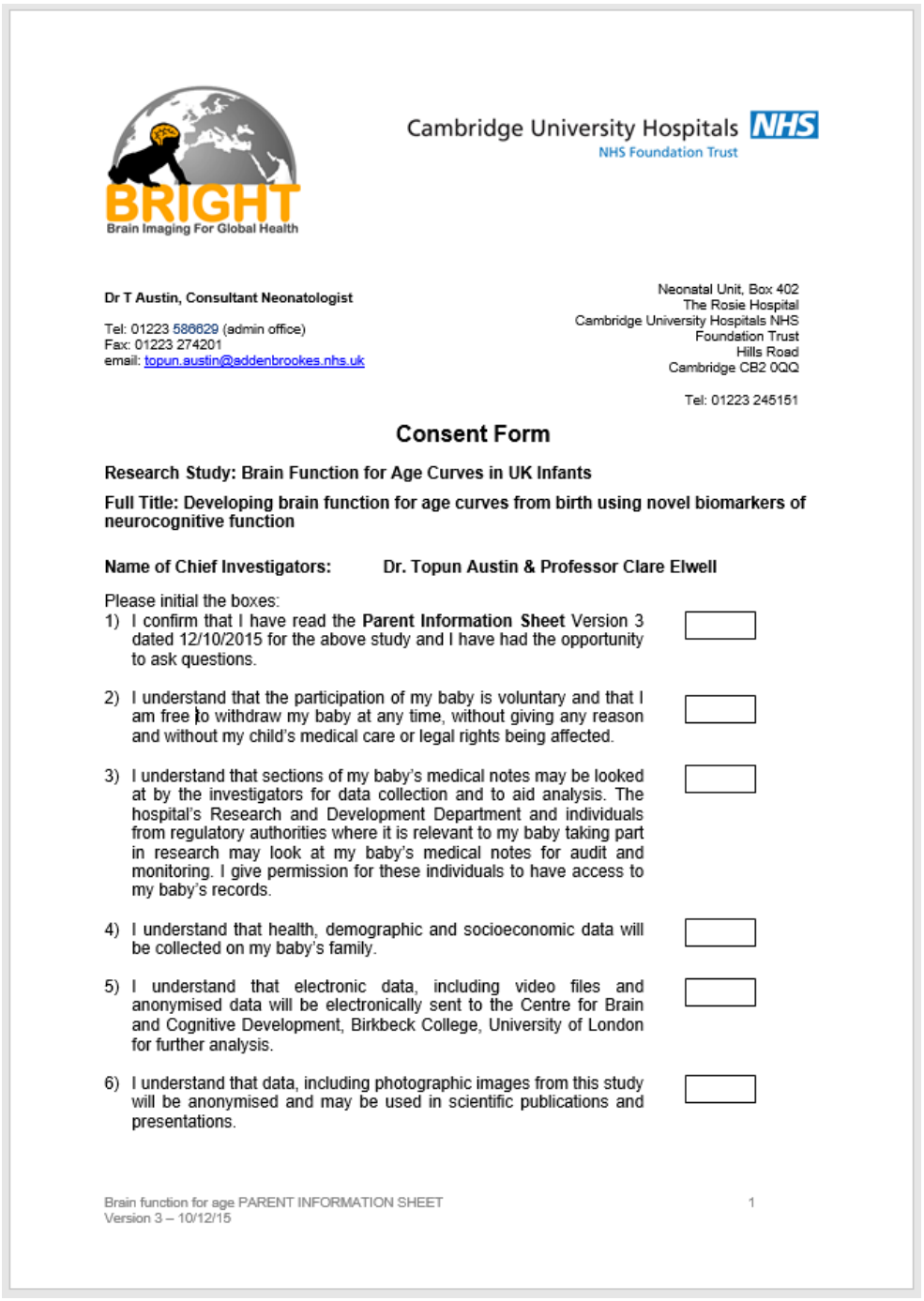
Patient Advice and Liaison Service
Box 53
Cambridge University Hospitals NHS Foundation Trust
Addebrooke's Hospital
Hills Road
Cambridge CB2 0QQ

Telephone: 01223 216756
e-mail: pals@addenbrookes.nhs.uk

Thank you again for taking the time to read through this sheet.

Dr Topun Austin
Consultant Neonatologist
Chief Investigator

2.4 Participant Consent Form – Cambridge



BRIGHT
Brain Imaging For Global Health

Cambridge University Hospitals NHS
NHS Foundation Trust

Dr T Austin, Consultant Neonatologist

Tel: 01223 586629 (admin office)
Fax: 01223 274201
email: topun.austin@addenbrookes.nhs.uk

Neonatal Unit, Box 402
The Rosie Hospital
Cambridge University Hospitals NHS
Foundation Trust
Hills Road
Cambridge CB2 0QQ

Tel: 01223 245151

Consent Form

Research Study: Brain Function for Age Curves in UK Infants

Full Title: Developing brain function for age curves from birth using novel biomarkers of neurocognitive function

Name of Chief Investigators: **Dr. Topun Austin & Professor Clare Elwell**

Please initial the boxes:

1) I confirm that I have read the **Parent Information Sheet** Version 3 dated 12/10/2015 for the above study and I have had the opportunity to ask questions. ☐

2) I understand that the participation of my baby is voluntary and that I am free to withdraw my baby at any time, without giving any reason and without my child's medical care or legal rights being affected. ☐

3) I understand that sections of my baby's medical notes may be looked at by the investigators for data collection and to aid analysis. The hospital's Research and Development Department and individuals from regulatory authorities where it is relevant to my baby taking part in research may look at my baby's medical notes for audit and monitoring. I give permission for these individuals to have access to my baby's records. ☐

4) I understand that health, demographic and socioeconomic data will be collected on my baby's family. ☐

5) I understand that electronic data, including video files and anonymised data will be electronically sent to the Centre for Brain and Cognitive Development, Birkbeck College, University of London for further analysis. ☐

6) I understand that data, including photographic images from this study will be anonymised and may be used in scientific publications and presentations. ☐

7) I understand that video data, will be collected during the assessments for later analysis of my baby's behaviour, and on occasion our interactions with each other. This data will be anonymised and may be used in scientific publications and presentations. ☐

8) I understand the objective of this study is to measure brain function and behaviour in infants from birth to 18 months of age. ☐

