

Robot-Assisted fMRI Assessment of Early Brain Development

PhD Thesis by Alessandro Allievi

**Imperial College of Science, Technology and Medicine
Department of Bioengineering, 2014**

Robot-Assisted fMRI Assessment of Early Brain Development

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Dissertation submitted in partial fulfillment of the requirements for the
degree of

Doctor of philosophy

Department of Bioengineering

Imperial College of Science, Technology and Medicine, 2014

Declaration of originality

The work presented in this thesis has been carried out at the Department of Bioengineering, Imperial College of Science, Technology and Medicine, and this document has not been previously submitted to any other university or technical institution for a degree or award. The thesis comprises only my original work, except where due acknowledgement is made in the text.

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Acknowledgments

Upon starting my Ph.D four years ago I was filled with the blissful excitement that, I have come to learn, can only be generated by the prospect of pushing the current boundaries of knowledge, to capture a glimpse of unexplored truth. With trepidation came apprehension, fuelled not only by the scientific uncertainties which are inextricably associated with cutting-edge research, but also by the self-doubt of a young graduate asked for the very first time to take his life in his own hands, and simply be up to the task. My initial fears were immediately mitigated by the extraordinary group of people, which I soon realized would provide me with their help, support and advise throughout my Ph.D. Retrospectively and cold-mindedly dissecting my professional and personal trajectory over these past four years, it is clear to me how these people were fundamental to all the work and scientific achievements reported in this thesis. I am therefore pleased to provide proper acknowledgement of their contribution in this section.

I would like to start by thanking my supervisors Etienne Burdet and David Edwards, for their unparalleled involvement and support in all aspects of my research. Not only have they granted me their outmost trust and respect, but they have constantly offered me scientific advice, as well as moral, financial and administrative support.

I would also like to thank all my colleagues and members of the the Human Robotics Group (HRG) at Imperial College. The wise and insightful advice provided by my first supervisor Alejandro Calderon-Melendez, and by the post-doctoral group members Nathanael Jarrassé, Nicholas Roach, Julius Klein, Sivakumar Balasubramanian and Ganesh Gowrishankar was paramount to the development of durable and reliable hardware and software early on into my Ph.D. This was fundamental in view of the ambitious goal of performing a developmental study of the preterm population during the relatively short time-frame at my disposal. I would also like to thank all of the Ph.D. and Master's students who have contributed to creating a friendly and motivating working environment, and with whom I have also established a relationship of friendship and mutual and unconditional support. In particular I need to thank Asif Hussein, Atsushi Takagi, Wayne

Dailey, Consuelo Granata, Ildar Farkhatdinov, Marcus Gardner, Mike Mace for the many inspiring, thought-provoking conversations, which have driven me to overcome practical challenges and better my understanding of scientific notions. I am also highly grateful to Andrew Pennycott, Carlo Bagnato, Alfredo Belfiori and Valeria Pupella, whom in addition to supporting me throughout the course of my Ph.D, have assisted me greatly during my thesis write-up.

I need to thank all the colleagues of the Centre for the Developing Brain, King's College London, and formerly of the Weston Group, Imperial College London, whom have assisted with the collection of the data reported in this thesis. In particular I would like to extend my thanks to the post-doctoral members Nora Tusor and Gianlorenzo Fagiolo for their practical help; and to the radiologists Joanna Allsop and Mathew Fox for their competence and assistance in performing fMRI acquisitions. I need to also thank the nursing team for carrying out the infants' preparation, and for their crucial collaboration in guaranteeing the subjects' safety at all times. I would also like to extend my gratitude to the infants' parents for accepting to take part in the study.

I would like to thank my main collaborator, friend, heart and soul of the studies Dr. Tomoki Arichi. In addition to his constant support, motivation and advice, I thank Tom for consenting the patients, assisting with their preparation and obtaining the ethical approval required to conduct our experiments. Furthermore I thank him for taking me under his wing and introducing me to all the relevant neuroscientific literature and notions, as well as to the acquisition and analysis of neonatal fMRI data. I consider myself extremely lucky to have had the opportunity to collaborate with Tom, as I consider his proactive attitude, our diversity of backgrounds, and our collaboration and involvement in all individual aspects of the studies as key elements to the success of the work presented in this thesis.

Last but not least I want to thank my parents Antonella and Gabriele, my sisters Giulia and Elisa, my family and my girlfriend Lucy for their unconditional support and love throughout these four years. Without them none of this would have been possible.

Abstract

Preterm birth can interfere with the intra-uterine mechanisms driving cerebral development during the third trimester of gestation and often results in severe neuro-developmental impairments. Characterizing normal/abnormal patterns of early brain maturation could be fundamental in devising and guiding early therapeutic strategies aimed at improving clinical outcome by exploiting the enhanced early neuroplasticity.

Over the last decade the morphology and structure of the developing human brain has been vastly characterized; however the concurrent maturation of brain function is still poorly understood. Task-dependent fMRI studies of the preterm brain can directly probe the emergence of fundamental neuroscientific notions and also provide clinicians with much needed early diagnostic and prognostic information. To date, task-fMRI studies of the preterm population have however been hampered by methodological challenges leading to inconsistent and contradictory results.

In this thesis I present a modular and flexible system to provide clinicians and researchers with a simple and reliable solution to deliver computer-controlled stimulation patterns to preterm infants during task-fMRI experiments. The system is primarily aimed at studying the developing sensori-motor system as preterm infants are often affected by neuro-motor dysfunctions such as cerebral palsy. Wrist and ankle robotic stimulators were developed and firstly used to study the emerging somatosensory “homunculus”. The wrist robotic stimulator was then used to characterize the development of the sensori-motor system throughout the mid-to-late preterm period. An instrumented pacifier system was also developed to explore the potential sensorimotor modulation of early sucking activity; however, despite its clear potential to be employed in future fMRI studies, results have not yet been obtained on preterm infants.

Functional difficulties associated with prematurity are likely to extend to multi-sensory integration, and the olfactory system currently remains under-investigated despite its clear developmental importance. A custom olfactometer was developed and used to assess its early functionality.

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Thesis Structure

Motivation and aims

The third trimester of gestation is a crucial period for the maturation of cerebral structure and function, and perinatal complications associated with preterm birth have the potential to alter this natural process ultimately leading to long-term neurodevelopmental impairments. There is a pressing need to gather early diagnostic and prognostic information, to allow an efficient allocation of resources, as well as the implementation of early interventional strategies aimed at improving clinical outcome by exploiting the enhanced neuroplasticity of the newborn brain. Characterizing early brain function could be crucial in complementing current clinical assessment tools and structural imaging measures. In view of its intrinsic safety, and good spatial resolution functional-MRI (fMRI) has the potential to be applied to the preterm and neonatal population in order to explore the emerging brain function. However, to date fMRI studies of the preterm population have been hampered by methodological challenges, including the inability to present infants with reliable and consistent patterns of sensory stimulation. This is especially true for studies which have attempted to assess the early functionality of the sensorimotor system; however these may of particular relevance given the susceptibility of preterm infants to neuromotor dysfunctions such as cerebral palsy.

The main goals of this thesis were to develop a standard approach and a set of tools to allow conducting task-dependent fMRI studies of the preterm population in a safe and reliable manner, and to utilize the systems developed to investigate early brain function, with a particular focus on the sensorimotor system.

Structure

Background and literature review

- Chapter 1 describes how preterm birth can interfere with the natural process of brain maturation normally occurring in-utero throughout the 3rd trimester of gestation, ultimately leading to a range of neurodevelopmental disorders, including neuromotor impairments.

The potential benefits of early assessment and intervention are then reported, with a focus on how functional-MRI (fMRI) can help complement current techniques and provide new important biomarkers. This will provide the reader with an understanding of the clinical motivation underlying the work of this thesis.

- Chapter 2 describes the general principles underlying blood oxygen level dependent (BOLD) fMRI, and the methodology with which BOLD fMRI data sets are analyzed within this thesis. This will enable the reader to interpret the results that will be presented in Chapters 4, 5 and 6.
- Chapter 3 reviews the current literature of neonatal fMRI studies, reporting on the challenges and limitations of current approaches, and on the ambiguity of the results that have so far been reported. This will provide the reader with the technical motivation underlying the work of this thesis

Work of this thesis

- Chapter 4 firstly presents the technical aspects of a wrist and ankle proprioceptive stimulator system, designed to meet the requirements specific to the neonatal population. Experimental results are subsequently presented, which reveal an emerging somatotopic arrangement of the sensorimotor cortices.
- Chapter 5 firstly presents the technical aspects of an orocutaneous and an olfactory stimulator. Experimental results are then reported, which suggest that the functionality of the olfactory system is already well established in by the time of birth.
- Chapter 6 presents the results of a developmental conducted with the wrist stimulator presented in Chapter 4, which was aimed at characterizing the maturational trajectory of the somatosensory system throughout the preterm period and how this can be influenced by extra-uterine exposure.

Conclusions and future work

- Chapter 7 presents the novel technical and neuroscientific contributions provided by the work of this thesis, discusses the potential clinical implications of the reported findings, and suggests the direction of future work.

Chapter 1: Early brain development and preterm birth

Overview - Infants born preterm are exposed to a markedly higher risk of suffering from long-term neurodevelopmental impairments, even in the absence of overt cerebral abnormalities or injuries. The work of this thesis was performed with the far-reaching goal of identifying new biomarkers of abnormal cerebral development, which can ultimately add to the diagnostic and prognostic information currently gathered from structural imaging and clinical assessment techniques. This could assist clinicians in developing, implementing and assessing early therapeutic strategies aimed at improving clinical outcome by exploiting the enhanced cerebral plasticity of the preterm brain.

With this in mind, in this chapter I will first describe the stages of cerebral development normally occurring in-utero throughout the third trimester of gestation, to then highlight how preterm birth can interfere with natural brain ontogeny and lead to cerebral abnormalities. I will report how normal development and aberrations can be detected in-vivo via magnetic resonance imaging (MRI), and the diagnostic and prognostic benefits that can be obtained combining structural findings, with other clinical assessment strategies. Finally, I will discuss how functional imaging techniques such as functional-MRI (fMRI) can add a further dimension to the early brain characterization, potentially providing complementary, if not crucial, information which may help devising new clinical biomarkers.

This Chapter contains excerpts from:

Allievi, A. G., Arichi, T., Gordon, A. L., & Burdet, E. (2014). Technology-aided assessment of sensori-motor function in early infancy. *Frontiers in Neurology*, 5. doi:10.3389/fneur.2014.00197

1.1 Preterm brain development: a structured sequence of maturational changes

1.1.1 Macroscopic morphological development

The first major neurodevelopmental milestone is “neurulation”, which occurs between the 3rd and 7th weeks of gestation, and leads to the establishment of the central nervous system (CNS), (Table 1.1, (Volpe, 2008)). In particular, brain development occurs via “primary neurulation”, which begins after approximately 18 days of gestation, when the extremities of a layer of germinal cells lift, fold and progressively fuse together forming the neural tube (Volpe, 2008). Through gradual differentiation of nerve cells, during the 5th and 6th week of gestation the neural tube is then compartmentalized into 3 primary sub-regions: the hindbrain, the mid-brain, and the forebrain (Lagercrantz and Ringstedt, 2001). During the 2nd and 3rd month of gestation, the forebrain is further subdivided into the telencephalon and diencephalon, which collectively give rise to all major cerebral structures prior to the 3rd trimester of gestation (Lagercrantz and Ringstedt, 2001).

An important substrate arising from the telencephalon is the cerebral cortex, which initially exhibits a liscencephalic appearance and gradually assumes its mature morphology following a preset process termed “gyrification”, during which the gyri and sulci are formed. Gyrification proceeds especially quickly throughout the 3rd trimester of gestation, peaking between gestation week 26 and 28 (Volpe, 2008). As a result, during this period, the increase in cortical surface area is exponentially greater than that of cerebral volume, with the ratio of their respective increases determined by an allometric scaling law (Volpe, 2008, Kapellou et al., 2006).

Finally, it is important to note that during the late preterm period and (predominantly) throughout the first 2 years of postnatal life, neural axonal projections become gradually coated with myelin; a fatty bilayer that provides axonal insulation and enhances electrical conductivity along the central and peripheral nervous networks (Volpe, 2008, Deoni et al., 2011, Battin and Rutherford, 2001).

Table 1.1: Summary table displaying the major events in human brain development and their peak times of occurrence (adapted from (Volpe, 2008)).

Major Developmental Event	Peak Time of Occurrence
Primary neurulation	3-4 weeks of gestation
Secondary neurulation	4-7 weeks of gestation
Prosencephalic development	2-3 months of gestation
Neuronal proliferation	3-4 months of gestation
Neuronal migration	3-5 months of gestation
Organization	5 months of gestation to years postnatally
Myelination	Birth to years postnatally

1.1.2 Development of the cerebral cortex

By the beginning of the 2nd month of gestation, the telencephalic primordium is entirely composed of neuroepithelial stem cells that form a proliferative layer referred to as the “ventricular zone” (VZ) (Bystron et al., 2008). The VZ becomes increasingly thicker until embryonic day 33, when neural stem cells downregulate their neuroepithelial characteristics, and instead begin to largely differentiate into multipotent radial glial cells (Bystron et al., 2008). Following this, “basal” neurogenic cells accumulate below the VZ forming a layer that has been termed the subventricular zone (SVZ). By gestation week 25-27 the SVZ keeps proliferating, effectively becoming the principal progenitor of cortical neurons in the expanded cerebrum as the VZ is downsized to a one-cell-thick layer (Bystron et al., 2008).

While neural stem cells proliferate and differentiate within VZ and SVZ, at around gestation week 7 ca. pioneering cortical neurons begin to emerge (probably via somal translocation) and accumulate to form a preplate (PP) layer above the VZ (Bystron et al., 2008, Volpe, 2008). At approximately gestation week 10, the cortical plate (CP) then begins to form within the PP, splitting it into an inner compartment of heterogeneous cell population, the intermediate zone (IZ), and a superficial cell-sparse layer, termed the marginal zone (MZ), which eventually develops into layer 1 of the mature cortex (Bystron et al., 2008). The remaining 5 layers of the cerebral cortex are then thought to be formed in an “inside-out sequence”, with newly born cortical neurons inhabiting

increasingly outer layers (i.e. from layer 6 to layer 2) of the CP (Bystron et al., 2008). The full layering of the cortex is thought to be achieved by gestation weeks 20 to 24 (Bystron et al., 2008, Volpe, 2008). It is important to note, however, that not all cortical regions mature synchronously (Bystron et al., 2008, Volpe, 2008).

1.1.3 Development of structural connectivity

In parallel to the progressive layering of the cortex, a connectivity-rich zone termed the subplate (SP) starts to differentiate from within the IZ. The SP first becomes discernible at approximately gestation week 10-12, to then peak at around gestation week 29 and fully regress by gestation week 38 (Kostovic and Jovanov-Milosevic, 2006, Bystron et al., 2008). This layer contains well-differentiated neurons with pre-, intra- and post- synaptic elements but also acts as a buffer wherein the sprouting axonal projections originating from the lower cerebral structures wait for their cortical targets to fully develop before growing into them (Kostovic and Jovanov-Milosevic, 2006).

After 26 weeks of gestation, the afferent fibres then gradually penetrate the CP to assume their laminar positions. The first synapses appear within the CP in a deep-to-superficial fashion (Kostovic and Jovanov-Milosevic, 2006). Consequently, in the preterm infant, there is a predominance of deep synapses and deep dendritic maturation as this precedes the gradual shift to superficial cortical layer penetration. Indeed, between 26 and 28 weeks of gestation, thalamocortical axons ascend through the developing layer VI and densely innervate layers IV and III, leading to significant synaptogenesis with the somatosensory, auditory, visual and frontal cortex and the formation of sensory-driven cortical function (Kostovic and Rakic, 1990, Kostovic and Judas, 2010). Between 31 and 34 weeks gestation, the thalamocortical terminals in layer IV become more elaborate and there is rapid growth of pyramidal neurons in layers III and V (Kostovic and Jovanov-Milosevic, 2006, Bystron et al., 2008). The following period (between 35 and 37 weeks of gestation) is marked by the growth of interhemispheric connections with the production of callosal axons that grow through the periventricular zone and penetrate the subplate to eventually invade the cortical plate (de Graaf-Peters and Hadders-Algra, 2006). The 50% increase in the surface and volume of cortical gray matter observed between 29 weeks and term, with an acceleration of its growth after 28 weeks, can

therefore largely be attributed to the ingrowth and branching of cortical afferents from the subplate, leading to the formation of corticocortical systems (Kostovic and Jovanov-Milosevic, 2006, Volpe, 2008).

Due to the important role of the subplate in the establishment of neural connectivity and, more generally its fundamental contribution to neurodevelopmental physiology (Volpe, 2008), several in-vivo studies such as electroencephalography (EEG) studies (described in Chapter 6) and those performed using MRI have often focused on capturing the dynamics of its maturation.

1.1.4 MRI of the developing preterm brain

A large number of studies have applied in-vivo MRI techniques in the foetal and preterm population, and have been able to devise clear signatures of the cytoarchitectural organization in addition to accurately characterizing the developing gross cerebral morphology (Battin et al., 1998, Battin and Rutherford, 2001, Kostović et al., 2002, Rados et al., 2006). Foetal studies have consistently identified the maturing cerebral layers including the CP, the SP, the IZ, and the SVZ from gestation week 20 to 23 ca. (Battin et al., 1998, Battin and Rutherford, 2001, Kostović et al., 2002, Rados et al., 2006). In particular, the transient subplate zone appears as a widening band of high T2 (and low T1) signal intensity.

From gestation week 24 to 32 (early preterm phase) synaptogenesis continues inside the subplate, while thalamocortical projections grow from the subplate into the frontal, somatosensory, visual and auditory regions of the cortical plate (Kostovic and Jovanov-Milosevic, 2006). This process gives rise to a hybrid framework of transient (subplate) and permanent (cortical) circuitry destined to last long into gestation (Kostovic and Jovanov-Milosevic, 2006). By gestation week 29, largely due to the continuous expansion of thalamocortical, callosal and cortico-cortical afferents projections, the subplate layer is seen to reach up to four times the thickness of the cortical plate (Kostovic and Jovanov-Milosevic, 2006, Kostovic and Judas, 2010). Non-myelinated callosal fibres form into large bundles (at ca. week 28) which are clearly identified by DTI and are associated with a subventricular zone of low-signal-intensity on MRI. The establishment of transient connectivity also marks the ingrowth of thalamocortical projections into the cortical plate, which enables the propagation of

sensory impulses originating from the periphery of the brain (Kostovic and Jovanov-Milosevic, 2006). It is at this stage that cortical development is thought to become “sensory-driven” (Kostovic and Jovanov-Milosevic, 2006), a notion compounded by electrophysiological and functional-MRI (fMRI) studies which have revealed clear cortical responses to external stimulations (this is discussed in depth throughout the rest of the thesis) in premature infants of equivalent gestational age. This is a fundamental principle when considering the effects that environmental interactions may have on the early cerebral development.

Gestation weeks 33-35 (late preterm phase) are characterized by the propagation of thalamocortical and long cortico-cortical pathways into the cortical plate, where incoming axons are processed to establish intracortical connectivity (Kostovic and Jovanov-Milosevic, 2006). This process leads to the progressive establishment of the cerebral cortex and its permanent network of functional circuitry, and is paralleled by a progressive recession of the transient subplate layer. MRI studies of preterm infants (Kostovic and Jovanov-Milosevic, 2006) have clearly demonstrated a concurrent thinning and decrease in T2 signal intensity of the subplate band starting from ca. 34 weeks gestation. MRI of the subplate dynamics is thus highly indicative of several underlying cerebral maturation processes, and is regarded as the most important biomarker for cortical development during the late preterm phase. These findings are summarized in Table 2.1 below. At this stage exogenous (sensory inputs) and endogenous (spontaneous movements) cerebral potentials are thought to significantly contribute to the developmental organization of cortical pathways (observed via diffusion anisotropy MRI) and, ultimately, of the cortex (Lagercrantz and Ringstedt, 2001, Kostovic and Jovanov-Milosevic, 2006). However, the possible influence of environmental factors on the organization of the cortex and specificity of the area of cortical response remains to be determined.