9) I agree for my baby to take part in the study. ☐

10) I agree to be contacted via phone, text and email ☐

Name of participant_____

Name of the Parent or Guardian (Relationship to participant)	Date	Signature

Name of Researcher Taking Consent	Date	Signature

2.5 Ethical Approval Letter – Keneba

Scientific Coordinating Committee
MRC Unit: The Gambia, Fajara
PO Box 273 Banjul, The Gambia
West Africa
Switchboard (+220) 4495442/6 Ext 2308
Fax (+220) 4495919/4496513
E-mail: scc@mrc.gm
Intranet: http://mrcportal/Committees/SCC/SitePages/Home.aspx
Webpage:https://mrcportal.mrc.gm/Committees/SCC/SitePages/Home.aspx

16 November 2015

Professor Clare Elwell
Department of Medical Physics and Bioengineering
University College London
Gower Street
London WC1E 6BT, UK

Dear Professor Elwell

SCC 1451v2, Developing brain function-for-age curves using novel biomarkers of neurocognitive development from birth in Gambian infants

Thank you for submitting your revised proposal and response letter both dated 10 November 2015 addressing the issues raised by the SCC at its meeting held on 02 November 2015.

I am happy to give Chair's approval for this project. Your proposal will now be forwarded to the Ethics Committee for further consideration at its meeting on 27 November 2015.

With best wishes

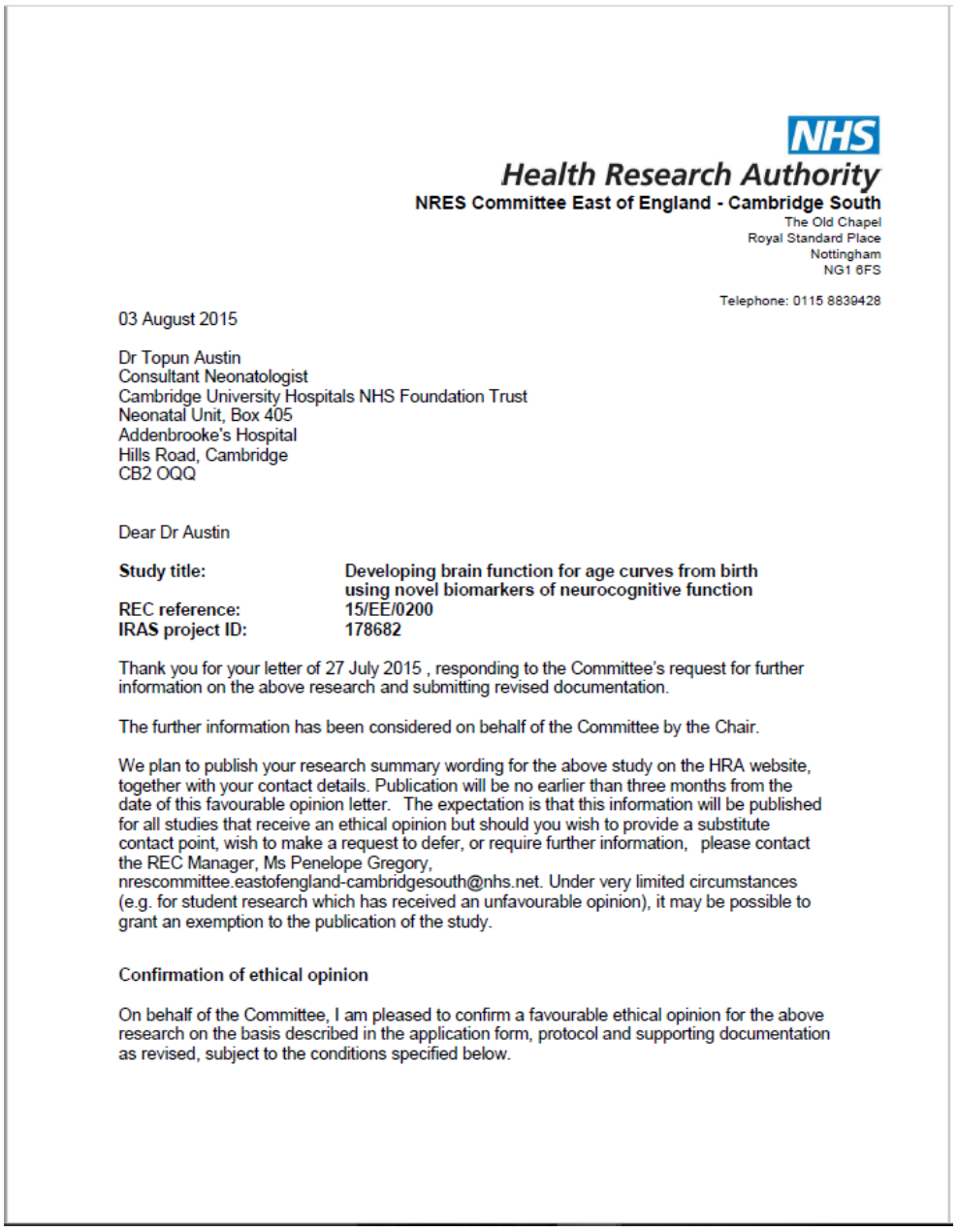
Yours sincerely

Dr Anna Roca
Acting Chair, Scientific Coordinating Committee

Documents submitted for review:-

- SCC Application Form, version 2.0 – 10 November 2015
- Response letter – 10 November 2015
- Informed Consent Document (adult), version 1.0 – 10 November 2015
- questionnaire

2.6 Ethical Approval Letter – Cambridge



NHS
Health Research Authority
NRES Committee East of England - Cambridge South
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Telephone: 0115 8839428

03 August 2015

Dr Topun Austin
Consultant Neonatologist
Cambridge University Hospitals NHS Foundation Trust
Neonatal Unit, Box 405
Addenbrooke's Hospital
Hills Road, Cambridge
CB2 OQQ

Dear Dr Austin

Study title: **Developing brain function for age curves from birth using novel biomarkers of neurocognitive function**
REC reference: **15/EE/0200**
IRAS project ID: **178682**

Thank you for your letter of 27 July 2015 , responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager, Ms Penelope Gregory, nrescommittee.eastofengland-cambridgesouth@nhs.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database. This should be before the first participant is recruited but no later than 6 weeks after recruitment of the first participant.

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from NRES. Guidance on where to register is provided on the HRA website.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see

"Conditions of the favourable opinion" below).

Non-NHS sites

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering letter on headed paper [Brain Function for Age_covering letter]	1	29 April 2015
Covering letter on headed paper [Brain Function for Age_covering letter]	1	09 July 2015
GP/consultant information sheets or letters [Brain Function for Age_LETTER TO GP_V1_090715]	1	09 July 2015
IRAS Checklist XML [Checklist_29042015]		29 April 2015
IRAS Checklist XML [Checklist_01052015]		01 May 2015
IRAS Checklist XML [Checklist_12052015]		12 May 2015
IRAS Checklist XML [Checklist_11072015]		11 July 2015
IRAS Checklist XML [Checklist_26072015]		26 July 2015
Non-validated questionnaire [Brain Function for Age CDI questionnaire_DRAFT_120515]	DRAFT	12 May 2015
Non-validated questionnaire [Brain Function for Age demointerview_v2_0605114]	2	06 May 2014
Non-validated questionnaire [Brain Function for Age demoquestions_v2_06052014]	2	06 May 2014
Non-validated questionnaire [Brain Function for Age family details form_v1_120515]	1	12 May 2015
Participant consent form [Brain Function for Age_CONSENT FORM_V2.1_260715]	2.1	26 July 2015
Participant information sheet (PIS) [Brain Function for Age_PARENT INFORMATION SHEET_V2.1 260715]	2.1	26 July 2015
REC Application Form [REC_Form_29042015]		29 April 2015
Research protocol or project proposal [Brain Function for Age_PROTOCOL_V1 200415]	1	20 April 2015
Summary CV for Chief Investigator (CI) [TA_CVshort march 15]		29 April 2015
Summary, synopsis or diagram (flowchart) of protocol in non technical language [Brain Function for Age_protocol summary table_v1_120515]	1	12 May 2015
Validated questionnaire [Brain Function for Age mullen scoresheet_concise]	1	12 May 2015
Validated questionnaire [Brain Function for Age oxford sleep diary_v1_050814]	1	05 August 2014
Validated questionnaire [Brain Function for Age milestones_parents_v1_011013]	1	01 October 2013

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website:
http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at
http://www.hra.nhs.uk/hra-training/

15/EE/0200	**Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project.

Yours sincerely

Dr Leslie Gelling
Chair

Email:nrescommittee.eastofengland-cambridgesouth@nhs.net

Enclosures: "After ethical review – guidance for researchers" *[SL-AR2]*

Copy to: *Mr Stephen Kelleher, Research and Development, Box 227*

2.7 R&D approval letter

UCL INSTITUTE OF CHILD HEALTH

Great Ormond Street Hospital for Children NHS Foundation Trust

Joint Research and Development Office
Division of Research and Innovation

020 7905 2698
Research.Governance@gosh.nhs.uk

12/02/2016

Dear Dr Michelle de Haan,

Project Title	Imaging brain and social-cognitive development in British and Gambian infants
R&D Number	15NP01
Protocol version	Ph.D Project Proposal
Protocol date	As Above
Funder	Child Health Research Charitable Incorporated Organisation
Sponsor	Cambridge University Hospitals NHS Foundation Trust

This project has been granted Management Approval by the Joint Research & Development Office.

Approval Conditions:

- You must submit an annual report which will be sent to you by the Joint R&D Office when it is due.
- The PI must inform the Joint R&D Office of any changes to the start and end dates of the project, or if there are any changes to the protocol or personnel. At the end of the study the PI will be sent a final report form to complete and return to the Joint R&D Office.

Please be aware that although you have been granted R&D approval you will not be authorised to spend against your award unless there is a signed contract with the research funder / lead site.

Please contact the Joint R&D Office if you require any further guidance or information on any matter mentioned above. We wish you every success in your research.

Yours sincerely,

Manju Agarwal
Research Management and Governance Officer
Joint Research and Development Office

cc: GOSH Finance and/or ICH Finance

Joint Research and Development Office
Division of Research and Innovation
UCL Institute of Child Health, 30 Guilford Street, London WC1N 1EH
Tel: 020 7905 2700 Fax: 020 7905 2201
www.gosh.nhs.uk

The child first and always

Page 1 of 1 ICH (Non-NHS) R&D Approval Letter v2.3 [09.01.15] 15NP01

Chapter 3. ERP study at 1 and 5 months

3.1 BRIGHT EEG/ERP standard operating procedure (SOP).

BRIGHT EEG SOP – all age points V3. 08-05-18

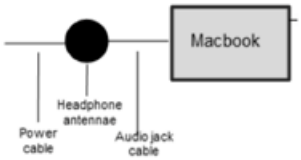
Equipment list (in bold all items relevant to BRIGHT):

In Neurolectrics box.	Other items:	Cleaning supplies.
- Sticktrodes (small bag in box, to be refilled from box in the cupboard) - Geltrodes: 2 bags with 30 each - 1 USB- power plug - 1 USB to micro USB cable for charge of the Necbox (orange) - 2 sets of fibre bundles - 1 micro USB to power plug - 1 Bluetooth USB - 1 NE USB stick(yellow) with software - 1 syringe - Tubes of gel - Bag of 4 Foretrodes (not in use) - 1 Reference ear clip (not in use) - 1 Foretrode strip (not in use	- Antennae for headphones - Wireless SONY headphones - Wireless overhead headphones (to be used at 5 and 18 months) - Soft caps of various sizes, in sets of inner grey and outer colourful cap: (35cm, 39cm, 42cm- labelled KS, 45cm, 48cm) One of each size in the UK, two sets in the Gambia	- Toothbrush - Sponges - Cotton buds - Wet wipes - Paper towel - Tape remover Additional items needed. - Measuring tape - Bubbles for 5/18 month testing

In preparation

1. Connect headphone antennae to power and to mac (see diagram below).

FOR 1M USE HEADPHONES IN CAP – FOR 5 AND 18M USE OVERHEAD HEADPHONES.

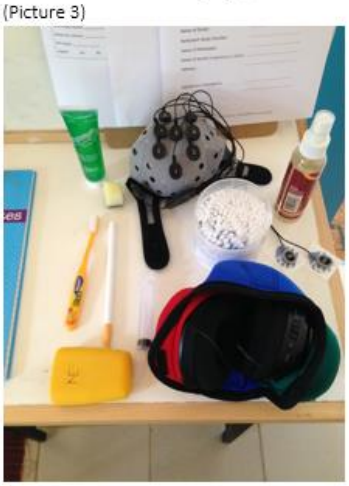


2. Make sure the Necbox's charge is at least 50%. It can be charged by connecting it to the PC or a wall socket via mini USB (orange cable).
3. Make sure the Geltrodes are slotted into the correct positions on the cap (PICTURE 1)
4. a) 1 and 5 month: Attach fibres to the Geltrodes (Picture 2).
   b) 18 month: Do not yet attach the fibres, as additional gel will be inserted through the wholes later on.