Table 2.1: Histology and concurrent magnetic resonance imaging (MRI) appearance of cerebral developmental schedule from gestation to term. Adapted from (Kostovic and Jovanov-Milosevic, 2006).

Developmental phases	Histological features	MRI correlates
Early preterm (24-32 PCW): th-cx fibers grow into cortical plate, synaptogenesis	VZ: gradual disappearance	Attenuation of the high T1 intensity along ventricles
	SVZ: thickening of callosal fibre-rich Zone	Low T1 intensity, thickening of the signal band
	IZ: formation of corona radiata & gyral white matter	Premyelination e anisotropy after 30th PCW 35
	SP: enlargement due to addition of callosal and other cortico cortical fibres, decrease of ECM	Gradual increase in T1 signal Intensity
	CP: formation of synapses in the deep portion	Anisotropy values high in DTI
Late preterm (33-35 PCW): cortico-corticals in CP	SP: gradual decrease in thickness and ECM content	Poorly delineated as transitional zone below CP
	CP: first lamination, development of dendrites	Decrease of T1 signal and DTI anisotropy
Neonatal phase: disappearance of transient patterns, rapid synaptogenesis, sensorydriven activity	SP: remnants of neurons ³	Not detectable
	CP: development of columnar circuitry	Anisotropy values approach zero in DTI
	Retraction of certain portion of the callosal axons	Decrease of callosal crosssectional area
	Short cortico-cortical connections	Visible tertiary gyri

VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone; AchE, acetylcholine esterase; CP, cortical plate; PCW, postconception time in weeks; MZ, marginal zone; ECM, extracellular matrix; SP, subplate zone; GABA, g-aminobutyric acid; DTI, diffusion tensor imaging.

1.2 Improving neurodevelopmental outcome of the preterm infant: early diagnosis and intervention

1.2.1 Preterm birth and clinical outcome

Largely due to the ongoing advances in neonatal care, the survival rate of infants born preterm (before the full 37 weeks of gestation) is steadily increasing. The sharp decrease in the minimum viable age for premature birth has resulted in the spurt of a very preterm population, which is highly susceptible to a broad new spectrum of physio-developmental impairments (Allen, 2008, Ment et al.,

2009a, Hack, 2009, Doyle and Anderson, 2010). Of these, neurological impairments are the most prominent and are especially common, with up to 50% of preterm infants born at very low birth-weight develop a spectrum of conditions that ultimately result in cognitive impairments, behavioural difficulties and a requirement for special educational support (Hintz et al., 2005, Allen, 2008, Larroque et al., 2008b, Beaino et al., 2010, Johnson et al., 2009, Johnson et al., 2010). Although the incidence and clinical implications of disabilities associated with preterm birth have been thoroughly reported, the concurrent underlying neuro-pathological correlates remain relatively poorly understood (Ment et al., 2009a, Volpe, 2009a). In recent years, changes in clinical practice have contributed to drastically reduce the incidence of severe forms of preterm brain injury (such as particular cystic-PVL and Haemorrhagic Parenchymal Infarction (HPI) (Platt et al., 2007, Robertson et al., 2007, Surman et al., 2009, Groenendaal et al., 2010, van Haastert et al., 2011)). However, even in the absence of overt cerebral abnormalities, preterm infants are often still affected by neurodevelopmental difficulties. This is thought to be indicative of a more generalized neuropathology specific to the preterm population that has been termed “Encephalopathy of Prematurity” (Allen, 2008, Volpe, 2009b, Volpe, 2009a).

1.2.2 Cerebral Palsy

Cerebral Palsy (CP) is a collective term describing neuromotor impairment resulting from a non-progressive neurological condition (Bax et al., 2005, Bax, 1964, Palmer, 2004). Although the aetiology of CP is likely to be complex and multi-factorial, in the majority of cases it results from pre/perinatally acquired brain lesions (Bax et al., 2005). Infants born preterm represent a high-risk phenotype, largely due to the wide range of associated perinatal complications, including the reported forms of white matter injury (such as PVL) and IVH. Ca. 9% of all live preterm births are affected by CP, and represent 40 to 50% of all CP cases (Ancel et al., 2006, Vincer et al., 2006). Patients affected by CP may require constant treatment on a multitude of orthopaedic and neurological fronts, which, in most cases, improve their motor skills along with their capability to enjoy near-normal lives. The long-term supportive care required for CP patients is multi-disciplinary, including hospital and community medical treatment as well as home adaptations and

social support. It is therefore a major healthcare concern with regards to the financial burden to both the NHS and families involved (Kruse et al., 2009, Mangham et al., 2009, Ment et al., 2009a).

1.2.3 Neuroplasticity of the preterm brain

While the resulting physical impairments can be managed, CP is currently non-curable and, most importantly, there appear to be no bio-physiological mechanisms in place for an efficient spontaneous healing or recovery. This apparent failure is perhaps surprising given that several popularized interpretations of the Kennard principle suggest neural plasticity (the potential to compensate for neural damage via the retention/formation of alternative axonal routes and synaptic connections) to be at its most mouldable state during early infancy (Kennard, 1936, Kennard, 1942, Schneider, 1979, Finger and Wolf, 1988, Dennis, 2010, Bennet et al., 2013, Teuber and Rudel, 1962, Butz et al., 2009). This notion is currently under close scrutiny, with much supporting/negating evidence provided by recent physiological studies comparing residual motor functionality after perinatal injury in adolescents born preterm to that of adolescents born at term. These have revealed that while premature birth appears to be associated with a decreased capability for long term depression (LTD)-like neuroplasticity (a key cellular mechanism underlying cortical plasticity) (Pitcher et al., 2012), both the preterm and term brain are able to self-reorganize following injury to reinstate at least some degree of somato-motor functionality. Some of the observed compensatory mechanisms entail the retention of contra-lesional primary motor pathways following perinatal brain injury, and the ability of somatosensory projections to bypass prenatal periventricular lesions to reach their original cortical destination (Staudt et al., 2006, Staudt, 2010, Arichi et al., 2014). Even where such events of neuro-reorganization are evident, patients still often exhibit severely impaired neuromotor function, suggesting that the neonatal brain may be incapable of autonomously “rewiring” itself in the most efficient manner to compensate for injuries. The process of plasticity-mediated recovery may however be enhanced by external therapeutic interventions.

Crucially, post-stroke rehabilitation of adult patients indicates that neuromotor recovery mainly takes place in the first months following the insult, and is largely dependent on the timely delivery of activity-guided therapies. In keeping with this notion, it could be argued that fine-tuned

interventional strategies delivered immediately after preterm brain injury would greatly enhance neuro-motor outcome, particularly if these were to be initiated before an acquired lesion is fully established (Johnston, 2009, Staudt, 2010, Hadders-Algra, 2011, Oberg et al., 2012). At present targeted activity-guided intervention is only initiated after clinical and behavioural abnormalities allow clinicians to accurately diagnose CP, which is often at around 18 months of age (Allen, 2002, Wood, 2006, Ment et al., 2009a). This highlights the need for novel tools able to both detect CP at an earlier stage, and dynamically evaluate the impact of the intervention strategies being implemented. This is especially important in view of the damage/formation of aberrant plasticity, which may result from inappropriate/noxious intervention; a risk that underlies the conservative minimal-touch-policies of current clinical care (Field et al., 2006).

1.2.4 Current clinical assessment tools

Current early clinical assessment tools typically involve a single or multiple assessors (usually health care professionals who have been specially trained to administer the test) observing or interacting with an infant, and subsequently scoring the motor behaviour or performance in particular tests. Scoring is generally done using ordinal scales which may be dichotomized (i.e. yes/no reflecting an infant's ability to perform a task), or have a range of values which reflect performance with reference to a predefined rank order (relative to a population appropriate spectrum of performance). Recent systematic reviews have identified neurobehavioral and neuromotor assessments suitable for use in infants and evaluated their validity and reliability at: (i) discriminating between individuals who are/are not affected by neurological or motor dysfunction, functional limitations, or disabilities at the time of assessment (discriminative ability), (ii) predicting future neuromotor performance, condition, or outcome based upon performance at the time of assessment (predictive ability), (iii) evaluating longitudinal changes in neuromotor performance, and the impact of intervention (evaluative ability) (Noble and Boyd, 2012, Bosanquet et al., 2013). Due to the current lack of a criterion standard for neonatal assessment, all available tools are criterion referenced, and no individual tool offers the best possible discriminative, (long and short term) predictive and evaluative properties (Noble and Boyd, 2012). While it is proposed that combining

assessments that measure different constructs may yield the best psychometric results, this approach is often impractical. A suitable tool should thus be chosen by considering the primary purpose of the (Noble and Boyd, 2012). In general, due to the statistical effects posed by the numerous environmental and developmental confounds, evaluative validity of current assessment tools has been sparsely and poorly reported (Campbell, 2005). Of the identified assessment tools, the Test for Infant Motor Performance (TIMP) was found to be a good all-round tool (and the only suitable evaluative assessment tool), and the assessment of General Movements (GM) to be the best long-term predictive assessment tool for the given age range (Noble and Boyd, 2012).

The TIMP was specifically designed for the assessment of infants between 34 weeks post-menstrual age (PMA) and 4 months post-term, and consists of two scales for rating both spontaneous motor behaviour and motor responses to stimulation (Campbell, 2005). Spontaneous motor behaviour is scored by observed items consisting of movements such as head centring, reaching, and finger movements, while the elicited motor behaviour is scored by performance in 31 items which assess the infants response to placement, handling, and visual or auditory stimulation (Campbell, 2005). Of particular importance, specific items in the TIMP have been formally assessed for their utility in the prediction of cerebral palsy, and have been found to correlate well with developmental outcome at 6 months of age as assessed by the Bayley Scales of Infant Development (Barbosa et al., 2005, Kim et al., 2011). However, while the TIMP has been found to have excellent sensitivity (with over 90% of infants correctly predicted to develop cerebral palsy) and good specificity (with 76% of infants correctly predicted not to develop later cerebral palsy) for the prediction of motor outcome at 12 months of age, approximately 35 minutes are required for an experienced practitioner to administer the test, and its predictive validity has been found to differ depending on the age of testing (Campbell et al., 2002, Barbosa et al., 2007). It has been suggested that the assessment of GMs may provide the most objective measurement of an infant's clinical status as it performed using a video recording of their spontaneous movements; thereby eliminating the need for patient handling and minimizing inter-rater variability (Prechtl, 1990, Hadders-Algra, 2004). The fundamental premise of GM assessment is that the quality and quantity of self-generated motor behavior is an accurate representation of the condition of the developing nervous foetal or infant nervous system

(Prechtl, 1990). GM assessment within the first 4 months of life has been found to predict cerebral palsy at 2 years of age with an excellent sensitivity (greater than 90%), and a good, but variable specificity (between 60%-100%).

Given the previously discussed implications of activity-dependent processes on the nervous system development, a patient group of particular interest is young infants at high risk of developing unilateral cerebral palsy, in whom an accurate measure of early bimanual function may enable the development of interventions using the apparent window of enhanced neuroplasticity in this period. Taking this into account, the mini-Assisting Hand Assessment (AHA) tool for infants between 8 and 18 months of age at high risk of unilateral cerebral palsy is currently under development (Greaves et al., 2013, Greaves et al., 2012). The aim of the mini-AHA is to transpose the established AHA clinical assessment for older children to this younger population, whilst maintaining the tool's capacity to accurately assess a child's ability to use each of their hands independently, and additionally to incorporate more cognitively demanding (and perhaps more discriminative) bimanual tasks (Greaves et al., 2012). Much like the AHA, the mini-AHA proposes to assess, discriminate and evaluate longitudinal changes in the usage and performance of the affected hand in children with unilateral cerebral palsy. This is done by observing the children play with a series of specifically designed toys, and scoring their ability (on a 4 point scale) to perform 20 increasingly difficult object-related manipulative tasks, ranging from simple holding of an item to bimanual toy interaction (Greaves et al., 2013). A preliminary internal-scale validation study has found that mini-AHA items can be ordered hierarchically using a Rasch model fit, such that a discrete monotonic increase is seen in both the item difficulty coefficient and the infant's ability to use their affected hand (Greaves et al., 2013).

Children were very finely separated according to their level of ability, and, crucially, test scores were found not to be affected by age. The mini-AHA and in general all observation-based clinical assessment tools however suffer from several limitations. Firstly, longitudinal studies on large population cohorts are required to validate the discriminative and predictive power of the tool. Before such tools can be made common place in clinical practice, all of the administering health care professionals must undergo extensive (and expensive) training sessions to minimize the effects that

subjectivity and resultant inter-rater variability may have on the final score. As the majority of clinical assessment tools are scored using categorical or ordinal scales, they all suffer from limited resolution, affecting their ability to provide new information about the disease mechanisms and natural history. Finally, the assessment procedures are often extremely time-consuming, resulting in prolonged and costly commitments of clinical staff and facilities.

1.2.5 Neuroimaging assessment

Of the previously described clinical assessment tools, by nature of its application and off-line scoring, perhaps the most amenable to combination with a technology-based solution for infants is GM assessment. Indeed, studies have shown that the predictive power of GM assessment is preserved even when the assessment is automated by fitting motion tracking sensors to the extremities of the infants (in addition to video recording the infant), and then performing motion feature extraction on a highly filtered motion image using custom computer software (Adde et al., 2013, Adde et al., 2010, Adde et al., 2009). The sensitivity and specificity of GM assessment for the prediction of cerebral palsy has also been found to be greatly enhanced by combination with MRI at term equivalent age (Skiold et al., 2012).

In comparison to other clinical assessment tools and commonly used neuroimaging modalities (such as cranial ultrasound), Magnetic Resonance Imaging (MRI) at term equivalent age has a relatively good evidence base supporting its use for the prediction of later cerebral palsy (Bosanquet et al., 2013). It has become the diagnostic investigation of choice in infants who have suffered brain injury at the time of birth, and can provide vital prognostic information through precise delineation of both the acquired lesion and its associated effects. In addition to standard anatomical images (usually T1 and T2 weighted), it is also worthwhile acquiring a diffusion weighted image (DWI), particularly for identifying areas of early ischemia before it can be readily seen on structural images. Following perinatal stroke, MRI has been shown to be highly predictive of later unilateral spastic cerebral palsy (in the side opposite to the brain injury) if abnormal signal is seen in the ipsi-lesional posterior limb of the internal capsule (PLIC), thalamus, and peri-rolandic cortex (Boardman et al., 2005); and if pre-Wallerian degeneration is evident on DWI in the contra-lesional cerebral peduncle

(de Vries et al., 2011). Similarly following Hypoxic Ischemic Encephalopathy (HIE), a number of studies have demonstrated that abnormal signal in the basal ganglia and thalami is highly predictive of dyskinetic or athetoid cerebral palsy (Cowan et al., 2003, Rutherford et al., 2004) injury to the basal ganglia, thalami, and brainstem are predictive of feeding and communication difficulties (Martinez-Biarge et al., 2011), and changes in myelination of the PLIC predictive of adverse motor outcome (Martinez-Biarge et al., 2011). Of further interest, a meta analysis of the predictive power of MRI techniques for predicting adverse outcome following HIE found that abnormal deep gray matter lactate/N-acetyl-aspartate (NAA) ratio on single proton MRS (magnetic resonance spectroscopy) has the highest pooled sensitivity (Thayyil et al., 2010). The predictive power of MRI may be further enhanced by the application of acquisition sequences which can also visualize other diverse aspects of brain tissue composition, microstructure, and function. A clear example of this is tractography derived from diffusion MRI data, which utilizes information about the random diffusion of water inside the brain to delineate the major axon fiber bundles within the brain's white matter (Mori and van Zijl, 2002). Using this approach it is possible to further increase the sensitivity of MRI for the prediction of later cerebral palsy by identifying subtle asymmetry in the microstructural integrity of the corticospinal tracts in infants with focal brain injury (van der Aa et al., 2011, Roze et al., 2012). When correlated with neurological or developmental outcome, these measures may therefore provide additional prognostic information, and can also be used as highly accurate cerebral biomarkers for studies of pathological effects and treatments (Ment et al., 2009b).

1.2.6 Understanding early brain function

With the aim of guiding and assessing early intervention strategies, as well as efficiently allocating streamlines of financial and clinical support, an on-going goal is to develop accurate assessment tools capable of providing enhanced diagnostic and prognostic power at an earlier developmental stage during infancy. Future assessment tools should not only have an increased specificity and sensitivity for predicting neurodevelopmental impairments (such as CP) over existing tools, but could also be predictive of the severity of such impairments (rather than just their

occurrence), giving clinicians much needed patient-specific information on their outlook, recovery and therapeutic effectiveness.

In this chapter I have highlighted how over the course of the latter decades, an extensive insight has been gained on the morphology and structure of the developing human brain. It is however becoming increasingly evident that structural information alone is not sufficient to explain much of the pathophysiology that underlies premature birth, and thus to predict future neurodevelopmental outcome. Although interlinked, cerebral structure and function develop separately (Smyser et al., 2010) such that functional impairments are not necessarily linked with structural aberrations (and viceversa). This indicates that abnormal patterns of brain function could underlie currently unexplained neuropathology. There is therefore an urgent need to develop precise methods and techniques of assessing early infant brain development and the impact of perinatal brain injury on the latter. Ideally, these imaging techniques would then be used as “biomarkers” during specific interventional brain stimulation strategies in order to monitor the benefits of these strategies and preemptively treat patients prior to the appearance of clinical pathology (Ment et al., 2009b).

With the advent of advanced imaging techniques such as fMRI it may be possible to finally gain an insight on the functional development of the preterm brain. One of the major aims of this thesis is to utilize robotic tools in conjunction with fMRI to provide a characterization of the normal preterm somatosensory development and associated effects of early external interactions. While somatosensory functionality is of particular interest due to its direct link with neuro-motor outcome (and thus with CP), functional difficulties associated with prematurity (including neuromotor) are likely to extend to multi-sensory integration and other forms of sensory processing. This thesis will therefore also aim to complement current knowledge and provide a qualitative representation of the emerging whole-body sensory map by studying the preterm brain response to several types of sensory stimulations.

Chapter 2: Advanced MRI techniques

Overview – Functional-MRI (fMRI) exploits the changing magnetic properties of haemoglobin to offer a non-invasive, although indirect, way of visualising and quantifying in-vivo brain activity. T2* weighted MRI images can capture the stimulus-triggered neurovascular response as a change in Blood Oxygen Level Dependent (BOLD) contrast. BOLD. fMRI responses to stimulations are reproducible across sessions and subjects, and the combination of data sets allows statistically inferring significant patterns of brain activity. fMRI offers a good spatial resolution, and is intrinsically safe as it does not produce harmful ionising radiations. In this thesis BOLD fMRI studies of the neonatal population were carried out in order to exploit the potential of this technique to offer a new window into the understanding of early brain functional development. To provide a general understanding of the methodological challenges, and aid the interpretation of the results obtained, the general principles underlying Magnetic Resonance Imaging, and the analysis and interpretation of fMRI data sets are presented in this chapter.