Picture 1


Picture 2

5. Attach two Sticktrodes to the two reference and ground electrodes (Picture 2)
6. Check that there is enough gel, and have the syringe and cleaning supplies at hand (Picture 3)


Picture 3. Items to have ready when testing: Signa Gel, prepared inner cap, cotton buds, tape remover, toothbrush, charged Necbox, outer cap with charged headphones
NOT IN THE PICTURE: Syringe and fresh tip.

7. Make sure the headphones are charged.

The headphones used at 1 month can be charged by connecting them to the antennae (Picture 4). The headphones used at 5 and 18 months are charged the same way, the same antennae can be used.

Picture 4. Charging of the headphones. Red light on antennae indicates that headphones are charging. Light turns green when antenna audio jack is connected to laptop and active

8. Launch the software on the mac by clicking the NIC software icon (Picture 5)

Picture 5. NIC software shortcut

9. Put gel into Geltrodes from the inside so that they are filled up to the maximum (Picture 6).

Picture 6. Geltrodes filled up with signa gel.

NOTE: LEAVE STEP 10 UNTIL RIGHT BEFORE CAPPING TO PREVENT GEL FROM DRYING OUT!

Capping

1 month.

1. Have two people there for the capping, one putting on the cap, the other supporting the infant's head. The infant should be placed on the knees of one researcher, in the same position as for the NIRS study. Stick the 2 reference electrodes onto the child's left mastoid bone. Put the cap gently but firmly on to the child's head, check the fit by gently pushing the electrodes against the head to feel if there is space- if there is space, adjust the fit by pulling the cap down!
2. Fasten chinstrap and check again if there is space between the electrodes and the head, if so adjust fit.
3. Put outer cap holding headphones onto infant's head.

5 month.

1. Have two people there for capping, one putting on the cap, the other distracting the baby with toys or bubbles. Stick the 2 reference electrodes onto the child's left mastoid bone.
2. Fasten chinstrap and check again if there is space between the electrodes and the head, if so adjust fit.
3. Place overhead headphones onto the infant's head, making sure the EARS ARE COVERED. Adjust size as needed.

Picture 7. Capping process

18 month.

1. Have two people there for capping. One needs to be distracting the toddler. Alternatively, toddlers can watch a cartoon on the tablet during capping
2. Put gel into the elecrodes from the inside of the cap as you would do in 1 month capping.
3. Have the baby turned away from you when putting the cap on from behind
4. Stick on sticky electrodes and close chinstrap (this way, the cap is tight for more gelling)
5. Gently push each electrodes down, if gel is coming out the small hole, there should be enough
6. Use the syringe to either add more gel, or, if their is enough, hold the electrode in place with one and and use the syringe tip to move the gel around inside the electrode. This helps to work through hair to make good contact between the gel and head

→ just adding more gel won't always help, so make sure to move the hair. Too much gel can cause other issued with the data, so getting the amount right is critical!

Gelling using the syringe: Use one syringe tip per baby, syringes themselves can be used many times. Pull up gel in the syringe before attaching tip. Then attach the tip to the syringe. Push down each electrode gently to make sure it is in close contact with the head. If gel is coming out, no more gel needs to be added, but the syringe still needs to be used to work through hair.

Insert the tip through the hole of the electrode and either add gel, or use the tip to work through any hair.

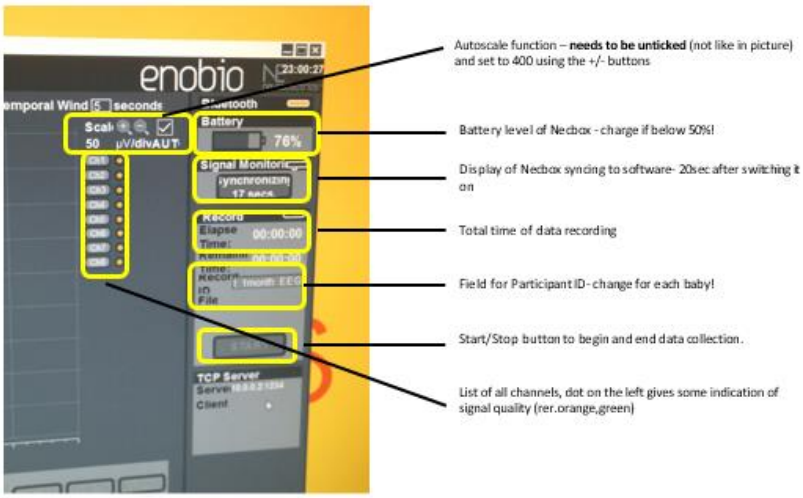
e) Clip on the electrode fibres.

f) Put the overhead headphones.

4. Connect the connection fibres to the Necbox and Velcro it on the cap (Picture 8). This is critical as excessive movement of the necbox affects data quality, so it need to be securely attached.

Picture 8. Fibres connected to Necbox, on/off switch in bottom right corner

5. Switch Necbox on - once switched on it will sync with the software (Picture 8 and 9).



6. Untick the box above the channel labels that regulates the autoscale function (labelled AUTO). And use the minus and plus buttons to set the scaling factor to 400. This will give you some consistency when evaluating the online trace during data collection of different babies.

Picture 9. Synchronisation process in the software. Display of scale, battery level, sync status, total recording time, Participant ID, start/stop button for data acquisition and channel quality

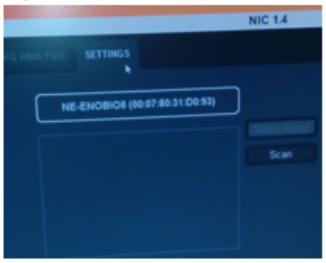
TROUBLESHOOTING: If device doesn't show, check Bluetooth settings of PC to make sure Bluetooth is enabled.

18 months.