2.1 Magnetic Resonance Imaging

While characterizing human brain function has long represented a major undertaking of neuroscientific research, it is only with the recent advent of functional imaging techniques that scientists have been provided with a powerful tool to infer brain activity in-vivo from direct and indirect physiological measures (Huettel et al., 2009). In the fully grown adult human brain the concept of brain “mapping” and functional localization has long been described, with Brodmann identifying the currently employed atlas of 52 distinct subdivisions of the cortex via histological studies (Brodmann and Garey, 1999). Although recent studies based on novel functional imaging techniques have confirmed the notion that cerebral substrates are involved in specific functional tasks, they have also revealed that brain function occurs as a co-ordinated activity of “networks” of cerebral regions (Bullmore and Sporns, 2012). In this respect, a true understanding of brain function is only likely to be gained by accurately describing both functional localisation and the whole-brain patterns of functional activity that result from underlying processes of neural-interactions, and correlating such functional information with structural landmarks. To achieve this complex task, researchers will likely be required to interpret and integrate the broad, but complementary range of information provided by the spectrum of available functional imaging techniques.

A trade-off between invasivity and spatio-temporal resolution is at the core of the diverse, though complementary nature of existing functional imaging techniques. Highly invasive techniques have the potential to measure depolarization of single neurons at a very fine timescale, but are rarely employed due to the induced tissue damage. Non-invasive in-vivo functional imaging techniques generally operate at a much lower spatial resolution, and are employed to infer activity of large neural populations (Huettel et al., 2009, Matthews, 2001). Due to their high dependence on the underlying imaging principle, both spatial and temporal resolutions vary greatly (and are generally inversely proportional) across the spectrum of non-invasive techniques. Whilst electrophysiology-based techniques are able to offer a very fine temporal resolution at the expense of spatial resolution, other recently developed imaging techniques such as functional-MRI (fMRI) have greatly enhanced spatial localization of functional activity at the expense of temporal resolution.

The principle underlying fMRI is that neural activity is coupled to metabolic processes, such as an activity-triggered increase in the demand for blood flow in specific areas of the brain (reviewed in (Buxton, 2009, Gjedde, 2001, Kim and Ogawa, 2012, Arichi, 2012)), which can be visualized via quickly acquired MR images to provide an indirect measure of brain function. Owing to its intrinsic safety (no ionising radiations), versatility (no need for dyes or radioactive isotopes) and its ability to offer spatially accurate whole-brain activity visualisation at a relatively fine temporal resolution (Matthews, 2001, Huettel et al., 2009, Buxton, 2009), fMRI is a very suitable technique for functional studies of the human brain. Since first being introduced over 20 years ago, fMRI has in fact provided a wealth of neuroscientific (and clinical) information concerning the functionality of the mature brain. These findings indicate that fMRI could also be employed to characterize the emergence and development of functionality during the early stages of human brain maturation, however a discouraging history of inconsistent findings and methodological challenges inherent to both data acquisition and analysis have so far limited fMRI studies within the preterm population (Arichi, 2012). To put these challenges into context, this chapter will aim at providing an understanding of the general principles that underlie fMRI acquisition and analysis. This will be done by firstly introducing the nuclear magnetic resonance (NMR) properties of the atoms, and detailing how these are exploited to generate MR images. Based on these general notions, diffusion tensor imaging (DTI) will then be discussed, as this advanced MRI technique has been used to map the anatomical fibre tract correlates of cerebral functional activity throughout the work presented in this thesis. Finally fMRI data acquisition, processing and analysis will be presented.

2.1.1 Nuclear magnetic resonance properties of the atom

MRI is based on the nuclear magnetic resonance (NMR) properties of the atoms that constitute organic tissues. In keeping with the mechanical quantization of particle physics, all atomic elementary particles are characterized by a (discrete) “spin” value, which parameterizes a form of intrinsic angular momentum. This physical property is specific to quantum mechanics and does not have a direct physical representation, but can be loosely interpreted as the rotation of a particle around its axis. Although the direction of spin can be altered (inverting the sign of spin value), its

magnitude is intrinsic to the type of elementary particle and cannot be changed in any known way (Buxton, 2009). Nuclei possess a net spin, which is the result of the linear combination of spin values of individual nucleons (protons and neutrons). While the effects of nucleons spinning in opposite directions cancel out, nuclei containing an odd number of nucleons will always have an unbalanced nuclear net spin. Owing to electromagnetic interactions, the spinning of an unbalanced, positively charged, proton generates an electric current that causes the nucleus to act like a magnetic dipole: a magnet with a north and south pole parallel to the spin axis. The nuclear dipole has an intrinsic magnetic moment, which causes it to experience a torque (and thus a change of orientation) when an external magnetic field is imposed on it (Buxton, 2009). In practice, due to the conservation of angular momentum, when a nuclear dipole is introduced into a magnetic field (referred to as the B_0 field in MRI) the nuclear spin axis is caused to wobble (precess) around the axis of the imposed field, while gradually (but never fully) aligning with it (Buxton, 2009, Westbrook et al., 2005, McRobbie et al., 2006, McRobbie, 2007). The frequency at which the nucleus precesses, commonly referred to as the Larmor frequency (ω_0), is constant for a given atomic nucleus and directly proportional to the intensity of an external field B_0 as described by the equation: $\omega_0 = \gamma B_0$, where γ is the atomic nuclei-specific gyromagnetic ratio (Buxton, 2009).

2.1.2 Principles of MR signal

Due to their abundant presence in all organic tissues of the human body and a nucleus formed of a single unbalanced proton, hydrogen atoms are the typical substrate of MRI studies. Upon the introduction of a tissue within a magnetic field B_0 , its hydrogen nuclei are forced into one of two possible configurations. The majority of protons are induced to precess parallel to B_0 in a “low energy state”, while some are caused to align anti-parallel to B_0 in a “high energy state”. As a result, the imaginary vector describing the “net magnetization” (M) of nuclear dipoles is brought into alignment with B_0 , such that M is predominantly described by its longitudinal component (M_z). MRI then works in two phases aimed at perturbing the magnitude and direction of M and measuring the current induced on an MR receiver coil while M returns to its equilibrium state (Buxton, 2009, Huettel et al., 2009).

During the first, or “transmit”, phase, an oscillatory magnetic field B_1 is produced by radio-frequency (RF) current pulses generated within the transmitter coil of an MR scanner. B_1 is tuned such that it is perpendicular to M (and B_0) and that its oscillatory frequency matches the Larmor frequency of the precessing hydrogen protons (Westbrook et al., 2005). While the energy introduced by the RF pulse is absorbed by the tissue increasing the number of high energy state dipoles, the B_1 oscillations serve to synchronize the spin of the precessing protons. As a result, M is “tipped away” from the B_0 alignment, and is made to precess at a flip angle (α), which is dependent on the amplitude and duration of B_1 . At this stage longitudinal magnetization M_z is reduced, in favour of the transverse magnetization component of M (M_{xy}). During the second, “receive”, phase of MRI, M_{xy} induces an electric current in the receiver MR coil. Upon removal of the RF pulse, a gradual recovery of M_z is paralleled by a (faster) loss of M_{xy} . This is primarily due to two mechanisms of energy dissipation which completely reverse the effects of the RF pulse (Buxton, 2009):

i) The magnetic interactions between spins of adjacent nuclei, which cause dipole spins to gradually dephase leading to a reduction of the transverse magnetization M_{xy} (Deoni, 2010, McRobbie, 2007). This process, termed free induction decay (FID), occurs exponentially, with the time taken for M_{xy} to drop to 37% of its initial magnitude referred to as T_2 , or the “spin-spin” relaxation time. In practice, in addition to random spin-spin interactions, imperfections in local field homogeneity (due to MR magnet imperfections, and differences in tissue susceptibility) also contribute to a faster FID; this decay is described by a time constant T_2^* (much lower than T_2);

ii) The influence of B_0 , which causes the transition of dipoles back from the high energy state (antiparallel to B_0) to a low energy state (parallel to B_0). As a result the longitudinal component of the net magnetization vector M_z is gradually recovered in the direction of the B_0 field. The time taken for M_z to recover to 63% of its initial magnitude has been termed the “spin-lattice” relaxation time T_1 , as this process involves the release of the energy previously absorbed by the dipoles into the surrounding tissue (McRobbie, 2007, Westbrook et al., 2005, Buxton, 2009, Deoni, 2010).

2.1.3 MRI contrast and acquisition

Since the loss of M_z is dependent on intrinsic properties of tissues (such as their molecular density and composition) measurement of the associated relaxation time constants T_1 and T_2 (derived from current profiles) provides tissue-specific signatures and is the principle that underlies image contrast in all MRI techniques. The fundamental process of MR acquisition involves “exciting” the samples being studied by means of RF pulses, and then measuring signal decay at set time intervals. In practice, several RF pulse sequences exist, in which pulse angle, intensity and delay are all optimized to delay (or tune) signal decay such that more subtle differences in T_2 or T_1 relaxation times can be identified and a better contrast is provided (Buxton, 2009, Westbrook et al., 2005, McRobbie, 2007). In a simple pulse sequence such as the gradient recalled echo (GRE) sequence, individual RF pulses are delivered at a constant time interval (the time to repeat (TR)) to induce a flip angle α (usually $\ll 90^\circ$). The FID signal is then measured at time TE after excitation (Buxton, 2009). In actual practice, measuring signal by FID signal alone is rather inefficient and particularly susceptible to both external and internal sources of magnetic field inhomogeneity which cause the signal to rapidly decay with a T_2^* time constant ($\ll T_2$). Clinical images are therefore usually acquired using spin echo (SE) sequences, which allow reversing time-invariant dephasing phenomena due to the constant magnetic field inhomogeneities, but do not reverse the effects of dephasing due to spin-spin interactions. T_2^* decay can thus be corrected, allowing T_2 decay extrapolation. In typical SE sequences this is achieved by applying further 180° RF pulses to the sample such that the transverse plane of precession is “flipped” inverting the order of the dephasing spinning dipoles. As the “fast-dephasing” protons (which were previously ahead and are now caused to lag behind the “slow-dephasing” protons) catch up and realign with the “slow-dephasing” protons, a new signal is produced at specific echo times (by convention also designated TE) (Buxton, 2009). The signals produced at TEs mirror the original FID, but their peak magnitude is reduced exponentially in accordance with T_2 decay (which is not reversed by spin echo). By inducing this particular trajectory of signal decay, spin echo sequences therefore allow measuring of tissue-specific T_2 time constants.

As previously described, the T2 “spin-spin” relaxation time is predominantly dependent on the strength of local magnetic field interactions, whereas the ability of atoms to release energy into surrounding structures underlies the (much longer) T1 “spin-lattice” relaxation time. As a result, free liquids (composed of relatively unbound molecules and thus presenting slowly dephasing protons) have a longer T2 than denser, macromolecular structures, while the converse is true for T1. MR image sequences exploit these differences to provide high-contrast T1 or T2 “weighted” images. Due to the time-course trajectory of transverse signal decay, good T2 image contrast requires a long (over 1500ms) TR and a long TE, such that liquid substrates with longer T2 appear brighter (due to a lower dephasing, and thus a higher residual signal), while denser tissues with faster dephasing protons appear darker. Conversely, T1-weighted images employ a short TR and a short TE, such that solid substrates with longer T1 appear brighter, while unbound substrates with faster “spin-lattice” energy dissipation appear darker (McRobbie, 2007, Westbrook et al., 2005).

2.1.4 Image formation

In order to create MR images, secondary magnetic field gradients are superimposed to B_0 , such that the NMR signal is spatially localized (Buxton, 2009). 3D spatial localization is achieved by means of 3 orthogonal z,y,x gradients, which respectively provide slice selection, phase encoding and frequency encoding (Buxton, 2009). In order to recognize which point in space is contributing to the recorded FID timecourse, data needs to be spatially encoded. This is done by first selecting a plane in 3D space, and assigning a known phase and frequency signature to each point on the plane such that signals can be differentiated. The gradient along z causes all protons within an x-y plane (slice) to precess in phase and, most importantly, at a particular Larmor frequency, at which they can be excited (and thus differentiated) by matching RF pulses. To better understand the principles underlying MRI spatial encoding, each slice image can be interpreted as an x-y grid (image space) of precessing tissue elements (dipoles), each contributing to the overall signal recorded at any point in time. The aim of MRI is to assign an intensity value to each grid element (pixel) such that a 2D slice image can be formed. Multiple 2D slices are then combined to obtain a 3D visualisation of the image (Buxton, 2009).

At any point in time the signal recorded by the MR receiver coil is the combination of that of all dipoles within a slice. To first differentiate between the signal contributions of individual grid elements, a transient “phase encoding” gradient is used to dephase precession of the dipoles along the y direction of a given slice. When the phase encoding gradient is removed the dipoles return to the base frequency, but a constant phase offset between their spins is maintained. For a single slice image, the whole-slice signal is acquired multiple times, each time increasing the intensity of the phase encoding gradient and thus the spin phase shift between adjacent grid elements (along y). During the readout phase of each signal acquisition, a further “frequency encoding” x gradient is turned on and superimposed onto the “rows” of phase encoded dipole elements (Buxton, 2009). As previously described, the spin of any dipole element can be represented as a precessing magnetization vector with characteristic phase and magnitude. The recorded FID signal can be viewed as the sum of projections of all magnetization vectors onto the axis normal to the receiver coil. At any specific time point during readout, the imaginary line joining the sequence of dephased dipoles’ magnetization vectors can be interpreted as a wave (or spiral) having a phase and a “pseudo-frequency” directly proportional to the rate of phase change (by definition $f = \Delta \phi$) between consecutive elements. Because this pseudo-frequency describes a pattern of spatial orientations of spins along the rows of the slice, it is also referred to as spatial frequency (Buxton, 2009). Low spatial frequencies can be regarded as a loosely wound spiral of dipoles’ magnetization vectors, where elements consecutive in space are likely to contribute equally to the whole-slice FID signal; conversely high spatial frequencies can be regarded as dipoles’ magnetization vectors configured as a highly wound spiral, where elements close in space are highly dephased and their contribution to the whole-slice FID signal is differently weighted. The FID signal contribution of dipoles configured in any given spatial frequency is therefore described by the equation (Buxton, 2009):

(2.1)

$$S_k = \int \rho(x) e^{ikx} dx, \quad \text{with} \quad k = \gamma G t$$

Where k is the spatial frequency, G is the magnitude of the frequency encoding gradient, γ is the Larmor frequency, t is time, $S(k)$ is a complex value describing the signal intensity (real part) and phase value (imaginary part) of a given spatial frequency k , and $\rho(x)$ is the density of dipoles at a given spatial location along the x -axis x .

The sustained frequency encoding gradient (during readout) causes the precession speed of dipoles to increase along the x -axis. This causes the phase shifts between consecutive row elements, and thus the spatial frequency (or winding of the spiral) of dipoles along the x axis of the slice to increase with time. Consecutive time samples of the whole-slice FID signal can thus be interpreted as describing the intensity (the density of dipoles aligned perpendicularly to the recording coil) and phase of signal contributions of increasingly higher spatial frequencies to the image slice, and correspond to $S(k)$ in equation 2.1 (Buxton, 2009). In summary, frequency encoding allows encoding spatial frequency content along the x -axis of the slice into its FID signal time course. Spatial frequency encoding along the y direction of a given slice is achieved via phase encoding and requires multiple acquisitions. Rather than frequency encoding dipoles in time during a single acquisition, phase encoding in fact relies on changing the phase encoding gradient at every scan such that a pseudo-frequency is induced between the magnetization vectors of the same dipole at equivalent readout times of repeated acquisitions.

The sequence of phase and frequency encoding gradients and RF pulses is predetermined, and is used to generate a spatial frequency domain representation of the acquired data known as k -space. Within the k -space, all information needed to reconstruct a 2D slice image is contained within a spatial “phase vs. frequency” matrix, which is filled row-wise with (complex) $S(k)$ values during acquisition. Each line of the k -space matrix contains the digitized spatial frequency intensity (and phase content) of the whole-slice FID signal acquired via spin-echo at a particular phase encoding gradient (Buxton, 2009). It is important to note that, as previously discussed, owing to the cumulative effects of the frequency encoding gradient, rows of the k -space can be filled directly with the digitized FID time course, which represents the digitized intensity of increasing spatial frequencies. More specifically, the spatial frequency sampling rate Δk_x is directly proportional to the temporal sampling rate of the FID signal such that $\Delta k_x \text{ (cycles/m)} = \Delta t_x \gamma G_x$; this also determines the

spatial field of view $FOV_{(m)}=1/\Delta k_x$ and the spatial resolution $\Delta x=1/2k_{max}$ (Buxton, 2009). While frequency encoding gradients are used fill a row of k-space, the phase encoding determines which row is to be filled, with steep positive phase encoding gradients selecting the top k-space rows, shallow phase encoding gradients the central rows and steep negative phase encoding gradients the bottom rows. One can thus recognise two fundamental spatial frequencies within the ordinate system of the k-space matrix: (i) a frequency-encoded spatial frequency along k-space rows, corresponding to spatial encoding along the x-axis of the space domain; (ii) a phase-encoded spatial frequency component across k-space columns, corresponding to spatial encoding along the y-axis of the space domain (Buxton, 2009). It is important to note that every point in the matrix contains spatial frequency information (intensity and magnitude) regarding the entire image, such that points in the k-space do not correspond to pixels in the spatial domain. Since outer rows (and columns) of the k space arise from steeper gradients which render consecutive elements (in the space domain) easily discernible, they encode higher frequency information such as the edges (rapid changes of image signal as a function of position) of the image. However, largely due to the dephasing (lack of precessing coherence) caused by large gradients, the signal intensity provided by the outer rows is rather low. The converse is true for elements positioned at the center of the k space, which arise from low gradients. These data points do not possess spatial resolution information, but due to a high precessing coherence (low dephasing gradients) provide most of the image signal (Buxton, 2009). The spatial frequency and phase information contained within all points of the k-space matrix can be extracted and converted into an image by performing an inverse 2D Fourier transform. During this conversion each k space element is turned into a 2D image (essentially a grid) of varying amplitude, peak-to-peak spacing, and direction in the spatial domain. All images are then assigned intensity based on their k-space value and then combined to form the final MR slice image (the magnitude and phase images of a slice are formed separately based on the magnitude of the real and complex $S(k)$ value respectively) (Buxton, 2009). Mathematically this process can be described by adapting equation 2.1 for a 2D k space (Buxton, 2009):

(2.2)

$$S_k = \int \rho(\mathbf{r}) e^{i\mathbf{k} \cdot \mathbf{r}} d\mathbf{r}, \quad \text{with } \mathbf{k} = \gamma \mathbf{G} t$$

Where $\mathbf{k}$ is the 2D spatial frequency, G is the net magnitude of the frequency and phase encoding gradients, γ is the Larmor frequency, t is time, $S(\mathbf{k})$ is a complex value describing the signal intensity (real part) and phase value (imaginary part) of a given 2D spatial frequency $\mathbf{k}$, and $\rho(\mathbf{r})$ is the density of dipoles at a given spatial location along a vector $\mathbf{r}$, spanning the x-y space plane.

Equation 2.2 shows that $\rho(\mathbf{r})$, the pixel intensity at any location on the space plane, is the 2D Fourier transform of $S(\mathbf{k})$. Pixel intensities (for the phase and magnitude image) can therefore be computed by an inverse 2D Fourier transform of the k-space (Buxton, 2009).

2.2 Diffusion MR Imaging

Water molecules within the highly dynamic extracellular fluids of organic tissues are subject to continuous tumbling and collisions, which confers them with a constant, but random, thermal motion. In addition to causing random changes in the relative orientation of hydrogen atoms, which gives rise to local field inhomogeneities and contributes to a quicker T2 decay, thermal motion also produces a random displacement of molecules in a process known as diffusion. By the effect of diffusion, molecules that are initially close together are therefore caused to move apart following random and independent trajectories of displacement over time. For free water self-diffusion, the displacement of any given molecule along an arbitrary direction x during a time interval T is described by a Gaussian probability spread function with variance σ_x (Buxton, 2009):

(2.3)

$$\sigma_x = \sqrt{2DT}$$

In which D is the medium-specific diffusion coefficient.

Since, in the brain, water molecules typically diffuse with a value of $D=0.001 \text{ mm}^2/\text{s}$., diffusion is a relatively quick method for molecules to travel very short distances, but its efficiency decays quickly at longer distances (Buxton, 2009).

2.2.1 Diffusion weighted imaging

Diffusion weighting (i.e. sensitivity to diffusion) can be added to MR images by complementing the imaging sequence with two strong linear field gradients of equal magnitude and duration, but inverse polarity. The effects of each pulsed gradient are similar to those of frequency encoding, with the precession rate of dipoles (within water molecules) increasing/decreasing linearly with their spatial distribution along the gradient. When the gradient is removed, dipoles revert to their original precession frequency, but their spins are dephased (Buxton, 2009). The amount of phase offset introduced is dependent on the net field strength experienced by the dipole throughout scanning. Since the diffusion sequence employs balanced gradients, the net field strength, and thus the phase

offset, experienced by dipoles is proportional to their displacement (in the direction of the gradient) between gradient pulses. This is described by the equation (Buxton, 2009):

(2.4)

$$\Delta\phi = \gamma G \delta t \Delta x$$

Where $\Delta\phi$ is the net spin phase shift of a dipole, γ is the gyromagnetic ratio, G is the gradient(s) pulse strength, δt is the gradient(s) pulse duration, and Δx is the net distance travelled by a spin between gradient pulses.

The intensity of each pixel within a diffusion weighted MR image results from the collective effects of the dipoles within it. Phase dispersions, and therefore local field inhomogeneities and the T2 rate of signal decay within a pixel are directly proportional to the net spatial dispersion (diffusion) of its dipoles. The T2-weighted signal intensity of each pixel is therefore a direct measure of the amount of diffusion occurring within it. For free diffusion, the distribution of spin phases is proportional to that of displacement (equation 2.4), and is therefore described by a Gaussian function with a variance σ_ϕ , which can be derived from equations 2.3 and 2.4, and the general equation of variance (Buxton, 2009):

(2.5)

$$\sigma_\phi^2 = 2(\gamma G \delta t)^2 D T$$

This Gaussian dispersion of phase results in an exponential decay of a given pixel's signal intensity, with an attenuation $A(D)$ which is described by the following equation (Buxton, 2009):

(2.6)

$$A(D) = e^{-bD}$$

While the local diffusion coefficient D characterises the intensity change within a pixel due to the effects of diffusion, b is a constant that relates the pixel's intensity change to the magnitude of phase offsets induced by the gradient pulses. b therefore incorporates all of the gradients' amplitude and

timing parameters and can be derived by comparing equations 2.5 and 2.6 and adjusting the result to account for the attenuation effects of diffusion that occur during the application of the gradient pulses (Buxton, 2009):

(2.7)

$$b = (\gamma G \delta t)^2 \left(T - \frac{\delta t}{3}\right)$$

The experimental parameter b can therefore be set such that the value of D can then be inferred from the signal attenuation $A(D)$. While a diffusion weighted image (DWI) can be acquired simply by setting a high value of b to a normal T2-weighted imaging sequence, the resulting attenuation is not a pure reflection of the effects of diffusion, as the signal is also dependent on the T2 rate of FID decay. To eliminate the effects of T2 decay, two versions of the same image are generally acquired: a T2-weighted “baseline” image, obtained by setting $b=0$ and a DWI obtained with a large b . The signal attenuation due to diffusion $A(D)$ is then computed as the ratio of the signals of the two images (Buxton, 2009). It is important that the two images are acquired at the same echo time (TE), such that the effects of T2 decay are identical for both and cancel out when the ratio is computed. A value of D is then finally obtained (as the slope of $\ln(A)$ vs b plot) for each pixel and displayed as a greyscale value on an image referred to as the apparent diffusion coefficients (ADC) map (Buxton, 2009).

While, in principle, diffusion weighting can be added to any imaging sequence, in practice it is limited by its need for a long TE to allow molecules a long enough time to diffuse before signal readout. Conventional SE and GRE sequences may however require a short TE, especially for imaging tissues with a fast T2 rate of signal decay. Imaging tissues with a short T2 and a small D is therefore usually achieved by means of particular stimulated echo (STE) sequences (Buxton, 2009). The basic mechanism underlying STE is interleaving the bipolar gradient pulses with further 90° RF pulses to temporarily “park” the transversal component of magnetization vector along the longitudinal axis after the first diffusion gradient is applied, and then tipping it back onto the transversal plane before the application of the second diffusion gradient. While the transverse component of the magnetization vector is parked longitudinally, it decays at rate T1, which is much slower than T2 and therefore allows molecules to diffuse over time with little magnetization being

lost. However both during the application of the diffusion gradient pulses and during signal readout the transverse component of the magnetization vector is brought back to its original position and a strong diffusion-weighted FID signal can be recorded (Buxton, 2009). Finally, the best sensitivity to diffusion is provided by steady-state free precession pulse (SSFP) sequences. These consist of a rapid/quasi-instantaneous succession of RF pulses delivered at TR intervals much smaller than T₂, and causing a low flip angle ($<90^\circ$) such that only part of the longitudinal magnetization is transferred onto the transverse plane. Since $TR \ll T_2$, each new RF pulse delivered acts on both the residual longitudinal magnetization vector and on the transverse magnetization vectors due to previous RF pulses which have not yet decayed. Additionally, all transverse magnetization vectors accumulate a constant phase offset during each TR period due to local field inhomogeneities and other gradient effects. Echoes of all previous magnetization vectors are therefore created at multiples of TR. The complex dynamics of this particular sequence eventually leads to a steady state signal, whereby a constant signal composed of all previous signal echoes and the new FID is produced just before and after each TR. The signal generated by an SSFP sequence is therefore a mixture of SE and STE effects and is highly sensitive to diffusion (Buxton, 2009).

2.2.2 The diffusion tensor model

Although so far only diffusion along a single axis has been discussed, in practice diffusion can occur in any direction within the 3-dimensional space. Diffusion within tissues is often anisotropic, meaning that the ADC value for a given voxel is dependent on the direction along which it is being measured. This is mainly the result of tissues containing oriented structures, such as nervous fibre tracts, whose cellular walls restrict diffusion in a direction-dependent manner. Diffusion tensor imaging therefore aims at characterizing diffusivity in space to infer both the presence and orientation of nervous fibre bundles, which are often too small to be otherwise detected by conventional MRI. To attain a full quantification of anisotropic diffusion in the 3 dimensional space, the diffusion coefficients D, or ADC, need to be measured along a minimum of 6 linearly independent directions (Buxton, 2009). To understand this requirement it is useful to note that diffusion within a voxel can be interpreted as resulting from the diffusion of particles along 3

principal axes, say $x'-y'-z'$ which are perpendicular to each other, but of unknown spatial orientation. If diffusion is measured along an arbitrary axis, the resulting coefficient D arises from the projection of diffusivities along each independent principal axis onto the measurement axis. This is expressed by the equation (Buxton, 2009):

(2.8)

$$D = D_1 \cos^2(\theta_1 - \phi_1) + D_2 \cos^2(\theta_2 - \phi_2) + D_3 \cos^2(\theta_3 - \phi_3)$$

Where the terms D_1 , D_2 , and D_3 represent the diffusion coefficients along the 3 principal axes, θ_1 , θ_2 and θ_3 represent the angle between each principal axis and a reference frame, and ϕ_1 , ϕ_2 and ϕ_3 represent the angle between the arbitrary axis of measurement and the reference frame. Since D_1 , D_2 , D_3 , θ_1 , θ_2 and θ_3 are unknown, 6 independent measurements of D along different arbitrary axes (6 different sets of ϕ angles) spanning an arbitrary space $x''-y''-z''$ are required to establish the magnitude and orientation of the diffusivity components in three dimensions. The graphical representation of anisotropic diffusivity in the 3D $x''-y''-z''$ space is therefore a “peanut-shaped” surface, where each point on the surface is the “tip” of a vector extending from the origin onto the surface (Buxton, 2009). The length of each vector is proportional to the value of net diffusivity D of all particles measured along the vector’s direction in 3D space, such that the outermost edges of the “peanut lobes” indicate directions of highest net diffusivity, and the “peanut indents” correspond to directions of restricted net diffusivity. It is important to note that, in the literature, the graphical representation of diffusivity (the diffusion tensor) takes the shape of an ellipsoid rather than that of a peanut. This is to visualize diffusion in terms of the final spatial distribution of molecules, and not as the map of ADCs measured along each direction in 3D space. In reality, these two representations are closely related as the peanut shaped map of diffusion coefficients fundamentally results from multi-axial imaging of the elliptical spread of particles (Buxton, 2009).

In practice, the voxelwise diffusion expressed in terms of the magnitude and direction of individual diffusivity components is not calculated using equation 2.8 directly, but is expressed via a 3x3 diffusion matrix termed the diffusion tensor. Under this notation, the measured value of D

along a three-dimensional diffusion sensitizing gradient vector $\mathbf{u}$ is expressed by the equation (Buxton, 2009):

(2.9)

$$D = (u_x, u_y, u_z) \begin{pmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{pmatrix} \begin{pmatrix} u_x \\ u_y \\ u_z \end{pmatrix} = \mathbf{u}^T \mathbf{D} \mathbf{u}$$

Where the diagonal elements of $\mathbf{D}$ are proportional to the ADCs along the three arbitrary orthogonal axes $x''-y''-z''$ that describe the vector $\mathbf{u}$ (the arbitrary measurement frame), and the off-diagonal elements of $\mathbf{D}$ describe the correlation between the ADCs along different axes. To fully define the arbitrary-space-specific diffusion tensor, all elements within the $\mathbf{D}$ matrix must be calculated. Since the tensor matrix is symmetrical, it contains 6 unknown elements, which, in principle, can be found via 6 measurements along linearly independent $\mathbf{u}$ vectors spanning the $x''-y''-z''$ space. In practical terms, the attenuation measured at a given voxel for an acquisition i is defined as (from equations 2.6 and 2.9) (Buxton, 2009):

(2.10)

$$A(D)_i = A(D)_o e^{-b(\mathbf{u}^T \mathbf{D} \mathbf{u}_i)}$$

$\therefore$

$$\ln(A(D)_i/A(D)_o) = -b(\mathbf{u}^T \mathbf{D} \mathbf{u}_i)$$

The voxelwise attenuation is typically measured with the same b parameter along 6 direction vectors $\mathbf{u}_{(1-6)}$, and the baseline measurement $A(D)_0$ is acquired by setting $b=0$. A total of 7 measurements are therefore required in order to fully define the 3x3 tensor $\mathbf{D}$ in an arbitrary $x''-y''-z''$ space. The spatial dispersion of particles associated with such tensor can be graphically represented via an ellipsoid whose hemiaxes are aligned with the axes of the arbitrary space (eigenvectors), with their respective lengths defined by the diagonal terms of the tensor matrix (eigenvalues). This ellipsoid is therefore a tilted and projected version of the diffusion ellipsoid that lies in the $x'-y'-z'$ principal space, and that directly describes the orientation of fiber tracts (Buxton, 2009). Ideally, the principal space ellipsoid could be measured directly if the orthogonal axes $x''-$

$y''-z''$ of the arbitrary measurement were overlaid exactly onto the $x'-y'-z'$ axes of the principal frame. Since, by definition, diffusion of particles along the principal axes are independent from each other, in this ideal case the ADC along each orthogonal axis is independent of the others and the diffusion tensor, termed the principal diffusion tensor, becomes (Buxton, 2009):

(2.11)

$$\mathbf{D}_{pa} = \begin{pmatrix} D_1 & 0 & 0 \\ 0 & D_2 & 0 \\ 0 & 0 & D_3 \end{pmatrix}$$

Where the matrix elements describing the correlation between ADCs along the principal (and arbitrary measurement) axes are all zero, and the diagonal elements D_1 , D_2 and D_3 describe diffusion along the principal (and measurement) axes and are termed the principal diffusivities. In practice, the arbitrary and principal space cannot be aligned as the orientation of the principal axes is unknown. As a result the principal diffusion tensor is not measured directly, but is usually obtained by fully defining a diffusion tensor in any arbitrary and then applying a rotation matrix $\mathbf{R}$ to it, such that its ordinate system $x''-y''-z''$ is brought into alignment with the $x'-y'-z'$ space. The correct rotation matrix $\mathbf{R}$ allows diagonalizing the tensor matrix, such that D_{pa} is obtained (Buxton, 2009):

(2.12)

$$\mathbf{D}_{pa} = \mathbf{R}\mathbf{D}\mathbf{R}^{-1}$$

The process involved in finding the rotation matrix $\mathbf{R}$ is equivalent to solving equation 2.8 for the 3 rotation angles θ_1 , θ_2 and θ_3 . Rotation and principal diffusion tensor matrices are found independently for each voxel. The final diffusion image can then be formed by assigning each voxel to an ellipsoid whose hemiaxes' magnitudes and directions are determined by the principal diffusivity coefficients (D_1, D_2 , and D_3) and principal axes orientations respectively (Buxton, 2009). Another means of displaying diffusion images is to only assign voxels to a single vector representing the principal axis of largest diffusivity. While full tensors representations are required to fully describe the direction and magnitude of diffusion, a simpler approach to constructing diffusion maps is to assign each voxel to one of two absolute measures: the tensor's trace and fractional anisotropy index (FA). The trace describes the isotropic component of diffusion and is computed as the average

of principal diffusivities: $D_{av} = (D_1 + D_2 + D_3)/3$, whereas the FA index quantifies the anisotropic component of diffusion and is computed as the ratio of standard deviations of principal diffusivities

and a measure of total diffusivity: $FA = \frac{\sqrt{\frac{3}{2} \frac{\sqrt{(D_1 - D_{av})^2 + (D_2 - D_{av})^2 + (D_3 - D_{av})^2}}{\sqrt{D_1^2 + D_2^2 + D_3^2}}}}{\sqrt{D_1^2 + D_2^2 + D_3^2}}$; a higher FA index

therefore indicates a larger difference across principal diffusivity components (Buxton, 2009).

2.2.3 Fibre tract mapping

Mapping of white matter fibre tracts via diffusion tensor imaging (DTI) is a promising technique, which represents a nicely complementary approach to mapping the functional cortical activity revealed by fMRI to anatomical structures (Buxton, 2009). In DTI, the orientation of axonal bundles within individual voxels is usually expressed by the single vector describing the largest axis of principal diffusivity (which is almost 10 times larger than the vectors describing the other principal components). The fundamental theory is that fiber tracts can be delineated starting from an arbitrary seed point and then connecting the principal diffusivity vectors voxelwise. Pathways can be reconstructed using one of two fundamental methods: deterministic or probabilistic tracing. While deterministic tracing involves adopting subvoxel step sizes to determine local fiber orientation and thus the next voxel belonging to the pathway, the probabilistic method deals with the added complexity of the uncertainties arising from potential sudden branching or deflection of pathways that may be missed by individual voxel tensors (Buxton, 2009). Mainly due to the limitations in the spatial resolution provided by MRI, a single voxel may contain several axonal fibre bundles. If the voxelwise bundles are not parallel to each other, these cannot be differentiated via the classic diffusion tensor model approach. For instance, if a voxel contained three sets of fibre bundles, all equal in size and perpendicular to each other, the diffusion tensor associated with the voxel would assume a spherical shape, falsely indicating an isotropic pattern of diffusion. This effect is inherent to the fundamental principles of diffusion imaging, whereby diffusivity measured along each arbitrary vector is the net projection of all particles positions onto it. To overcome this limitation of DTI, the voxelwise signal attenuation is modelled to incorporate the effects of water compartmentalization on the net variance of displacements. It is in fact assumed that water molecules are contained in several fibre bundles (compartments) with different structural

characteristics, and that therefore independently affect the spatial variance of molecular distributions (Buxton, 2009). Under the assumption that the rate of water exchange between compartments is slow, such that molecules remain in either compartment throughout image acquisition, an example of voxel's attenuation due to fibre bundles crossing at right angles is described by a biexponential decay (Buxton, 2009):

(2.13)

$$A(\phi) = v_1 e^{-b(D_1 \cos^2 \phi + D_2 \sin^2 \phi)} + v_2 e^{-b(D_1 \sin^2 \phi + D_2 \cos^2 \phi)}$$

Where b is the diffusion sensitivity used to measure the signal attenuation A along an axis at an arbitrary angle ϕ , and v_1 and v_2 represent the molecular fraction of water contained in either fibre bundle. With large values of b the biexponential decay becomes prominent and diffusivity can be assessed independently along 2 directions in space. This approach is useful in distinguishing crossing fibers, but requires measuring attenuation in several directions (usually between 32 and 64) to be able to produce a 3D diffusion surface for each voxel (Buxton, 2009).

2.3 Functional magnetic resonance imaging

The NMR signal captured by MRI is extremely sensitive to inhomogeneities of the magnetic field. While at first all NMR fluctuations were treated as detrimental noise, achieving a better understanding of the physiological processes underlying specific patterns of field inhomogeneities has led to the development of advanced MRI techniques such as functional magnetic resonance imaging (fMRI) (Buxton, 2009, Arichi, 2012). Although alternative sources of fMRI contrast have been sparsely reported, the work within this thesis is based on fMRI acquisitions performed with the commonly adopted Blood Oxygen Level Dependent (BOLD) contrast (Ogawa and Lee, 1990, Ogawa et al., 1990b, Ogawa et al., 1990c, Kim and Ogawa, 2012, Le Bihan et al., 2006, Jasanoff, 2007). BOLD fMRI is based on the notion that an indirect measure of functional brain activity can be drawn from the quantitation of transient local field inhomogeneities due to changes in the local level of blood oxygen content. In this passage the fundamental principles underlying the blood flow increase associated with brain activity and the source of net magnetic field interference due to changes in blood oxygenation will be described before introducing the standard methodologies of acquisition and analysis of the BOLD signal.

2.3.1 Neurovascular coupling

Under resting state conditions, neuron cells settle into a high energy state determined by a large unbalance between intracellular and extracellular ionic concentrations, and by a slightly negative transmembrane resting potential. Excitatory (or inhibitory) synaptic impulses increase membrane permeability to specific ions (i.e Na^+), which, driven by the forces produced by an electrochemical gradient, then rapidly diffuse across the cellular membrane. This leads to the generation of an electric current proportional to the net flux of (charged) cations, and, crucially, to the depolarization (and eventually hyperpolarization) of the cellular membrane through an inversion in the transmembrane polarity termed action potential. Having travelled across an individual cell body, action potentials induce a neurotransmitter-mediated increase in the local concentrations of intracellular Ca^{2+} at the level of the pre- and post-synaptic junctures, which allows them to continue propagating across neighbouring cells (Buxton, 2009). The spikes of electrical activity produced by

the propagation of depolarizing action potentials across interconnected neuron cells is commonly detected by electrophysiology techniques and used as a means of directly inferring cerebral activity. Following depolarization, ionic impermeability of the membrane is quickly reinstated, cations are pumped (i.e. via the sodium potassium pump) in direction opposite to their natural drift to restore the ion gradients, and neurotransmitters are repacked into vesicles by the action of astrocytes. The energy required for the neuron cells to recover their resting steady state is provided through the consumption of adenosine triphosphate (ATP), whose level is maintained via an enhanced supply of glucose and oxygen to the local cellular mitochondria for oxidative phosphorylation (Buxton, 2009). Since the blood stream is known to be the primary means by which both glucose and oxygen are transported around the human body, the local vasculature is responsible for the provision of the metabolic substrates in the amount required by neural activity (Attwell and Iadecola, 2002, Arichi, 2012, Logothetis and Pfeuffer, 2004, Cauli and Hamel, 2010, Kim and Ogawa, 2012, Jones and Rabiner, 2012). This coordinated activity of neurons and local vasculature is achieved via a chain of physiological processes, which are collectively referred to as neurovascular coupling (Buxton, 2009). Astrocytes, once considered normal glial cells with mainly a structural functionality, have recently been found to play a fundamental role in the bridging of the neurovascular unit as they possess processes extending both into the inter-neural synaptic space and into vascular muscle cells. The basic principles of neurovascular communication involve astrocytic projections (or end feet) surrounding the synaptic junction being excited by the release of neurotransmitters, leading to an increase in the astrocytic intracellular CA^{2+} , which in turn cause astrocytic feet in contact with the local vasculature to release vasoactive chemical agents. This cascade of physiological processes leads to synaptic activity contributing directly to the relaxation of the smooth muscle of the adjacent vasculature (or constriction, depending on the base state of the muscle). This leads to an increase in the local blood flow, through which the required glucose and oxygen amounts are provided to neurons (Buxton, 2009, Arichi, 2012).