7. Once the signal is displayed, check if any electrodes are bad, and consider detaching the fibre and re-gelling if the toddler is happy

Starting the study

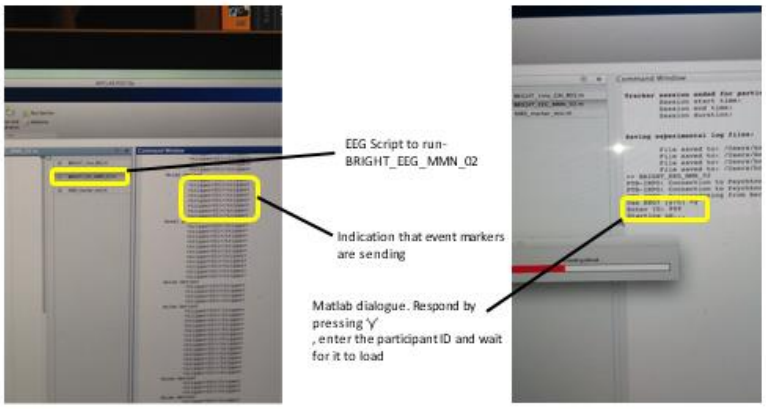
1. Select the system in the Enobio Software under the Settings tab at the top (Picture 10).



Picture 10. Settings Tab to scan for and select software.

2. Start data acquisition within the software by clicking the START button (Picture 9).
3. Run script through MATLAB ('BRIGHT_EEG_MMN_02') (Picture 11).
4. Answer the MATLAB dialogue boxes as follows:(Picture 11)

- Use EEG (y/n): put in 'y' and press enter
- Enter Participant ID



5. After script loaded and study began, check the child's headphones to make sure the sound is coming through.
6. The study is running for a total of 10min at 1 month, 15min at 5 and 18m

For 5month: Try to keep the baby happy as well as limit excessive movement as it will cause artifact/noise in the data. Try and limit interaction to quiet or silent gestures etc. It is key to limit movement and speech/noise as it interferes with the study. Tell the parent this also.

For 18 month: Try to keep toddler happy while limiting excessive movement. This can best be achieved by having the toddler on the parent's lap leaning against them (avoiding rocking back and forth movements) or in a high chair. Infants can either watch toys, bubbles, gesture games or a **silent** cartoon on the tablet.

After the study

1. Stop acquisition in the software by pressing the START/STOP button on the right hand side of the user interface/software (Picture 9).
2. Switch off the Necbox and remove from fibres.
3. Take off the outer cap.
4. Undo chinstrap of inner cap
5. Use a cotton bud soaked with tape remover to remove Sticktrodes. Have one experimenter distract the baby while they are taken off.
6. Remove grey cap.
7. Wipe the child's mastoid and the leftover gel on the top of the head with a wet wipe.

CLEANING

NOTE: Cleaning the kit is essential to ensure good data quality. Leftover gel causes deterioration of the electrodes and will eventually cause poor data quality. Please take extra care and plan time for this right after the study.

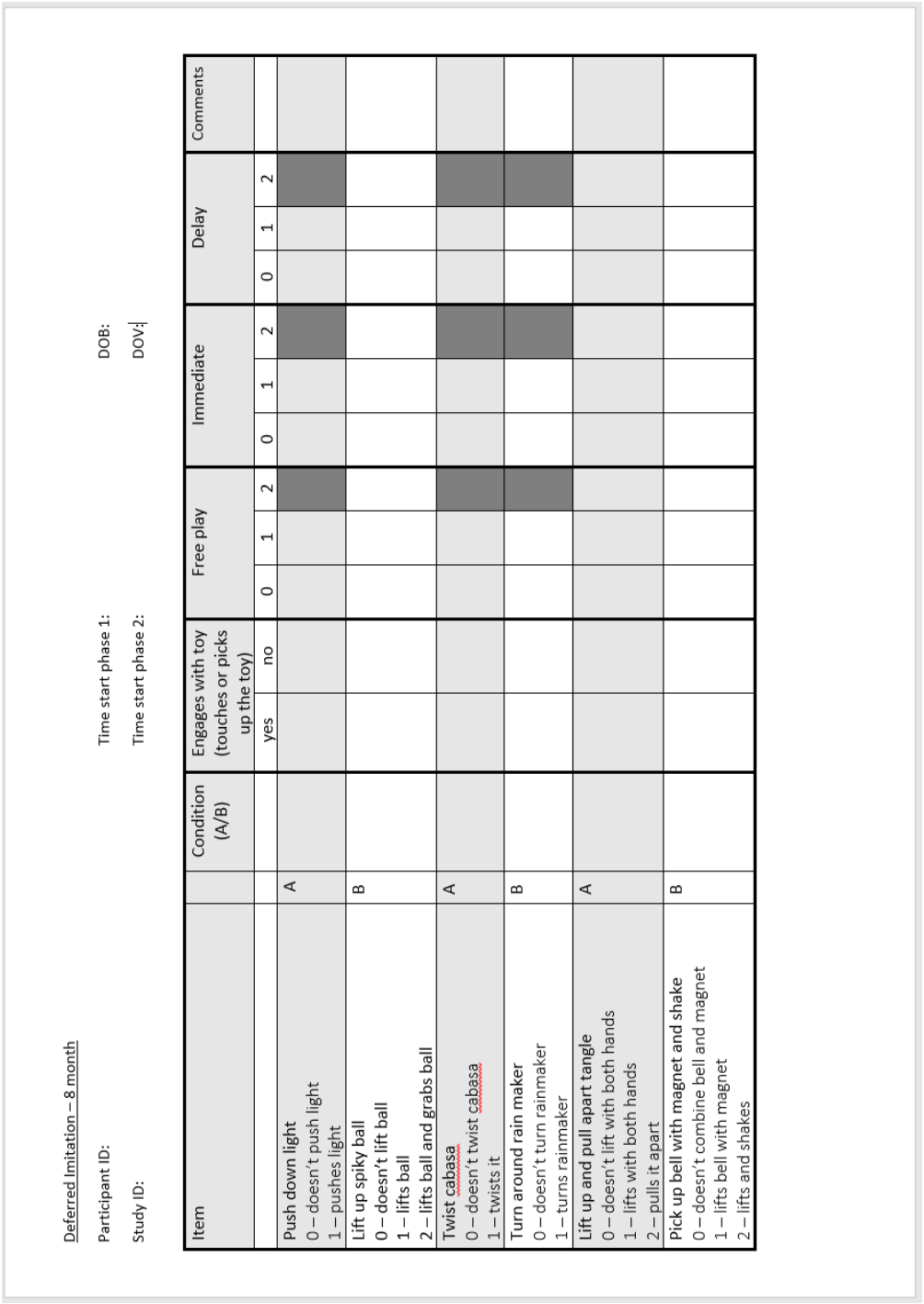
8. Detach the fibres from the Geltrodes.
9. Clean Geltrodes with soft bristle toothbrush. Make sure there is no gel on the inside or outside and the little hole is free! Dry each one with a cotton bud.
10. Clean the electrode ends of the fibres with cotton buds.
11. Clean cap carefully from any gel spillages and let it dry before using it again.