It is important to note that neural activity results in a transient state of functional hyperhaemia, in which the enhanced local cerebral oxygen metabolic rate ($CMRO_2$) of the tissue is accompanied by a far superior increase in both the local cerebral blood flow (CBF) and cerebral blood volume (CBV,

the fraction of tissue volume occupied by blood vessels). Owing to this imbalance between metabolic requirements and oxygen supply, the proportion of oxygen consumed by cerebral tissue, termed the oxygen extraction fraction (OEF), is reduced in areas of neural activity. Crucially an oversupply of highly oxygenated blood is therefore found in correspondence of active cerebral areas, and this phenomenon is exploited by fMRI to infer activation (Arichi, 2012, Buxton, 2009).

2.3.2 NMR properties of haemoglobin

As described in sections 2.1.2, partially due to static local field inhomogeneities causing a quicker loss of precessional coherence across excited protons, the experimental FID signal decays within an apparent transverse relaxation time T_2^* much shorter than the expected tissue specific transverse relaxation time T_2 (Westbrook et al., 2005, Buxton, 2009, McRobbie, 2007), such that the true exponential rate of signal intensity decay (I) is expressed by the equation:

(2.14)

$$I(t) = I_0 e^{-\frac{t}{T_2^*}}$$

Paramagnetic molecules, or elements that contain unpaired electrons and are thus slightly positively susceptible (attracted) to magnetic fields, are abundantly present in air pockets, dense bone, and ferromagnetic blood breakdown by-products and are sources of enhanced T_2^* induced image distortions (McRobbie, 2007). Paramagnetic agents have been widely implemented in MRI as a means of exploiting local signal intensity dropouts to enhance the visualization of vascular-rich areas (Westbrook et al., 2005). Crucially, haemoglobin (Hb) (the protein expressed by red blood cells responsible for the transportation of the vast majority of intravascular oxygen) is known to change its magnetic properties depending on its oxygen binding state. While oxygenated Hb is diamagnetic (not susceptible to magnetic field forces), deoxygenated Hb (d-Hb) is paramagnetic as it contains a pair of unbound electrons (Pauling and Coryell, 1936, Thulborn et al., 1982, Ogawa et al., 1990c, Ogawa et al., 1990b, Ogawa and Lee, 1990) and therefore causes a faster NMR signal decay.

Although the paramagnetic properties of dHb have long been established, it is not until Ogawa and colleague's seminal work that dHb was identified as an intrinsic contrast agent sensitive enough

to reveal in-vivo changes in blood oxygenation levels (Ogawa et al., 1990c, Ogawa and Lee, 1990). This was done by using a simple GRE MRI sequence to image a rat's brain at different levels of cerebral blood oxygenation, which were induced by altering the oxygen concentration in the air inhaled by the animal. The experiment revealed stark signal changes within the large vessels of the brain, an effect that was termed BOLD contrast (Ogawa et al., 1990c, Ogawa and Lee, 1990). It was also observed that further BOLD contrast changes were associated with hypoglycaemia and sedation, suggesting that the technique could potentially be employed to infer the intensity of brain activity (Ogawa et al., 1990b). Owing to the effects of neurovascular coupling, which determine a proportionate decrease of paramagnetic d-Hb (and therefore a higher signal) within areas of enhanced neural activity, a local increase in BOLD contrast was observed in human subjects performing a simple motor task (Kwong et al., 1992, Bandettini et al., 1992) and in response to a visual stimulus (Kwong et al., 1992, Ogawa et al., 1992).

2.3.3 Biophysical basis of BOLD signal

Although it is widely accepted that a proportionate decrease of local deoxygenated haemoglobin produces an enhanced positive BOLD contrast, modelling the BOLD percent signal change is a challenging task that ultimately requires quantifying the effects of numerous experimental, biophysical and physiological parameters on the T_2^* time of signal decay (Buxton, 2009). In general, stronger B_0 fields increase the magnetic susceptibility of the d-Hb contained within a voxel, improving tissue contrast and signal-to-noise ratio (SNR) of BOLD experiments, with the net BOLD percent signal change increasing linearly to supralinearly with field strengths up to 7T (Krüger et al., 2001, Peters et al., 2007, Schäfer et al., 2012, Harmer et al., 2012). BOLD contrast is also modulated by the interplay between the strength of the B_0 field and the specific anatomic features of the vasculature contained within a voxel, which may include any combination of arteries, capillaries and veins. For field strengths normally adopted in human studies (up to 3T), the intravascular blood compartment contributes significantly to the BOLD signal, despite representing only a small proportion of the total tissue volume (about 4%) (Krüger et al., 2001, Schäfer et al., 2012). BOLD contrast is predominantly derived from the venous vasculature, since veins are the largest blood

vessels, undergo the largest dilation during increased blood flow, and sustain the largest shift in oxygenation during brain activity (Buxton et al., 1998, Buxton et al., 2004). Additionally, due to their large diameter, veins are less subject to the effects of the spin dephasing, and thus to BOLD signal destruction, induced by extravascular water molecules diffusion alongside blood vessels within a given voxel (Ogawa et al., 1993, Buxton, 2009).

2.3.4 Neural activity and BOLD response

Within an imaging voxel, neural processing can be seen as the complex integration of highly correlated synaptic and spiking neural activity, arising from a balance between excitatory and inhibitory inter-neural modulation. While it has been proposed that CBF may be driven by synaptic activity, while CMRO₂ is representative of the overall energy cost, which also includes spiking activity, the precise correlation between BOLD response and the underlying neural activity is still unclear. Determining precisely which aspects of neural activity are measured by BOLD is a challenging task, but a preliminary understanding has been achieved via primate studies combining direct electrode recordings with fMRI (Stefanacci et al., 1998, Logothetis et al., 1999). In their study Logothetis and colleagues compared BOLD responses with simultaneous recordings of local field potentials (LFP), low-frequency fluctuating potentials associated with synaptic activity, and multi-unity activity (MUA), higher frequency potentials associated with spiking activity (Logothetis et al., 1999). The time course of LFPs closely matched that of the BOLD response, indicating that BOLD signal is predominantly a reflection of synaptic activity.

2.3.5 The Haemodynamic response function

Although initial fMRI experiments focused on characterizing BOLD contrast within cerebral regions of interest (ROI) that were arbitrarily selected based on the type of stimulus, a precise model of the full time-course of the BOLD signal response allows identifying areas of functional activity throughout the whole brain. This is fundamentally possible because the BOLD response time-course to a given stimulation was found to be highly reproducible within the same subject (Aguirre et al., 1998b, Miezin et al., 2000, Handwerker et al., 2004, Handwerker et al., 2012), and despite subtle

differences, to retain the same morphology across brain regions and subjects (Friston et al., 1994a, Friston et al., 1995b, Handwerker et al., 2012, Arichi, 2012, Hoge and Pike, 2001). Due to its fundamental dependence on the transient features of local deoxygenated blood content, the BOLD signal time-course that follows neural activity has been termed the haemodynamic response function (HRF) (Hoge and Pike, 2001, Friston et al., 1994a, Buxton, 2009, Glover, 1999, Arichi, 2012). As previously mentioned, BOLD contrast is the net result of a stimulus-induced hyperaemia via neurovascular coupling, and ultimately results from local increases of CBF and CBV accompanied by a lower increase in $CMRO_2$. Most currently accepted haemodynamic models of BOLD response are therefore based on the timecourse differences across these three physiological parameters (Buxton et al., 2004, Buxton et al., 1998, Mandeville et al., 1998).

The first comprehensive biophysical model of the observed HRF is referred to as the “balloon model” (Buxton et al., 1998). Fundamental to this model is the analogy between the venous vessels and a balloon with equivalent biomechanical properties. The balloon responds to changes in liquid influx with a delayed and slower variation in volume that is modulated by the viscoelastic properties of its membrane. The balloon model describes a physiological process in which the active contraction of arterioles produces enhanced local CBF, which acts to passively stretch the local venous vasculature leading to a delayed venous CBV response (Buxton, 2009, Buxton, 2012, Buxton et al., 2004, Buxton et al., 1998). Although the balloon model rather accurately approximates experimental HRF measurements, further studies have revealed that a comprehensive descriptive model may be difficult to formulate as HRF features may arise from the interplay of several complex physiological processes underlying neurovascular coupling. Although several interpretations have been proposed for each of its features, the canonical HRF is described as a sequence of three fundamental epochs: an initial dip, a positive peak and a negative undershoot (Buxton, 2009, Arichi, 2012, Buxton, 2012, Buxton et al., 2004, Buxton et al., 1998).

1. The initial dip is a low amplitude decrease in BOLD contrast observed immediately post-stimulus. This effect is better visualized by increasing the B_0 field strength and the intensity of the stimulus and has been associated with a quick initial rise in local metabolic demands (and thus

CMRO₂) that is not yet matched by an increase in the supply of oxygenated blood (Ernst and Hennig, 1994, Hu and Yacoub, 2012, Yacoub and Hu, 2001, Hu et al., 1997, Yesilyurt et al., 2008, Buxton, 2009, Buxton et al., 2004, Arichi, 2012). As a consequence, the proportion of dHb is increased, which diminishes BOLD contrast (Malonek and Grinvald, 1996, Buxton, 2009, Buxton et al., 2004, Hu and Yacoub, 2012, Arichi, 2012). As the increase in CMRO₂ is thought to be more spatially specific than increases in either CBF or CBV, the initial dip has been hypothesized to be the transient BOLD signal feature offering the best localization of functional activity in fMRI experiments (Malonek and Grinvald, 1996).

2. The positive peak is the result of the BOLD signal rising due to a quick increase in CBF, followed by an increase in CBV (Hillman et al., 2007). These changes induce a state of local functional hyperaemia, whereby the tissue is oversupplied with oxygenated blood. The peak is observed approximately 5-7 seconds post-stimulus (Friston et al., 1994a, Friston et al., 1995b, Buxton, 2009, Buxton et al., 2004, Buxton et al., 1998, Glover, 1999, Handwerker et al., 2004, Yesilyurt et al., 2008, Arichi, 2012) and, despite subtle inter-subject and inter-region differences, its amplitude typically rises to approximately 2-3% above the baseline BOLD signal level (Friston et al., 1995b, Glover, 1999, Handwerker et al., 2004, Arichi, 2012). The peak amplitude was shown to be enhanced by both the duration and intensity of the stimulus (Yesilyurt et al., 2008, Hirano et al., 2011, Arichi, 2012).

3. The post-stimulus negative undershoot is the result of the BOLD signal dropping to below baseline after the peak has been reached, and then slowly recovering its baseline intensity. The BOLD signal falls below baseline approximately 10-20s after stimulus cessation, and the undershoot lasts up to 60s (Frahm et al., 1996, Buxton, 2009, Buxton et al., 2004, Buxton et al., 1998, Mandeville et al., 1998, Chen and Pike, 2009a, van Zijl et al., 2012, Arichi, 2012). This effect is due to a rise in local d-Hb which has been hypothesized to originate from one of three potential sources: (i) an increased OEF due to the enhanced CMRO₂ lasting longer than the transient increase in CBF (Dechent et al., 2011, Frahm et al., 2008, Frahm et al., 1996, Arichi, 2012), (ii) a mismatch in the

timing between the faster CBF and the slower CBV BOLD response which leads to an increase in deoxygenated venous blood (Buxton, 2009, Buxton et al., 2004, Buxton et al., 1998, Mandeville et al., 1998, Arichi, 2012), or (iii) a rapid undershoot in the local CBF following its previous sharp rise (Chen and Pike, 2009a, Chen and Pike, 2009b).

A fundamental concept to fMRI methodology is that the BOLD response resulting from a prolonged stimulation, or a train of stimulations, is predicted by the linear addition of the overlapping HRFs induced by successive stimulations. This until the signal plateaus at its ceiling value corresponding to full oxygenation of local haemoglobin (Friston et al., 1994a, Boynton et al., 1996, Dale and Buckner, 1997, Arichi, 2012). This property, in conjunction with the retained characteristic HRF morphology across subjects and experiments, is of fundamental importance to fMRI analysis as it allows relating BOLD response to stimulus design via a simple linear time-invariant transform model, essentially linearising the highly non-linear direct relationship between a stimulus and the induced neural response (Boynton et al., 1996, Arichi, 2012). In practice, the BOLD signal can be modelled as the convolution of the stimulation paradigm, which includes the onset and duration of the stimulus, and the canonical HRF. Since the magnitude and duration of neural intensity appear to independently determine the respective parameters of BOLD response (Boynton et al., 1996, Donaldson and Buckner, 2001, Logothetis, 2008, Arichi, 2012), the intensity of stimulation (in addition to its duration) is also likely to be an important experimental confound. A precise quantitative relationship between stimulation intensity and neuronal activation has however not yet been determined (Logothetis and Wandell, 2004).

While the assumption of BOLD signal linearity is largely accepted and commonly used in fMRI analysis, recent studies using event-related protocols have highlighted that below a given threshold (4s in the visual cortex) stimuli of decreasing durations lead to an increasingly non-linear BOLD response. Typically, this results in short stimulus HRFs often overestimating the amplitude of the BOLD response to longer stimulations (Buxton, 2009, Buxton et al., 1998, Friston et al., 1998b, Mandeville et al., 1998, Yesilyurt et al., 2008, Hirano et al., 2011, Arichi, 2012). Neuronal adaptation has been proposed to potentially underlie these non-linearities (Boynton et al., 1996), as it would cause neural activity to increase steeply immediately after stimulation, and then decline to a

plateau as stimulation is sustained. Short stimulus responses, which capture little or no adaptation, may therefore overestimate the response to longer stimuli.

2.3.6 fMRI methodology

The notion that BOLD response produced by neural activity follows a predictable HRF in response to specific parameters of stimulation is fundamental to fMRI experimental design, and data analysis. While whole-brain imaging represents one of the major advantages fMRI, analyzing the large amount of data produced is a computation-intensive statistical process that requires accounting for the noise introduced by multiple sources (Friston et al., 1994a, Huettel, 2004, Buxton, 2009, Monti, 2011). With the aim of removing potential sources of data bias, while preserving BOLD contrast sensitivity to the underlying neural activity, standard pipelines of fMRI data processing and analysis steps have been developed (Friston et al., 1994a, Smith et al., 2004a, Monti, 2011) and can be implemented via dedicated software packages such as AFNI, BrainVoyager, SPM and FSL (Ashburner, 2012, Cox, 2012, Goebel, 2012, Jenkinson et al., 2012). In keeping with the previous work of our group, in this thesis fMRI analysis was performed with the tools within the FMRIB's software library (FSL) (<http://www.fmrib.ox.ac.uk/fsl/>) (Smith et al., 2004a).

2.3.7 Image acquisition

BOLD fMRI requires a relatively rapid sequence of whole-brain image acquisitions, such that dynamic changes in signal can be used to localize areas of functional activity (Ogawa et al., 1990b). Further to increasing temporal resolution, shorter image acquisition times also help minimising potential motion-induced artefacts (Bandettini, 2001, Huettel, 2004, Buxton, 2009). While temporal resolution is important, fMRI sequences should also aim at optimizing spatial resolution and SNR (Bandettini, 2001, Buxton, 2009); unfortunately a clear trade-off exists between these three parameters (Westbrook et al., 2005, McRobbie, 2007), such that for a given SNR increasing image acquisition speed requires sacrificing spatial resolution (increasing voxel size) (Elster and Burdette, 2001, Westbrook et al., 2005). This is described by the SNR equation of a 2-Dimensional (2D) MR image:

(2.15)

$$\text{SNR} = K * (\text{voxel size}) * \frac{(\sqrt{\text{measurements}})}{(\sqrt{\text{bandwidth}})}$$

Where the constant K is directly proportional to the strength of the main field B_0 , “measurements” is the amount of signal components used in the Fourier reconstruction of a voxel, and “bandwidth” is the range of frequencies used to sample the MR signal (Elster and Burdette, 2001).

Typical fMRI experiments employ Echo-Planar Imaging (**EPI**) sequences, also referred to as “single-shot” techniques, since these allow acquiring whole-brain images much more rapidly (within a few seconds or less) than the standard sequences previously described. This is achieved via quick gradient inversions, to produce a train of gradient echoes able to phase-encode an entire 2D slice image following a single excitation RF pulse (Mansfield, 1977). Sampling typically begins from the lower left corner of k-space and, owing to the rapid switching of gradients, subsequent phase-encoded lines are acquired in alternating direction, such that the full k-space is rastered with a Cartesian trajectory (Mansfield, 1977, Westbrook et al., 2005, McRobbie, 2007). While the enhanced data sampling rate, and consequent reduction of image artefacts due to physiological sources of motion (Bandettini, 2001, Buxton, 2009), are key advantages of EPI sequences, these also suffer from lower spatial resolution and poorer SNR compared to standard MRI sequences, and are inherently susceptible to other image artefacts such as ghosting, regional signal drop-outs, and image distortions (Jezzard and Clare, 1999, Westbrook et al., 2005, Buxton, 2009, McRobbie, 2007). GRE EPI, as first implemented by Ogawa and colleagues, is still the most commonly used imaging sequence in fMRI experiments (Ogawa and Lee, 1990, Ogawa et al., 1990b, Ogawa et al., 1990c, Ogawa et al., 1992, Bandettini, 2001, Harmer et al., 2012), and is effective at enhancing the $T2^*$ effects, and thus BOLD contrast, arising primarily from large venous vessels (Ogawa et al., 1993, Bandettini, 2001, Silvennoinen et al., 2003, Buxton, 2009). Conversely, less commonly implemented SE EPI reduce $T2^*$ contrast due to static sources of field inhomogeneities such as large vessels, and are thus more sensitive to signal changes caused by smaller vessels, in theory providing a better localization of functional activity (Bandettini, 2001, Harmer et al., 2012). Due to their

reduced sensitivity to overall BOLD signal changes, SE EPI sequences however require very high field strengths (at least 7T) to detect statistically significant patterns of functional activation (Harmer et al., 2012).

2.3.8 Data pre-processing

To better understand the principles of data pre-processing and statistical analysis, it is useful to note that an fMRI dataset is composed of a sequence of whole-brain BOLD contrast weighted images also termed “volumes”, sampled at a rate defined by the repetition time TR (Friston et al., 1994a). A time series for any spatial location is then defined as the BOLD signal time course of the corresponding voxel across volumes (Friston et al., 1994a, Monti, 2011).

fMRI data pre-processing is of fundamental importance as noise fluctuations alone can induce BOLD signal changes of as much as 0.5-1%, while activity-induced BOLD contrast typically measures a signal change of between 0.5-5% from baseline level (Donaldson and Buckner, 2001, Smith, 2001b). In addition to removing noise patterns from the signal time series, fMRI data pre-processing also aims to eliminate potential sources of data biasing prior to statistical analysis (Friston et al., 1994a, Smith, 2001b, Monti, 2011). Throughout the work of this thesis, fMRI data was pre-processed in accordance with the default FSL pipeline; this section will therefore describe each pre-processing step as implemented therein.

Slice acquisition and time correction

Each individual volume is formed by multiple slices sequentially acquired at equivalent time intervals throughout the total volume acquisition time TR (Smith, 2001b, Huettel, 2004). While in practice this implies that, for instance, the final slice is acquired TR seconds after the initial slice, without slice acquisition time correction, FSL assumes that the entire volume was acquired at a specific time point, halfway through the TR interval (Smith, 2001b), which may severely affect later statistical analysis. In FSL slice acquisition time correction uses acquisition parameters to appropriately phase shift the voxels’ signal time series slice-wise, using Hanning-windowed sinc signal interpolation (Smith, 2001b, Huettel, 2004).

Motion correction

Head motion is a major potential source of signal bias in fMRI experiments, as it violates the fundamental premise that equivalent voxels in successive volumes corresponds to the same spatial location within the brain. Fluctuations in a voxel's time series may thus be artificially induced by a change in the tissue being imaged, rather than by BOLD signal response alone (Brammer, 2001, Huettel, 2004); this is especially true around high contrast tissue boundaries such as CSF/grey matter and bone/cortex junctions. If correlated with the timing of stimulation patterns, even small head motions have been shown to cause detection of false activations (Hajnal et al., 1994). The first approach to managing head motion involves video-looping through the volumes such that gross movements can be identified, and data discarded or truncated accordingly (Brammer, 2001). Smaller movements are then corrected for by considering the head as a rigid body, and applying an appropriate set of transforms to change the orientation and position (but not the shape) of each volume such that it is brought into alignment, or co-registered, with an arbitrarily chosen reference, or template, volume (Friston et al., 1995a, Friston et al., 1996, Brammer, 2001, Jenkinson et al., 2002, Huettel, 2004). In FSL, this process is implemented via a dedicated motion correction tool MCFLIRT (Motion Correction using FMRIB's Linear Image Registration Tool), which computes a set of (usually) 3 translation and 3 rotation transformation parameters (one for each x,y,z direction) required for co-registering each volume to the template volume (arbitrarily selected as the data set's central volume) (Friston et al., 1995a, Brammer, 2001, Jenkinson et al., 2002). In addition to realigning images, the absolute and direction-specific estimates of head displacement can be used to systematically truncate datasets, and/or as a series of additional confounds in the data analysis to explain (and regress out) temporal changes in image intensity that are linearly associated with the measured head motion (Brammer, 2001, Jenkinson et al., 2002). This approach is however limited, as the relationship between head motion and the resulting aberrant pattern of BOLD contrast is highly non linear, and it fails to correct for physiological motion patterns, such as pulsations, that violate the rigid body assumption (Brammer, 2001, Beckmann and Smith, 2004). An alternative method, which was implemented throughout the work of this thesis is to perform blind source separation via Independent Component Analysis (ICA), which allows removing coherent patterns of

noise due to physiological sources and/or motion (described in section 2.3.10) (Beckmann and Smith, 2004).

Spatial re-alignment of fMRI volumes does not however compensate for more complex effects of head motion, especially those arising from through-plane movements (such as nodding) between volume acquisitions (Friston et al., 1996, Muresan et al., 2005). The BOLD signal produced by the tissue within a voxel is in fact a function of the tissue's spin excitation history, which depends on all of its previous positions during scanning. In practice, motion between volume acquisitions may lead to the same tissue substrate being excited again before it is predicted by the EPI sequence, fundamentally altering its steady state magnetization, and therefore modifying the BOLD signal intensity it produces. This "spin history" artefact will affect all subsequently acquired volumes until the tissue reaches a new steady state (Friston et al., 1996, Muresan et al., 2005). A change in head position during the acquisition of an individual volume (i.e. inter-slice head motion) would produce similar spin history artefacts, and additionally would require realignment of slice stacks. Several data processing methods have been proposed to perform slice stack realignment (Kim et al., 1999) as well as spin history correction (which can also be done online via Prospective Motion Correction (reviewed in (Maclaren et al., 2013))), however these corrections are not carried out in FSL due to the intricate, and often unpredictable, correlation between whole-brain signal intensities and patterns of head motion (Friston et al., 1996, Brammer, 2001, Jenkinson, 2001, Beckmann and Smith, 2004).

Distortion correction

During EPI acquisitions, boundaries between tissues with different magnetic susceptibilities tend to create local field inhomogeneities shifting the apparent position of image pixels (image distortions/warpings), and giving rise to regions of signal drop-out (Jezzard and Clare, 1999, Westbrook et al., 2005, Buxton, 2009, McRobbie, 2007). These artefacts can be corrected by first calculating the pixel-wise phase difference (and thus spatial shift induced by Fourier image reconstruction) between a reference high resolution GRE image and EPI images acquired at different echo times. A field map characterizing the spatial distribution of B_0 field inhomogeneities can thus be obtained, and used to unwarp individual EPI images (Jezzard and Balaban, 1995). The primary

responsible of image distortions in the adult brain are air tissue boundaries, such as sinuses (Jezzard and Clare, 1999, Buxton, 2009), which however are not yet pneumatized in preterm and term infants, and the implementation of this pre-processing step was therefore found not to be necessary for the data analysed within this thesis (Adibelli et al., 2011).

Intensity normalisation

Several factors, including the scanner's inability to provide a stable signal, can induce variations of the global signal intensity across subsequently acquired volumes. To compensate for this effect, the mean intensity of each volume can be normalized to match an arbitrary value, and the volume-wise BOLD signal be rescaled accordingly (Friston et al., 1994c, Smith, 2001b). While this pre-processing step is implemented by default in other fMRI analysis packages, it is not automatically enabled in FSL, and was not performed throughout the work of the thesis. Generally, global intensity normalization is not recommended, since it does not account for the potentially large shifts in intensity produced by stimulus induced activation, which can significantly increase the mean intensity of the corresponding volume, effectively reducing the whole-volume scaling factor. This may artificially induce a negative correlation between the stimulus and areas that do not exhibit functional activity (Aguirre et al., 1998a, Smith, 2001b). Global intensity variations are instead corrected via high-pass temporal filtering of the dataset to reduce large scale signal drifts. Additionally a simple grand mean scaling is performed, whereby the mean of all voxels in all volumes in a scanning session is computed, and individual volumes are then normalized by this common scaling factor. This normalization is necessary to allow inter-session and inter-subject comparisons, but does not affect intra-session analysis (Smith, 2001b).

Spatial filtering

Low-pass spatial filtering of fMRI volumes is typically performed, effectively removing high spatial frequency components, whose contribution amounts largely to spatial resolution rather than signal intensity (see Section 2.1), such that images are blurred/smoothed (Smith, 2001b, Huettel, 2004). In FSL this is done by replacing the intensity of each voxel with a weighted average of neighbouring intensities obtained via the convolution between the data volume and a 3D Gaussian

filter, whose width and magnitude determine the neighbourhood size and weightings respectively (Smith, 2001b). In this process spatial resolution is sacrificed to increase the data SNR, and to reduce the number of data points, which, if exceedingly large, can lead to multiple comparison statistical problems and increase false positive discovery rates (Friston et al., 1994d, Huettel, 2004).

Temporal filtering

Low-pass or high-pass temporal filters can be applied to the data set to remove high and low frequency artefacts from the voxels' signal timecourses (Friston et al., 1994c, Smith, 2001b, Huettel, 2004). High-pass filtering can be used to remove the low frequency signal intensity fluctuations induced by scanner drift, slow head movements and aliased breathing motion; and is routinely performed in FSL as an alternative to global intensity normalization (as described in Section 3.6.5 of Chapter 3) (Friston et al., 1994c, Smith, 2001b). While low-pass filtering is sometimes used to reduce high frequency noise components induced by vascular pulsations as well as vibrations of the scanner bed, it tends to dampen and smooth the BOLD signal changes associated with rapid neural responses, also affecting time-series/HRF correlation and drastically reducing the statistical power of later statistical analysis (Smith, 2001b). For these reasons it is not implemented by default in FSL, and was not carried out throughout the work of this thesis, wherein patterns of high frequency noise were removed via ICA blind source separation instead (Beckmann and Smith, 2004).

2.3.9 Study design

The assumption of BOLD response linearity renders fMRI experimental design a highly flexible process, however care should be taken in ensuring that the stimulation paradigm is able to repeatedly produce robust patterns of BOLD contrast changes that are easily discernible from noise artefacts (Bandettini et al., 1993, Friston et al., 1994c, Donaldson and Buckner, 2001). In general, the BOLD signal time series in response to stimulation paradigms can be modelled as the convolution of a canonical HRF with a simple (often binary) representation of the stimulus timecourse (Friston et al., 1994c). The type of paradigm and associated parameters are usually chosen depending on the aim of

the study, but experimental paradigms can be broadly classified in two main categories (Donaldson and Buckner, 2001, Huettel, 2004, Amaro and Barker, 2006, Monti, 2011):

(i) *block design paradigms*: in which the subject is cyclically presented with time periods in which he is continuously stimulated (/or asked to perform a specific task) interspersed with periods of rest. Whole-brain BOLD signals associated with stimulation can then be contrasted to those associated with rest, such that cerebral regions of functional activity can be statistically identified (Bandettini et al., 1993, Donaldson and Buckner, 2001). In these regions, the expected BOLD signal response to blocks of continuous stimulation is roughly modelled as the linear combination of overlapping HRFs (Friston et al., 1994c, Boynton et al., 1996, Dale and Buckner, 1997). Particular attention must however be paid to the possible effects of subject habituation to the stimulus/task. Due to its repetitive nature, this experimental paradigm is generally not suited for studies that aim at characterizing higher cognitive processing and sensory integration in which unpredictability of stimulation is an important confound (event-related paradigms are used instead) (Poellinger et al., 2001, Donaldson and Buckner, 2001, Huettel, 2004, Amaro and Barker, 2006).

(ii) *event-related Paradigms*: in which the subject is presented with brief stimulations at unpredictable time intervals. Areas of functional activity are then statistically identified as those in which BOLD signal response to stimulation consistently follows that predicted by a canonical HRF (Buckner et al., 1996, Dale, 1999, Bandettini and Cox, 2000, Donaldson and Buckner, 2001). If assumptions are made with respect to the region of interest in which the BOLD response to stimulation/task is likely to be observed, event-related paradigms can also be used to characterize the HRF response (Buckner et al., 1996, Friston et al., 1998a, Dale and Buckner, 1997, Buxton et al., 2004).

In summary, block stimulation paradigms produce higher amplitude responses and are thus most suited for detecting patterns of functional activation, whereas event-related paradigms are most suited for evaluating haemodynamic responses in predetermined regions of interest (Dale, 1999, Buxton, 2009). In keeping with the principles of characterization of electrophysiological responses, an accurate representation of HRF morphology can be achieved via time locked-averaging of the signal within a cluster of activated voxels, which reduces the random noise effects on individual

post-stimulus responses (Buckner et al., 1996, Dale and Buckner, 1997, Miezin et al., 2000). However, particular care should be taken when designing event-related paradigms since these tend to suffer from poor sensitivity and efficiency, due to the inherently lower amplitude of BOLD response, and to the relatively sparse pattern of stimulation. Several approaches have been proposed to improve event-related designs including: setting a regular inter-stimulus interval (ISI) of 12-15 seconds (Dale, 1999, Bandettini and Cox, 2000, Miezin et al., 2000) randomise ISIs to increase the number of events and give rise to higher amplitude overlapping responses (Dale and Buckner, 1997); jittering the stimulus within a single TR interval to generate a controlled composite haemodynamic response which can then be deconstructed (Miezin et al., 2000, Donaldson and Buckner, 2001).

Obtaining an accurate HRF representation is also important for block design experiments, where the plateau of the induced BOLD response is proportionate to the area under the HRF time course, while the rate at which the BOLD signal changes, especially during stimulus/rest transitions, is proportionate to the HRF morphology (Buxton, 2009).

2.3.10 Data Analysis

To better understand the principles underlying data analysis, it is useful to remember that fMRI data is formed by a set of volumes, each acquired at a specific time interval TR, and each composed of a set of spatially equivalent voxels. Any voxel within the data set is therefore fundamentally characterized by a BOLD signal time series acquired at sampling frequency $1/TR$, in which the n^{th} timepoint corresponds to the voxel signal intensity within the n^{th} volume (Friston et al., 1994a, Monti, 2011). Areas of functional activation can then be localized to the individual voxels (or clusters thereof) whose BOLD signal time course increases following stimulation in a manner predicted by the HRF (with a delay of 5 to 8 seconds) (Bandettini et al., 1993). Owing to this reproducibility of BOLD response, statistical analysis of stimulus correlates are usually performed via the implementation of a General Linear Model (GLM) (Friston et al., 1995a, Friston et al., 1994b), which does pose some constraints, but its simplicity and flexibility make it a suitable choice for most task-driven fMRI data analysis (Monti, 2011, Poline and Brett, 2012).

First level analysis

Within FSL, first level analysis refers to the within-voxel (univariate) and across-voxel (multivariate) timeseries analysis required to infer brain activity from a session-specific set of 4D fMRI data. Under the General Linear Model (GLM) assumption, the measured time course of fMRI data is explained by the sum of the time courses of known explanatory variables, each weighted by an arbitrary coefficient (Poline and Brett, 2012). At the first stage of statistical analysis, FSL uses a GLM (implemented via the FILM (FMRIB's Improved Linear Model) tool) to explain fMRI data voxelwise, such that the intensity timeseries $y(t)$ of any given voxel in response to a single pattern of stimulations is expressed by the equation:

(2.16)

$$y(t) = \beta_1 * x_1(t) + \varepsilon(t)$$

where $x_1(t)$ is the explanatory variable obtained via the convolution between the design paradigm and the predicted HRF waveform; β_1 is a the parameter estimate used to scale the explanatory variable $x(t)$ to the observed intensity timeseries $y(t)$; and $\varepsilon(t)$ is the residual error in the fit accounting for the discrepancies between the actual response $y(t)$ and predicted response $x_1(t) * \beta_1$ (Friston et al., 1995a, Friston et al., 1994b). This model can be extended to include further explanatory variables, which may represent stimulation patterns as well as nuisance confounds (such as head motion), each scaled by a corresponding parameter estimate (Friston et al., 1994b, Friston et al., 1998a, Smith, 2001a). The voxel timeseries determined by “n” explanatory variables is then expressed as:

(2.17)

$$y(t) = \beta_1 * x_1(t) + \dots + \beta_n * x_n(t) + \varepsilon(t)$$

or

$$\begin{pmatrix} y(t_1) \\ \dots \\ y(t_N) \end{pmatrix} = \begin{pmatrix} x_1(t_1) & \dots & x_n(t_1) \\ \vdots & \ddots & \vdots \\ x_1(t_N) & \dots & x_n(t_N) \end{pmatrix} \begin{pmatrix} \beta_1 \\ \dots \\ \beta_n \end{pmatrix} + \begin{pmatrix} \varepsilon_1(t_1) \\ \dots \\ \varepsilon_n(t_N) \end{pmatrix}$$

where individual columns of the design matrix $\mathbf{X}$ represent separate explanatory variables, each scaled by the corresponding element of the parameter estimate vector $\boldsymbol{\beta}$ (Friston et al., 1994b, Smith,

2001b). GLM statistical analysis attempts to identify the set of parameter estimates β that provide the best fit between the predicted response and the measured response (Monti, 2011). A common approach to this problem is to employ a linear regression method such as Ordinary Least Squares (OLS), whereby the parameter estimates providing the best model fit are estimated via an iterative process aimed at minimising the sum of the squared residuals (ϵ). Fundamental to this method is the assumption that the error around each observation of y be uniformly, independently, and normally distributed (Woolrich et al., 2001). It is important to note that following OLS model fitting, each parameter estimate β therefore indicates how well the associated model explains the observed data, with greater β values indicating a better fit. In FSL prior to performing OLS regression, the voxel timeseries is pre-whitened to improve the efficiency of GLM estimation. Prewhitening is carried out via non-parametric estimation of timeseries autocorrelation, such that statistical dependence of subsequent samples due to patterns of structural noise is corrected for (Worsley and Friston, 1995, Woolrich et al., 2001, Bullmore et al., 1996). Following timeseries pre-whitening, the voxel-wise parameter estimates are computed by FSL, first converted into a t-statistic (by dividing each β by its standard error (SE), a measure of ϵ), and then further converted into a z-score via standard statistical transformation (Smith, 2001b, Woolrich et al., 2001). A z-statistical parametric map of the brain is then generated, in which each spatial voxel is assigned with an intensity value proportionate to its z-score (Friston et al., 1994c, Smith, 2001b). Areas of functional activity are identified by thresholding the z-statistical parametric map at a significance level that, while relatively arbitrary, should be standardized across similar studies (Genovese et al., 2002, Smith and Nichols, 2009, Nichols, 2012). Finally, a multiple comparisons correction is performed via cluster-extent based thresholding, whereby the chance of false positives is controlled based on the assumption that clusters of activated voxels are more likely to indicate functional activity, than isolated voxels alone. This correction is implemented via random field theory, as this method provides good sensitivity to weak and diffuse signals, and is able to account for the spatial smoothness of the data (Smith, 2001b, Smith et al., 2004b, Genovese et al., 2002).

Higher level analysis

In FSL higher level analysis refers to the statistical comparison between intra-subject or inter-subject multi-session data to either improve intra-subject experimental sensitivity, or to reveal and contrast patterns of functional activity across populations and stimulation types (Holmes and Friston, 1998). Before higher level analysis can be performed, all data sets need to be spatially registered to a standard template, such that homologous anatomical regions are brought into alignment (Jenkinson, 2001, Brett et al., 2002). FSL then allows performing higher level statistical analysis via fixed-effects or mixed-effects statistical modelling of the data, as well as by implementing random permutation methods. Since each analytical approach is based upon inherently different assumptions that determine the statistical inference that can be drawn from its results, choosing a method of higher level statistical analysis ultimately requires a careful consideration of the study population and the hypothesis being tested (Nichols and Holmes, 2002, Mumford and Nichols, 2006, Mumford and Poldrack, 2007):

A fixed-effects statistical model is normally applied to within-session data. This model assumes that a fixed relationship exists between the stimulus and the “real” activation (the effect), such that the within-session “measured” effects sequence is modelled as having a mean determined by the real effect, and a variance introduced by the errors of individual measurements (i.e. due to time varying noise). Significance of the effect is then evaluated by comparing the mean response to its within-session variance, or in essence the effect is deemed to be “*significant in relation to the precision with which it can be measured*” (Friston et al., 2005). While fixed-effects group analysis can be performed by cumulatively modelling cross-subject (and cross-session) responses in terms of an overall mean and a variance, in reality this approach does not model how the response changes across different data sets due to several unobserved factors (random effects). This implies that patterns of activation inferred via fixed-effects analysis are only representative of the studied group, but cannot be generalized to the wider population from which subjects are drawn as this requires modelling the cross-subject (and cross-session) variance (Mumford and Nichols, 2006).

Mixed-effects models include the variance arising from both within session (the fixed-effects) and cross-subject measurements (the random-effects), and thus allow verifying hypotheses which

require findings to be amenable to the wider population (Beckmann et al., 2003, Mumford and Nichols, 2006, Mumford and Poldrack, 2007). This approach is more conservative than fixed-effects analysis alone as it accounts for a greater overall variance, determined by the sum of the fixed-effects and random-effects variances. It is however unsuitable for small group analysis, in which the cross-session variance tends to be overestimated (Mumford and Poldrack, 2007).