CHARGING AND REFILLING

1. Charge of Necbox: charge fully once per week
2. Charge of headphones: charge fully once per week
3. Sticktrodes: Check regularly how many Sticktrodes are left, so that we can order replacement well in advance!
4. Gel: When starting a new tube, check how many are left in case we need to order and ship replacement!
5. The data is saved under Program files/NIC/data

Chapter 6: Deferred Imitation Study at 8 and 12 months.

6.1 Deferred Imitation Score Sheet 8 months



Deferred Imitation – 8 month

Participant ID: Time start phase 1: DOB:

Study ID: Time start phase 2: DOV:

Item		Condition (A/B)	Engages with toy (touches or picks up the toy)		Free play			Immediate			Delay			Comments
			yes	no	0	1	2	0	1	2	0	1	2	
Push down light 0 – doesn't push light 1 – pushes light	A													
Lift up spiky ball 0 – doesn't lift ball 1 – lifts ball 2 – lifts ball and grabs ball	B													
Twist cabasa 0 – doesn't twist cabasa 1 – twists it	A													
Turn around rain maker 0 – doesn't turn rainmaker 1 – turns rainmaker	B													
Lift up and pull apart tangle 0 – doesn't lift with both hands 1 – lifts with both hands 2 – pulls it apart	A													
Pick up bell with magnet and shake 0 – doesn't combine bell and magnet 1 – lifts bell with magnet 2 – lifts and shakes	B													

6.2 Deferred Imitation Score Sheet 12 months

Deferred Imitation – 12 month

Participant ID: Time at phase 1: DOB:

Study ID: Time at phase 2: DOV:

Item		Condition (A/B)	Engages with toy (touches or pick up the toy)	Free play				Immediate				Delay				Comments
				0	1	2	3	0	1	2	3	0	1	2	3	
Throw ball in cup 0 – doesn't throw ball into cup 1 – throws ball in cup	A															
Stroke frog with stick 0 – doesn't stroke frog 1 – strokes frog	B															
Turns around timer 0 – doesn't turn timer 1 – turns timer	A															
Stamps and lifts up 0 – doesn't stamp 1 – stamps but doesn't lift 2- stamps and lifts	B															
Picks up accordion and pushes 0 – doesn't pick up with both hands 1 – picks up with both hands 2- pushes	A															
Puts ball in box, closes lid, shakes 0 – doesn't combine actions 1 – put ball in box 2- closes lid 3 - shakes	B															

6.3 BRIGHT Deferred Imitation SOP

SOP I Behavioural Memory task I Deferred imitation

Rationale

This behavioural task will be introduced at the 8m time point and repeated at 12m. Infant's memory capacity increases dramatically over this time, which is why it is interesting to use this task at 8 and 12 months. While it is a behavioural assessment, it is done during the NIRS session, so NIRS data will be recorded while the task is being administered.

The task is designed to measure infants' ability to remember a novel action or action sequence over a short time delay. To standardise the delay the task will be administered before the NIRS habituation task, the test phase will take place after the NIRS script finishes. This will lead to a delay of 20min, if the NIRS session is performed as expected.

There are 12 actions, 6 to be administered at 8 months, and another 6 at 12months. The actions are summarised in table 1.

Table 1: Overview of actions for both time points.

	8 month		12 month	
1.	Push down light	1	Throw ball in cup	1
2.	Lift spikey ball, grab juggling ball	2	Stroke frog with stick	1
3.	Twist cabasa	1	Turn around sand timer	1
4.	Turn around rain maker	1	Stamp and lift up	2
5.	Pick up tangle with both hands- pull apart	2	Lift accordion at both sides - push together	2
6.	Pick up bell with magnet and shake	2	Put ball in box – put lid on - shake	3

Scoring

Each action will be scored as pass/fail. For two and three step action sequences each step will be scored pass/fail. Examples of what constitutes a score of 0, 1, 2 and 3 are given on the score sheet and in the detailed description of how each item should be administered below.

Example: Stamp
Baby pushes down stamp and leaves it there – score of 1
Baby pushes down stamp **and** lift to show picture - 2

We are not measuring the babies' motoric abilities! It is important to score by babies' intent, not the outcome!
If a baby if clearly trying to perform an action, the attempt receives the full score. Example: baby pushes light but isn't quite strong enough to make it light up – full score

Phases of the Assessment

There are 4 different phases to the study:

1. Free play: the experimenter presents the baby with the object(s) necessary for the action and gives them explore them for roughly 30 seconds. If any of the target actions is performed spontaneously, it is scored as in any other phase. This will allow us to determine how much of their behavior is due to memory and how much due to their spontaneous behavior.
2. Presentation: The experimenter takes the toy from the baby and off the table, then puts it in front of him-/herself on their side of the table, clearly marking the beginning of a new phase. Each action is demonstrated 3 times, while the experimenter narrates what he/she is doing. Scripts for each action are specified below.
3. Immediate imitation: For each baby, half of the objects are given back to the baby immediately after the demonstration for them to copy. It is determined by the condition (A/B) which objects will be immediately given to the baby (detailed explanation below). In this phase the experimenter again presents the baby with the object(s) and lets them try and imitate for roughly 30s, any target behaviours are scored.
4. Deferred/delayed imitation: This phase takes place after the 20min delay. Each object is handed to the baby and they can play with them for 30s. Any target behaviours are scored.

Preparation

For each assessment, half of the objects will be given to the infant immediately after the presentation phase. Which toys are given for the immediate imitation will vary between infants. The experimenter should decide beforehand if the infant is tested in condition A or condition B. If the infant is in condition A all objects marked A on the scoresheet should be given to the infant for immediate imitation. If the infant is in condition B the other three objects should be imitated immediately. Half of the infants should end up in each condition. Parents are not supposed to help the infant during the imitation even though that might feel unnatural to them. **It is worth pointing out to the parent, that this is a very hard task that many babies don't manage to copy the action.** It is also worth explaining that to us it is much more interesting to see how they go about it than pushing them to successfully complete the action.