An alternative is to perform group analysis via a non-parametric permutation method which, contrarily to fixed-effects and random-effects models, makes no assumptions about the distributions of the data and noise. Instead this method is based on the null hypothesis that if no correlation exists between an observed effect and an underlying experimental condition (such as stimulation), then correctly or randomly assigning observations to experimental conditions would produce no significant difference on the distribution of values. A measure of the significance of the experimental effect can then be drawn by comparing the variance associated with the correct assignment of observations to that associated with multiple random permutations of observations (Nichols and Holmes, 2002). This approach is particularly suitable for cases in which non-standard statistical analysis is required, and in some cases has been shown to outperform more standard parametric methods (Nichols and Hayasaka, 2003, Smith et al., 2006). In FSL, nonparametric permutation inference is performed via the randomise tool, which can be applied to higher level modelling and inference (Winkler et al., 2014).

Independent Component Analysis (ICA)

While the GLM approach allows for a relatively simple and flexible multiple linear regression framework whereby the significance of each confound is evaluated (via null-hypothesis testing) as its relative ability to explain an expected pattern of signal changes, this method of analysis is limited by the accuracy and validity of the assumed spatio-temporal characteristics of signal changes. Another potential drawback arises from the possible biasing of parameter estimates induced by structured patterns of signal noise that are temporally non-orthogonal to the regression model. An alternative approach to the GLM technique is to consider the fMRI signal timeseries as a sequence of randomly observed variables, which result from the linear combination of statistically

independent source signals (i.e. due to nuisance or functional processes). Separating and identifying such source signals is a typical blind source separation (BSS) problem, which can be addressed using independent component analysis (ICA). Similarly to principal component analysis (PCA), ICA models the time course of the separate independent sources $\mathbf{S}$ as (Kim and Kim, 2012):

(2.18)

$$\mathbf{S} = \mathbf{XW}$$

$$\begin{pmatrix} \mathbf{S}_{1,1} & \cdots & \mathbf{S}_{1,T} \\ \vdots & \ddots & \vdots \\ \mathbf{S}_{N,1} & \cdots & \mathbf{S}_{N,T} \end{pmatrix} = \begin{pmatrix} \mathbf{X}_{1,1} & \cdots & \mathbf{X}_{1,T} \\ \vdots & \ddots & \vdots \\ \mathbf{X}_{N,1} & \cdots & \mathbf{X}_{N,T} \end{pmatrix} \begin{pmatrix} \mathbf{W}_{1,1} & \cdots & \mathbf{W}_{1,T} \\ \vdots & \ddots & \vdots \\ \mathbf{W}_{T,1} & \cdots & \mathbf{W}_{T,T} \end{pmatrix}$$

Where the matrix $\mathbf{S}$ is filled column-wise with the timeseries (with N samples) of each independent source signal; the matrix $\mathbf{X}$ is filled column-wise with the timeseries of T observation variables (i.e. voxels); and $\mathbf{W}$ is a $T \times T$ unmixing matrix (Kim and Kim, 2012). In PCA the unmixing matrix is referred to as the loading matrix and is computed as the eigenvectors of the covariance matrix of $\mathbf{X}$. The loading matrix transform allows determining signal sources $\mathbf{S}$ that are uncorrelated and, under the assumption of multivariate normality, independent. However, in fundamental contrast to PCA, ICA does not assume multivariate normality (but only non-colinearity) of latent signal components, in which case independence of non-correlated components is not guaranteed and cannot be fully explained by the covariance structure of the $\mathbf{X}$ matrix alone (Kim and Kim, 2012). The unmixing matrix is instead derived by optimizing a specified criterion of statistical independence between ICs. Optimization algorithms may range from stochastic gradient descent to fixed-point iteration, and criterion optimization can involve minimizing measures of data co-linearity such as minimizing likelihood or mutual information, or maximizing measures of data non-gaussianity such as neg-entropy.

Maximization of non-gaussianity to retrieve ICs is motivated by the central linear theorem, which implies that the signals associated with linearly independent sources are less complex and Gaussian-distributed than the signal produced by any of their random mixtures (Kim and Kim, 2012). In keeping with this assumption, ICA seeks a mixing matrix that minimizes the gaussianity of the

probability distribution functions of each individual IC. This principle is exploited in FSL, where the MELODIC (Multivariate Exploratory Linear Optimised Decomposition into Independent Components) tool (Beckmann and Smith, 2004) is used to perform neg-entropy maximization via fixed point iteration (FastICA). The entire fMRI acquisition dataset is “unwrapped” into a single 2D matrix (X), which is filled column-wise with the time course of each individual voxel. ICA is then performed on the data, and signal sources S are output as pairs of sub-matrices: a first matrix containing the time course of each independent component column-wise, and a second matrix containing the spatial map (coordinates) of voxels expressing each component row-wise (Beckmann and Smith, 2004). Spatial maps are then converted into Z-statistic maps based on the residual error, with significant voxels thresholds defined by applying a Gaussian Mixture Model to the spatial distribution of intensity values (Beckmann and Smith, 2004). Prior to ICA, FSL additionally utilizes probabilistic PCA to define a linear sub-space of reduced dimensionality within which the source signals are contained (Beckmann and Smith, 2004).

As suggested by Beckmann and colleagues (Beckmann and Smith, 2004, Beckmann et al., 2005, Beckmann, 2012), the resulting spatial components may represent i) a map of stimulus related functional activity, ii) task-unrelated intrinsic brain activity, iii) or possible sources of artefact. ICA techniques are particularly useful to identify and remove artifactual signals in task-driven fMRI experiments. Artefacts include signals likely to be related to intrinsic physiological factors (vessel pulsation, respiratory movement) and head motion (Beckmann and Smith, 2004). Furthermore, ICA techniques represent a valuable application in the identification and study of resting state networks, for which even under conditions where no stimulus or task has been performed by the subject, coherent patterns of low frequency correlated activity in fMRI data are seen (Biswal et al., 1995, Beckmann et al., 2005).

Chapter 3: fMRI studies of the newborn brain

Overview - Functional MRI has the great potential to enhance our understanding of early brain development. Studies have demonstrated the feasibility of the technique, and that functional localization is present in the immature brain. However, a limited number of BOLD fMRI studies on the neonatal population have been carried out over the last two decades. A possible cause can be found in the great deal of uncertainty that remains about the nature of BOLD responses in the neonatal subjects. In this chapter, a review on the fMRI studies of the newborn brain is presented. It is also suggested that the ambiguity on the nature of neonates' BOLD responses is likely to be multi-factorial, with aspects of study design, image acquisition, and data analysis potentially determining the high degree of uncertainty so far observed.

3.1 fMRI studies of the newborn brain: potential and current limitations

Since first being introduced, fMRI has not only been used to extensively characterize resting state networks of functional connectivity across the adult human brain, but also to delineate cerebral areas responsible for task planning and execution, as well as for the processing of various types of stimulations (Ogawa et al., 1990a, Kim and Ogawa, 2012, Arichi, 2012). As highlighted in Chapter 1, mapping and characterizing the functional brain development throughout the early preterm period could lead to important advancements in the prognosis, diagnosis and ultimately, management and prevention of neurodevelopmental disorders such as CP. Combining a good spatial resolution, the ability to offer whole brain imaging, an intrinsic safety, and a non-invasive nature, fMRI would seem as an ideal technique to image the preterm population and provide clinicians with an all-important understanding of developing brain functionality (Arichi, 2012).

However, to date a somewhat surprisingly paucity of studies have utilized fMRI to characterize resting state connectivity, and, especially, to study functional responses to sensory stimulation in infants born preterm (reviewed in (Seghier et al., 2006, Seghier and Hüppi, 2010, Arichi, 2012)). Even within this small body of work, the reported results have often been inconsistent in terms of both the localization and magnitude of cerebral activation, with some studies reporting negative (decreased) BOLD responses (Arichi, 2012); a phenomenon rarely observed in adult subjects during equivalent experiments. While these findings may be representative of underlying physiology, the heterogeneity of results is more likely due to either loose exclusion criteria concerning the subjects' age or cerebral integrity, leading to statistical misrepresentation of patterns of functional activity in such a fast-growing and vulnerable population, or to dramatic methodological inconsistencies.

In fact experimental paradigms have been poorly and superficially reported throughout the majority of the literature. This is clearly surprising since in fMRI studies (and generally in all experimental studies) an accurate definition of the equipment and methods is paramount to the interpretation of scientific results. In task-dependent fMRI experiments it is fundamental that stimuli are time-locked to image acquisitions, and delivered in a robust and repeatable fashion; however in the vast majority of studies these methodological details have been omitted, and/or not implemented. Another important notion is that an age appropriate HRF needs to be used for GLM-based statistical

inference of the BOLD response (Arichi, 2012). Throughout the neonatal fMRI literature however, an adult HRF was convolved into the GLM. Finally, if inter-experimental results are to be compared, it is also important that the imaging sequence and field strength are standardized (Arichi, 2012).

These methodological challenges clearly need to be addressed before fMRI can be used to reliably characterize the development of functional connectivity throughout the preterm population. The research conducted by our group prior to the work within this thesis, has begun to standardize the methodology for task-dependent fMRI studies of the preterm somato-sensory cortex (Arichi et al., 2012, Arichi, 2012, Arichi et al., 2010). This was done by developing a preliminary systematic, objective and repeatable experimental protocol for somato-sensory stimulation (Arichi et al., 2010), which was used in a cohort of preterm infants to characterize the preterm-specific HRF (Arichi et al., 2012, Arichi, 2012); an essential analytical tool that will also be used throughout the work of this thesis.

In this chapter the relatively small, though rapidly growing body of literature concerning fMRI studies of the preterm and newborn (up to 12 months of age) population will first be reviewed, to then highlight the substantial methodological advancements introduced by our group. The merits and drawbacks of this new approach will then be discussed, with a particular focus on the enhancements that stimulation hardware will require before studies aimed at the longitudinal characterization of the neonatal homunculus can be performed.

3.2 fMRI studies of the newborn brain: review of the literature

The studies that have so far explored functional responses in the preterm and newborn (up to 12 months of age) population using resting-state or task-dependent fMRI have been comprehensively reviewed by Seghier and colleagues in 2006 and 2010 (Seghier et al., 2006, Seghier and H  ppi, 2010) and, more recently, by Arichi, Tusor and colleagues and Tao and Neil (Arichi, 2012, Tao and Neil, 2014, Tusor et al., 2014). To identify additional studies that may postdate or may have been missed by these reviews, a PubMed search was performed by setting the earliest publishing date to 1/1/1990 and the search term to: ("functional MRI" OR "fMRI" OR "functional MR") AND (infant OR newborn OR neonate OR preterm). The search returned a total of 277 articles, of which 35 were considered after discarding non-relevant studies (preterm-born adult/adolescent/children studies, animal studies, phantom studies, fetal studies, non-fMRI studies). Only 2 articles were not covered by previous reviews (Graham et al., 2013, Smyser et al., 2013). The studies retrieved were subcategorized according to their target population, with studies including preterm or young (<12 months of age) ex-preterm subjects summarized in Table 3.1, and those including young (<12 months of age) term-born subjects summarized in Table 3.2.

3.2.1 Studies of the preterm population

Following a first feasibility study which demonstrated that fMRI was cable of capturing dynamic BOLD changes associated with hyperventilation-induced decreases in pCO₂ in the newborn brain (Toft et al., 1995), a further 13 studies have used fMRI to reveal patterns of cerebral activity within the preterm or young ex-preterm population. Of these, 6 studies (47%) have aimed at characterizing resting-state functional networks, while the remaining 7 studies (53%) used task-dependent protocols based on visual (3 studies, 23%), somato-sensory (3 studies, 23%) and auditory (1 study, 7%) stimuli. Somato-sensory stimulations were delivered manually at approximate timings, while in no instance has a mechanism to time-lock the stimulus to the onset of TRs explicitly been reported. Amongst the 7 task-dependent studies, positive and negative BOLD responses have been reported in almost equal quantities, with 1 (visual stimulation) study reporting only positive BOLD responses, and the remaining 6 studies reporting mixed positive and negative BOLD responses. An

overall scarceness of preterm subjects scanned prior to term equivalent age is especially noticeable throughout task-dependent fMRI studies, and may be due to the enhanced challenges posed by this technique as compared to resting state fMRI. In fact the only study conducted on a large preterm population was reported by Lee and colleagues (Lee et al., 2012), in which activation in response to visual stimulation was however only identified in 4 of the 65 preterm subjects included. Collectively, all other task-dependent fMRI studies have (successfully) attempted to characterize responses to visual, auditory and somato-sensory stimulation on a total of only 6 preterm infants. Despite sign inconsistencies, BOLD responses appear to be far more robustly associated with non-visual stimuli in the preterm population (Arichi, 2012), perhaps due to an underlying immaturity of functional and structural visual connectivity or to limitations inherent to the experimental methodology (such as inappropriate light flickering frequencies). Compared to task-dependent fMRI studies, resting state studies have achieved a markedly higher recruitment rate of preterm infants, thereby even allowing for a longitudinal characterization of resting state functional connectivity throughout the preterm period (Doria et al., 2010). Finally, it should be noted that sedation was required throughout the vast majority of resting state and task-dependent fMRI studies of the preterm population, and was primarily performed via chloral hydrate administration.

3.2.2 Studies of young infants born at term

A total of 4 resting state and 19 task-dependent studies including term-born subjects of up to 1 year post-natal age (PNA) were identified in the literature. The proportionate increase of task-dependent protocols may be partly explained by their safer implementation in this less fragile population, but can also be attributed to a gradual introduction of task-dependent fMRI within the spectrum of pre-surgical assessment methodologies for older infants (i.e. epilepsy pre-surgery evaluation). Amongst task-dependent studies, 6 (32%) aimed to characterize neural responses to auditory (language or music) stimuli, and a further 13 (68%) to visual stimuli. No relevant study performing somato-sensory stimulation was identified. Although a direct cross-study comparison is rendered difficult by the evident heterogeneity in clinical conditions, anaesthetic agents, and acquisition parameters, the findings have highlighted consistent patterns in stimulus-specific

responses. Auditory stimulation was found to predominantly induce positive BOLD responses, while response to visual stimulation was reported to switch from positive to negative at around 8 weeks PNA.

Table 3.1: Studies of preterm or young ex-preterm subjects, adapted and expanded from (Arichi, 2012).

Study	Stimulus		Subjects included (discarded) type (PMA at scan) <i>*MR scan clinically required</i>	Sedation type (subjects)	MR sequence parameters					BOLD responses identified, amplitude
	Type	TR time-lock (hardware)			B ₀ field (T)	Spatial resolution x*y*z (mm)	TE (ms) TR (ms)	Flip Angle (°)	Sequence duration (min:sec)	
Toft et al. 1995 (Toft et al., 1995)	Changes in pCO ₂ induced by hyperventilation	-	1 preterm (31 wks) <i>*4 term-born (39-47 wks)</i> <i>*all subjects ventilated</i>	n/a	1.5	0.8*1.6*3 (single slice)	60 82	40	21:00 - 29:00	5/5 neg.
Anderson et al. 2001 (Anderson et al., 2001)	Auditory	n/a (pneumatic headphones)	5 preterm (33-36 wks) 1 ex-preterm (37 wks) 8 term-born (39-47 wks) Total: 14 (6)	None	1.5	3.125*3.125*n/a	60 5000	n/a	12:00	<i>Preterm: 3/5 pos., 2/5 neg.</i> <i>Ex-preterm: 1/1 neg.</i> <i>Term-born: 2/8 pos., 6/8 neg.</i>
Born et al. 2000 (Born et al., 2000)	Visual	n/a (strobelight & mirror, Kunelco A/S)	1 preterm (32 wks) 8 ex-preterm (38-61 wks) 8 term-born (54-179 wks) 14 visual deficit (52-338wks)	Chloral hydrate (24/31)	1.5	1.5-1.8*1.5-1.8 *4	66 2500	90	4:00	<i>Preterm: 1/1 none</i> <i>Ex-preterm: 3/8 pos., 3/8 neg., 2/8 none</i> <i>Term-born: 2/8 pos., 5/8 neg., 1/8 none</i> <i>Visual deficit: 1/14 pos., 13/14 neg.</i>
Lee et al. 2012 (Lee	Visual	n/a (strobelight	65 (19) preterm (28-33 wks) 23 (5) ex-preterm (38-43	None	1.5	2.5*2.5*4	60/130 3000	90	3:00-3:30	<i>Preterm: 4/65 pos., 61/65 none</i> <i>Term: 19/26 pos.,</i>

et al., 2012)		outside scanner)	wks)							7/26 none
Erberich et al. 2003 (Erberich et al., 2003a)	Visual Somato-sensory (R/L hand)	No - manual flickering/pumping (gantry light/ rubber bulb)	<i>*1 preterm (29-36 wks)</i> <i>*5 ex-preterm (37-45 wks)</i> <i>*1 term-born (58 wks)</i> Total: 6 (1)	Chloral hydrate (7/7)	1.5	3*3*3	40 3000	85	3:00	<i>Visual: 6/6 pos. & neg.</i> <i>Somato-sensory: 1/6 pos., 5/6 pos. & neg.</i>
Erberich et al. 2006 (Erberich et al., 2006)	Somato-sensory (R/L hand)	No - manual pumping (rubber bulb)	24 (18) ex-preterm (38-49 wks) 6 term-born (3-9 mths)	Chloral hydrate (50/50)	1.5	2.8*2.8*3	60 3000	90	3:00	30/30 pos. (mainly contralateral) & neg. (mainly ipsilateral)
Heep et al. 2009 (Heep et al., 2009)	Somato-sensory (forearm)	No - manual flexion/extension (wristbands, Infant limb holder J.T. Posey Company)	5 (3) ex-preterm (38-39 wks)	Chloral hydrate (8/8)	3	1.88*1.88 *3.59	35 2600	90	3:20	1/5 pos., 4/5 neg.
Doria et al. 2010 (Doria et al., 2010)	Resting state	-	17 early preterm (29-32 wks) 21 preterm (33-36 wks) 24 ex-preterm (39-43 wks) Total: 62 (12) 8 (4) term-born (39-43 wks)	Chloral hydrate (30/74)	3	2.5*2.5*3.25	45 1500	90	6:34	-
Smyser et al. 2010 (Smyser et al., 2010)	Resting state	-	10 early preterm (<30 wks) 16 preterm (30-34 wks) 36 late preterm (34-38 wks)	None	3	2.4*2.4*2.4	28 2910	90	10:00	-

28 ex-preterm (38-42 wks)

10 term-born (term age)

Total: 90 (8)

Fransson et al. 2007, 2009, 2011 (Fransson et al., 2007b, Fransson et al., 2009b, Fransson et al., 2011)	Resting state	-	12 ex-preterm (39 - 44 wks)	Chloral hydrate	1.5	2.8*2.8*4.5	50	80	10:00	-
			19 (2) term-born (39-41 wks)	(ex-preterm only) (12/12)			2000			

Smyser et al. 2013 (Smyser et al., 2013)	Resting state	-	*14 (10) ex-preterm (36-39 wks)	n/a	3	2.4*2.4*2.4	28	90	10:00	-
			25 ex-preterm (36-40 wks)				2910			
			25 term-born (37-41 wks)							

Abbreviations: PMA – post-menstrual age.

Table 3.2: Studies of term-born subjects, adapted and expanded from (Arichi, 2012).