Regardless of condition all objects will be tested in the delayed test phase!

Setting the event markers

As we are recording NIRS during this task it is crucial to set event markers so we can analyse the data. Insert a marker by clicking the 'event mark' button in the NTS software. This needs to be done when giving the first item to the baby to explore, when taking it from them to demonstrate its use and again for the imitation. This is illustrated below:

Give toy	Demonstration	IF APPLICABLE Immediate imitation	...	Give toy after delay
Mark when toy touches table	Mark when you put the toy on the table in front of you	Mark when toy touches table		Mark when toy touches table

Administration

The mum and infant should be turned away from the screen and a small table should be put up in between the infant and the experimenter. Before presenting any of the novel objects, the experimenter should play with the infant very briefly with a ball to establish a turn- taking situation. The ball should be rolled towards the infant on the table and the infant should be encouraged to roll it back. Once you feel as if the infant is engaging with you and paying attention to the play situation, you can move to the presentation on the novel objects.
The experimenter first lets the baby play with each object for approximately 30seconds. This way, the baby gets a chance to explore the toys. It also gives the experimenter a chance to judge how likely the baby is to perform the target action spontaneously. This will be helpful during scoring, as it will allow us to determine if an infant remembered and imitated an action, or whether they are just playing with the object as they would have anyway. The free play, presentation and immediate test phase (if applicable) will always be performed in a row for each object, meaning that the infant can have a look at object 1, then the experimenter presents it, then a test phase happens.

After this initial free play phase the experimenter takes the toy and demonstrates the target action to the infant while narrating it (script for each object below). This is done three times.

It is important to try and maintain the infants' attention to the demonstration throughout.

Whenever an object from the immediate imitation list was presented, it is given straight back to the baby after demonstration and the baby gets a chance to imitate the action for approximately 30 seconds.
Once all objects have been presented, the mum and infant should be turned back to face the screen for the rest of the NIRS study.

Test phase I Immediate

The three objects that were determined to be tested immediately will be handed to the infants right after the experimenter demonstrated the target action. The baby will be allowed to play with the object for approximately 30 seconds. The experimenter scores if the infant initiates the action, attempts it or completes it successfully (examples of what each score might entail are given below).

Test phase I Delayed

After the NIRS session is finished the delayed test phase will take place, **while the infant is still wearing the headgear.** Again, the infant will be given each object for approximately 30

seconds. The experimenter will judge whether the action was initiated, attempted or completed.

Script for administration

8 month	12 month
Push down light 'Look, I push it down!'	Throw ball in cup 'Look, I throw it in!'
Lift up spiky ball and pick up ball 'Look, I lift it up and take the ball!'	Stroke frog with stick 'Look, I stroke it'
Twist cabasa 'Look, I twist it!'	Turns around timer 'Look, I turn it around'
Turn around rain maker 'Look, I turn it around!'	Stamps and lifts up 'Look, I push it down and there's a picture!'
Lift up and pull apart tangle 'Look, I lift it up and I pull it!'	Picks up accordion and pushes 'Look I pick it up and push it!'
Pick up bell with magnet and shake 'Look, I lift the bell and I shake it!'	Puts ball in box, closes lid, shakes 'Look, I put the ball in, I close the box, and I shake it!'

Scoring examples

Examples of what constitutes a score of 0, 1, 2 and 3 are given below. All non- target actions should be scored 0, such as chewing the item, dropping it to the floor etc. If an item is misplaced (i.e. dropped to the floor) you can pick it up to give the baby another go.

8 month	12 month
Push down light 0 – doesn't push light 1 – pushes light	Throw ball in cup 0 – doesn't throw ball into cup 1 – throws ball in cup
Lift up spiky ball 0 – doesn't lift ball 1 – lifts ball	Stroke frog with stick 0 – doesn't stroke frog 1 – strokes frog
Twist cabasa 0 – doesn't twist cabasa 1 – twists it	Turns around timer 0 – doesn't turn timer 1 – turns timer
Turn around rain maker 0 – doesn't turn rainmaker 1 – turns rainmaker	Stamps and lifts up 0 – doesn't stamp 1 – stamps but doesn't lift 2- stamps and lifts
Lift up and pull apart tangle 0 – doesn't lift with both hands 1 – lifts with both hands 2 – pulls it apart	Picks up accordion and pushes 0 – doesn't pick up with both hands 1 – picks up with both hands 2- pushes
Pick up bell with magnet and shake 0 – doesn't combine bell and magnet 1 – lifts bell with magnet 2 – lifts and shakes	Puts ball in box, closes lid, shakes 0 – doesn't combine actions 1 – put ball in box 2- closes lid 3 - shakes

Item administration

8 month

1. Push down light

Place light within reach of baby.
Let them play with it for 30s
Say: 'Can I show you/can I have a go?'
Take light off table.
Place it in front of you.
Press it down with two or three fingers while saying: 'Look, I push it down'
Repeat the action 3 times, there is no need to take the light off the table between trials.
If immediate imitation present light to baby again and say 'You have a go'/ 'You do it'
Give them approximately 30 seconds for imitation. If baby is performing the action but cannot make switch the light on, you can help them during this phase.
Deferred imitation: Put light in front of baby, let them have a play for 30s, if they throw down light pick it up again and place it in front of them.

Score 1 if baby attempts to push light with their hand.

2. Lift up spikey ball and grab small ball

Put small ball (the hacky sack/ juggling ball type one) into spikey ball and place it on table in front of baby.
Let them play for 30s, if either object falls down, try putting the original set up back together.
Take the ball and hacky sack, take both off table.
Put the hacky sack into ball again and place it in front of you.
Lift the spiky ball and reach for sack while saying: 'Look, I lift the ball and take the ball!'
Put the sack back into the ball under the table, place it in front of you and repeat the action 3 times.
Immediate and deferred imitation: put both objects in front of baby, let them have play for 30s, if items are misplaced, bring them back into original set up.

Score 1 if baby lifts ball enough to make sack fall out. Score 2 if they consecutively reach for sack.

3. Twist cabasa

Put cabasa with its handle pointing upwards, (so that it stands on the flat side) on table in front of baby.
Let the baby play for 30s, if it falls down, try putit back the way you started.
Take the cabasa off the table, then place it in front of you.
Hold the cabasa by the handle then twist it, by placing one hand on the side and pulling the hand towards you while saying 'Look! I twist it!'
Immediate and deferred imitation: cabasa in front of baby, let them have play for 30s, if it gets misplaced, bring it back into original set up.

Score one if baby tries to twist the cabasa with their hand. Score 0 if they pick up, drop, bang or mouth cabasa.

4. Turn around rain maker

Put rainmaker in front of baby, standing upright.
Let them play for 30s, if it falls down, put it back in front of them.
Take the rainmaker off the table.
Put rainmaker in front of you. Turn it around while saying: 'Look, I turn it upside down!' and let all 'rain' run through. Repeat this 3 times.
Immediate and deferred imitation: put rainmaker in front of baby upright, let them have play for 30s, if misplaced, put it back.

Score 1 if baby tilts it enough to make the rain sound. Score of 1 is acceptable when the baby first lays the rainmaker onto its side and picks it up again to turn it! Score 0 if they instead mouth, drop, or bang the object on the table.

5. Lift up and pull apart tangle

Wrap tangle around your thumb to begin with, out of the baby's sight. Then put in front of them on the table.
Let them play for 30s, if it falls down wind it up again and put it back in front of them.
Take the tangle off the table and put it back into it's original configuration.
Put tangle in front of you. Pick it up at both end with each hand and pull it apart while saying 'Look, I pick it up, and I twist it' Take the tangle off the table, get it back to starting position, then repeat 3 times.
Immediate and deferred imitation: put tangle in front of baby, let them have play for 30s, if it gets misplaced or out of configuration, you can wind it up under the table to help them.

Score 1 if baby picks is up with both hands. Score 2 if they pull it apart. Score 0 for all other actions.

6. Pick up bell with magnet and shake

Place magnet and bell next to each other within baby's reach.
Let them play for 30s, if something it dropped, put it back in front of them.
Take bell and magnet off the table.
Put bell and magnet in front of you. Pick up magnet, bring it on top of bell to pick it up, then shake it. Say: 'Look, I pick it up and I shake it!'
Take the bell and magnet off the table, take them apart out of baby's sight, then repeat the presentation 3 times.
Immediate and deferred imitation: put bell and magnet in front of baby, let them have play for 30s, if something gets misplaced, bring it back into the original set up.

Score 1 if baby picks up bell with magnet. Score 2 if they shake it. Score 0 for only shaking the bell, mouthing either object or looking at objects separately.

12 month

1. Throw ball in cup

Put cup with ball next to it in front of baby.
Let them have a play for 30s and pick up either items, should they drop them.
Take items off the table, then put them in front of you.
Take the ball and throw it into the cup while saying: 'Look, I throw it in!'
Take the ball out of the cup under the table, then repeat 3 times.
Immediate and deferred imitation: put both items in front of baby again and let them have a go for 30s. Put any misplaced items back the way you started.

Score 1 if baby picks up ball and throws it in cup. It is acceptable to score 1 if baby puts ball into cup but doesn't let go off it and takes it back out again. Score 0 for other actions.

2. Stroke frog with stick

Put frog with **stick next to it** on the table.
Let baby play for 30s then take both items off the table, before putting them in front of you.
Pick up stick and stroke the frog's back, back and forth a few times. Say: 'Look, I stroke it!'
Put the stick back next to frog before repeating 3 times.
Immediate and deferred imitation: put both object in front of baby again. If they drop either item, pick it up for them.

Score 1 if they bring stick towards frog and move it, score 0 for all other actions.

3. Turn around timer

Put timer in front of baby, with all liquid in the bottom half.
Let them play for 30s, if it falls down, put it back in front of them.
Take the timer off the table, making sure all liquid is at one end.
Put timer in front of you. Turn it around while saying: 'Look, I turn it upside down!'
Take timer off table and let the liquid run back to one side (tilting it slightly will do that for you pretty quickly) and repeat the demonstration 3 times.
Immediate and deferred imitation: put both objects in front of baby, let them have play for 30s, if items are misplaced, bring them back into original set up.

Score 1 if baby tilts it enough to make the liquid run down. It is acceptable to score if they arrive at this in two steps (lying timer on its side and then turning it).

4. Stamp and lift up

Place stamp onto post it block in front of baby

Let them play with it for 30s
Place stamp on post its in front of you.
Press it down and then lift it from the block while saying: 'Look, I push it down, and lift it up!'
Repeat the action 3 times, take the block and the stamp off the table after each trial and remove the top post it to start with a clean page each time.
Immediate and deferred imitation: put stamp on block in front of baby, give them approximately 30 seconds for imitation. If baby is performing the action but cannot get the stamp to appear you may help them during immediate imitation.

Score 1 if baby pushes the stamp, score 2 if they consecutively lift it up. Score 0 if they only lift the stamp, mouth it, drop it or don't use the two objects together.

5. Pick up accordion and push

Put accordion in front of baby, handles facing left and right.
Let them play for 30s, if it falls down, put it back in front of them.
Take the accordion off the table, then put it in front of you.
Pick it up at both end with each hand and push it together while saying 'Look, I pick it up, and I push it' Put the accordion back down to the table, then repeat the action 3 times.
Immediate and deferred imitation: put accordion in front of baby, let them have play for 30s, if it gets misplaced, you can put it back into starting position.

Score 1 if baby picks it up with both hands. Score 2 if they push it. Score 0 for all other actions.

6. Put ball in box, close lid, shake

Put open box and ball next to each other in front of the baby on the table.
Let them play for 30s, if something falls down, put objects back in front of them.
Take the box and ball off the table, then put them in front of you.
Pick up ball, put it into box, close the lid and shake the 'rattle' while saying 'Look, I put the ball in the box, I close the lid and I shake it.' Take the 'rattle' under the table in order to undo it. Repeat 3 times.
Immediate and deferred imitation: put ball and open box in front of baby and let them have a play for 30 seconds.

Score 1 if baby puts ball into box. Score 2 if baby puts ball in box and closes lid. Score 3 if baby puts ball in box, closes lid and shakes. Score 0 for all non-target actions, and for actions in wrong sequence (i.e. only closing lid and shaking).

Appendix

Pictures of all items and how they should be presented to baby

8 month

1. Light

2. Expandable ball and small ball

3. Cabasa

4. Rainmaker

5. Tangle

6. Bell and magnet

12 month

1. Cup and ball

2. Frog and stick

3. Liquid timer

4. Stamp and post its

5. Accordion

6. Box with lid and ball

Appendix

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