Study	Stimulus		Subjects included (discarded) (PNA at scan)	Sedation type (subjects)	MR sequence parameters					BOLD responses identified, amplitude
	Type	TR time-lock (hardware)			B ₀ field (T)	Spatial resolution x*y*z (mm)	TE (ms) TR (ms)	Flip Angle (°)	Sequence duration (min:sec)	
*MR scan clinically required										
Dehaene- Lambertz et al. 2002. (Dehaene- Lambertz et al., 2002)	Auditory (language)	n/a (piezoelectric headphones)	20 (10) (2-3 mths)	None	1.5	3.1*3.1*4	60 3333	n/a	3:00	20/20 pos.
Perani et al. 2010 (Perani et al., 2010)	Auditory (music perception)	Yes – (DAP Center Mark II, MR Confon) (piezoelectric headphones, MR Confon)	18 (3) (0-3 days)	None	1.5	3.75*3.75*3	40 3000	n/a	7:00	15/18 pos. 3/18 neg.
Blasi et al. 2011 (Blasi et al., 2011b)	Auditory (sounds)	n/a (piezoelectric headphones, MR Confon)	21 (3 to 7 mths)	None	1.5	3.5*3.5*5	57 3000	90	16:00	21/21 pos.

Perani et al. 2011 (Perani et al., 2011a)	Auditory (language)	n/a (piezoelectric headphones, MR Confon)	15 (0-3 days)	None	3	2.81*2.81*3	40 3000	n/a	8:03	15/15 pos.
Graham et al. 2013 (Graham et al., 2013)	Auditory (language)	n/a (pneumatic headphones, Bilsom)	20 (19) (3-6 mths)	None	3	3.125*3.125*4	30/80 2000		9:28	20/20 pos.
Born et al. 1996 (Born et al., 1996)	Visual	n/a (n/a)	7 (6 wks-36 mths)	chloral hydrate (6/7)	1.5	3.125*3.125*4	66 4000	n/a	6:30	6/7 neg., 1/7 n/a
Yamada et al. 1997 (Yamada et al., 1997)	Visual	n/a (strobelight & mirror)	*6 (<8 wks) *9 (8-54 wks)	pheno-barbitone (15/15)	1.5	1.7*1.7*5 (5 slices)	50 3000	90	5:06	<8 wks: 6/6 pos. >8 wks: 9/9 neg.
Born et al. 1998 (Born et al., 1998)	Visual	n/a (LED goggles/ strobelight & mirror, Grass Instruments Co./ Kunelco A/S)	17 (5) (3 days - 48 mths)	chloral hydrate (9/12)	1.5	3.125*3.125*4	66 4000	n/a	6:30	1/12 pos., 10/12 neg., 1/12 none
Martin et al. 1999 (Martin et al., 1999)	Visual	n/a (LED goggles, Grass Instruments Co.)	22 (1-12 mths) 29 (1-6 yrs) 7 (6-12 yrs) Total: 58 (17)	pheno-barbitone (28/58) chloral hydrate	2	1.7-1.9*1.7-1.9*5	40 100	20	8:00	0-4 mths: 6/18 pos., 5/18 neg., 7/18 none 4-40 mths: 4/25 pos., 12/25 neg., 9/25 none

				(12/58) halothane (9/58)						40+ mths: 13/25 pos., 8/25 neg., 4/25 none
Morita et al. 2000 (Morita et al., 2000)	Visual	n/a (strobelight & mirror)	*8 (<60 days) *8 (60-361 days)	pheno- barbitone (16/16)	1.5	3.4*3.4*6-7	50 3000	90	2:06	LGN: 16/16 pos. Primary visual cortex: <60 days pos., >60 days neg.
Yamada et al. 2000 (Yamada et al., 2000)	Visual	n/a (strobelight & mirror)	*16 (<8wks) *11 (8-22 wks)	pheno- barbitone (27/27)	1.5	1.7*1.7*5 (5 slices)	50 3000	90	5:06	<8 wks pos. >8 wks neg.
Altman & Bernal 2001 (Altman and Bernal, 2001)	Visual (31) Auditory (38) (language)	n/a (Visual: LED goggles, Grass Instruments Co.) (Auditory: generic headphones)	38 (2) (2mths-9 yrs)	chloral hydrate (10/38) pheno- barbitone (28/38) propofol (1/38) alprazolam (2/38)	1.5	3.75*3.75*n/a	60 3750	60	3:07	Visual: 1/31 pos., 28/31 neg., 2/31 none Auditory: 26/38 pos., 12/38 none
Born et al. 2002 (Born et al., 2002)	Visual	n/a (strobelight & mirror, Kunelco A/S)	4 (1 visual deficit) (4- 71 mths)	chloral hydrate (4/4)	1.5	1.8*1.8*4	66 2500	n/a	4:00	4/4 neg.
Konishi et al. 2002 (Konishi et al., 2002)	Visual	n/a (strobelight & mirror)	*16 (<8 wks) *11 (8-22 wks)	pheno- barbitone (27/27)	1.5	3.4*3.4*6-7	50 3000	90	2:06	Lateral Geniculate Nucleus (LGN): 27/27 pos. Primary visual cortex: <60 days

										pos., >60 days neg.
Muramoto et al. 2002 (Muramoto et al., 2002)	Visual	n/a (strobelight & mirror)	*10 (<8 wks) *10 (8-32 wks) Total:20 (22)	pheno- barbitone (20/20)	1.5	1.72*1.72 *6	50 3000	90	5:06	<8 wks pos. >8 wks neg.
Marcar et al. 2004 (Marcar et al., 2004)	Visual	n/a (projector & mirror)	11 (<5 yrs) 10 (5-12 yrs)	midazolam, sevoflurane anesthesia	2	2*4.1*5.5	58 2000	90	3:20	Pos. & neg. (proportion not documented)
Seghier et al. 2004 (Seghier et al., 2004)	Visual	n/a (n/a)	*1 (13 wks) *arterial stroke	chloral hydrate	1.5	1.9*1.9*4	40 2000	80	4:00 (repeated 3 times)	*Negative responses, and “inverse gamma” impulse response in event-related experiment *responses contralateral to stroke
Li et al. 2013 (Li et al., 2013)	Visual	Yes - Neurobehavioral Systems software (projector, screen & mirror)	*11 (1-11 yrs)	propofol (11/11)	1.5 3	3*3*5	50/30 2060	90	n/a	1/11 neg. 1/11 pos. & neg. 9/11 n/a
Lin et al. 2008 (Lin et al., 2008)	Resting state	-	38 (2-4 wks) 26 (1 yr) 21 (2 yrs)	None	3	4*4*4	32 2000	n/a	5:00	-
Gao et al. 2009 (Gao et al., 2009)	Resting state	-	20 (12-36 days) 24 (1 yr) 27 (2 yrs)	None	3	4*4*4	32 2000	n/a	5:00	-

Gao et al. 2011, 2013 (Gao et al., 2013, Gao et al., 2011)	Resting state	-	51 (11-35 days)	None	3	4*4*4	32	n/a	5:00	-
			50 (1 yr)				2000			
			46 (2 yrs)							

Abbreviations: PNA – postnatal age.

3.2.3 Task-dependent fMRI studies: visual stimulation

Materials and methods

A total of 16 visual fMRI studies have been reported in the literature, 3 of which were performed on the preterm and young ex-preterm population and 13 on young term-born infants only. Since first being introduced by Born and colleagues in 1996 (Born et al., 1996), the vast majority of subsequent studies have used a block stimulation paradigm in which periods of stroboscopic light flickering at 8Hz were interspersed with periods of darkness. Having initially set the flickering frequency to 8Hz as this rate had previously been shown to induce the largest CBF response in adult subjects (Fox and Raichle, 1986, Hagenbeek and Van Strien, 2002, Vafae and Gjedde, 2000), in their second study, Born and colleagues (1998) found that lowering the flickering frequency from 8Hz to 1Hz produced a variable, but marked effect on the extent of cerebral activation observed in newborns (Born et al., 1996). A further source of neural response variability may be the reduced ability of the immature visual system to process higher temporal frequencies (Marcar et al., 2004) with premature infants also found to exhibit more robust patterns of visual evoked potentials at lower stimulation frequencies (Pike et al., 1999). In an attempt to maximize response, 3 of the 16 studies therefore used lower stimulation frequencies, ranging between 2 and 4Hz (Seghier et al., 2004, Marcar et al., 2004, Erberich et al., 2003b), and 1 study performed stimulations at both 1Hz and 8Hz (Lee et al., 2012).

Throughout the majority of studies the equipment used to deliver visual stimulation was similar to that first described by Yamada in 1997 and Born and colleagues in 1998 (Yamada et al., 1997, Born et al., 1998); a photic stimulator was placed outside the scanner and the light reflected towards the patient's eyes via a mirror accurately positioned within the scanner coil. Stroboscopic lamps were by far the most common light source, except in the work of Marcar and colleagues (Marcar et al., 2004) where a projector was used to display image patterns. The alternative approach of delivering photic stimulation directly to the subjects via LED goggles was also attempted by Born and colleagues (Born et al., 1998), Martin and colleagues (Martin et al., 1999, Martin et al., 2000) and Altman and Bernal (Altman and Bernal, 2001); this method however proved to be particularly cumbersome, especially amongst younger populations (Born et al., 1998).

Except for the study performed by Erberich and colleagues (Erberich et al., 2003b), in which gantry light flickering was manually performed at approximately 2 Hz, all other studies report precise timings for both flickering frequency and stimulation paradigm epochs. While a constant flickering rate can be reliably attained with stroboscopic lights and other dedicated hardware, most studies fail to describe the method with which the timings of paradigms are robustly reiterated, and it is unclear whether photic stimulations are TR locked. This is perhaps surprising since the systematic and repeatable presentation of stimuli is fundamental to task-dependent fMRI experiments, in which a precise characterization of stimulation parameters and timings is required to allow inter-subject and intra-subject statistical inference. Only the recent study by Li and colleagues (Li et al., 2013) explicitly reports the stimulation paradigm to be synchronized with image acquisitions. This was done by TR synchronous triggering of the paradigm implemented via commercial stimulus presentation software (Neurobehavioral Systems, <http://www.neurobs.com>), using the transistor-to-transistor-logic (TTL) pulses produced by the scanner with each TR.

In addition to within-study and across-study inconsistencies regarding stimulation paradigms, the results produced by visual fMRI studies are also likely to have been limited by several confounding effects inherent to the heterogeneity of subjects included in a single study population (Arichi, 2012). Infants have often been grouped together across a broad age range, with some studies even including newborns and infants of up to 3 years of age in a single population (Born et al., 1998, Born et al., 1996); age is however an important confound, as maturational changes are known to affect neural response, especially in young populations (Arichi, 2012). Another potential source of bias is the wide variety of clinical subjects included in single groups (Arichi, 2012), with some studies which have grouped healthy term-born infants with infants born preterm, or even with infants suffering from a variety of severe clinical conditions including cerebral damage (Born et al., 2000, Born et al., 2002, Born et al., 1998, Born et al., 1996, Yamada et al., 1997, Morita et al., 2000, Yamada et al., 2000, Altman and Bernal, 2001, Konishi et al., 2002, Muramoto et al., 2002). Comparing results obtained by different research groups is further complicated by the inconsistent use of anaesthetic agents (Arichi, 2012), with the majority of studies utilising either chloral hydrate (Born et al., 2000, Born et al., 2002, Born et al., 1998, Born et al., 1996, Seghier et al., 2004) or intravenous

phenobarbitone (Yamada et al., 1997, Yamada et al., 2000, Morita et al., 2000, Konishi et al., 2002, Muramoto et al., 2002), while only a single study was fully performed on un-sedated infants (Lee et al., 2012).

Results

Visual fMRI studies have reported highly variable results, with contrasting findings concerning both the site and amplitude of neural responses. While in adult subjects, visual stimulation has been shown to elicit a positive BOLD response at the level of the visual cortex and along the entire calcarine fissure (Arichi, 2012), in their first study Born and colleagues reported a task-dependent decrease in BOLD contrast restricted to the anterolateral region of the calcarine fissure in all 6 studied infant subjects (Born et al., 1996). Identically framed studies performed by the same authors reported similar findings across populations of term-born children and amongst subjects with visual deficits (Born et al., 2000, Born et al., 2002, Born et al., 1998), while the sign of BOLD response was much more variable in the preterm and ex-preterm population (Born et al., 2000). Born and colleagues suggested that negative BOLD responses may be characteristic of the early stages of brain development, when due to the enhanced synaptic density (peaking at 8 months of age in the human visual cortex), stimulation induces a great increase in metabolic demands that is not matched by an equivalent increase in regional CBF, thus leading to a greater proportion of deoxygenated blood in correspondence of areas of neural activity (Born et al., 2000, Born et al., 1998, Born et al., 1996). Perfusion MRI studies conducted by the same authors provided some evidence in support of the alternative hypothesis that negative BOLD may be induced by a counter intuitive decrease in CBF of the primary visual cortex due to infants' sedation (Born et al., 2002). While the potential effects of age/sedation on the nature of the response remain unclear, a negative BOLD response was interestingly also noted in adults during natural slow-wave sleep, suggesting the behavioural state of the subject should at least be closely monitored (Arichi, 2012, Graham et al., 2013).

In contrast with the findings of Born and colleagues, a number of concurrent studies consistently reported a positive BOLD response in correspondence of the lateral geniculate nucleus (LGN), as well as a BOLD response at the level of the calcarine fissure changing from positive to negative after

the 8th week of postnatal age (PNA) (Yamada et al., 2000, Yamada et al., 1997, Morita et al., 2000, Muramoto et al., 2002, Konishi et al., 2002). In keeping with one of Born's first hypotheses, some authors have suggested that the change in response observed at 8 weeks PNA exclusively along the calcarine fissure, may be explained by a concurrent increase in synaptic density (and thus in metabolism) limited to the primary visual areas (Yamada et al., 2000, Morita et al., 2000).

An important study was also conducted by Seghier and colleagues in 2004, in which a sedated 13 week infant with a perinatal arterial stroke of the left hemisphere exhibited no BOLD on the affected side, in which the optic radiation was also not found via DTI, while a negative BOLD response was observed in the intact hemisphere (Seghier et al., 2004). By subsequently performing an event-related experiment, the authors were also able to precisely characterise the subject-specific HRF. The response was described as an inverse gamma function, corresponding to an inverted mirror image of the established canonical adult response, with a time to (negative) peak of approximately 6 s (Seghier et al., 2004).

Lee and colleagues recently reported a visual fMRI study performed on a large cohort of preterm and term-equivalent (ex-preterm) infants (Lee et al., 2012). Positive only BOLD responses were identified in merely 4 of the 65 preterm subjects and 17 of the 23 term-equivalent subjects, with no activity detected in all other subjects (Lee et al., 2012). Since visually evoked potentials have been reliably identified in infants starting from 24 weeks PMA, and newborns generally exhibit behavioural signs that indicate visual attention, the authors suggested that their findings may be due to fMRI not being able to capture visual function in preterm infants (Lee et al., 2012).

While it is clear that methodological differences may underlie the heterogeneity of visual fMRI results, these may also arise from genuine differences amongst subjects that have been sampled at various stages of visual development (Johnson, 1990, Arichi, 2012). In fact it has been posited that processing of visual information in the newborn may initially occur at a subcortical and extra-geniculo-calcarine level, with visual areas progressively developing over the course of the first 2 to 3 months of PNA (Johnson, 1990).

3.2.4 Task-dependent fMRI studies: auditory stimulation

Materials and methods

A total of 7 auditory fMRI studies have been reported in the literature, with only a single study performed on the preterm and young ex-preterm population by Anderson and colleagues in 2001 (Anderson et al., 2001). All studies have consistently presented subjects with a type of auditory stimulus, broadly subcategorised into music, speech and tone, via a block experimental paradigm. A tone stimulus was only adopted in the work reported by Anderson and colleagues, in which the frequency and amplitude of the tone were tuned to 60–80 dB and 0.3–2.3kHz respectively to match the infants' optimal sensitivity range (Anderson et al., 2001, Ruben, 1995). Including the first auditory fMRI study conducted by Altman & Bernal in 2001 where infants were presented with epochs of normal mother speech vocalizations (Altman and Bernal, 2001), a total of 5 studies have investigated and contrasted the responses of term-born infant subjects to various types of auditory stimulations including: normal and nonsensical speech vocalizations (Dehaene-Lambertz et al., 2002); non-speech vocalizations and non-voice sounds (Blasi et al., 2011b); speech vocalization in native language with varying emotional intonation (Graham et al., 2013); normal, hummed and flattened speech in native language (Perani et al., 2011b). A single study further aimed to characterize infants' neural responses to classical and altered music stimulations (Perani et al., 2010). Auditory stimuli were all recorded and digitized; when required, audio files were pre-processed via dedicated software packages (i.e. for speech flattening and humming (Perani et al., 2011b)), before being presented to infants throughout experimental paradigms via commercial MR compatible piezo-electric (i.e. MR Confon; <http://www.mr-confon.de> (Perani et al., 2010, Perani et al., 2011b, Blasi et al., 2011b)) or pneumatic (i.e. Bilsom (Graham et al., 2013)) headphones. In one occasion, the authors reported using generic headphones, connected to shielded speakers located within the scanner room via insulating tubes (Altman and Bernal, 2001). Only Perani and colleagues explicitly reported the presentation software and hardware used to design and TR lock stimulation paradigms, both supplied by MR Confon together with the MR compatible headphones (Perani et al., 2010). However in a later study, although utilising the same headphones, the authors failed to report the (presumably identical) approach to stimulus design and synchronization (Perani et al., 2011b).

Results

Nearly all studied subjects exhibited positive BOLD responses to auditory stimulations, predominantly at the level of the primary auditory cortex, (Altman and Bernal, 2001, Anderson et al., 2001, Dehaene-Lambertz et al., 2002, Perani et al., 2010, Perani et al., 2011b, Blasi et al., 2011b, Graham et al., 2013). Interestingly, statistical comparisons of patterns of neural activation revealed that infants' responses are sensitive to subtle differences between auditory stimuli, such as the emotion conveyed by both pitch and intonation of voice (Perani et al., 2011b, Graham et al., 2013), the slight variations of musical compositions (Perani et al., 2010, Perani et al., 2011b), and the vocal nature of (non-speech) sounds (Blasi et al., 2011b).

In 2001 Altman and Bernal conducted an important study in which concurrent visual and auditory stimulation in a population of 38 infants aged between 2 and 9 months allowed for a direct comparison of responses (Altman and Bernal, 2001). In agreement with previous findings, strobe light stimulation was associated with prevalently negative BOLD response, while exclusively positive BOLD contrast was observed in response to an auditory stimulus consisting of mother speech vocalizations. Overall, these findings together with those reported by other auditory fMRI studies suggest that even during the early stages of life, the neonatal auditory system is already capable of finely differentiating stimuli, and that maturation of human sensory systems may follow markedly different developmental trajectories, with the auditory system seemingly maturing before the visual system (Arichi, 2012).

3.2.5 Task-dependent fMRI studies: somatosensory stimulation

A total of 3 studies have reportedly investigated fMRI responses to somatosensory stimulation in (term-born control) preterm and ex-preterm subjects, in all instances subjects were sedated via chloral hydrate administration (Erberich et al., 2006, Erberich et al., 2003a, Heep et al., 2009). The first study conducted by Erberich and colleagues in 2003 on a population of 1 preterm and 5 ex-preterm infant subjects (34-58 wks PMA), all suffering from intracerebral pathology (Erberich et al., 2003a). The experiment consisted of a block paradigm where somatosensory stimulation was delivered for approximately 30 seconds via periodical inflations of a rubber bulb placed within the

infant's hand. The bulb was manually actuated by the experimenter to open/close the hand at an approximate frequency of 1 Hz. Both the left and right hand were separately and subsequently stimulated in 4 out of the 6 infant subjects, while only the right hand was actuated in remaining 2 subjects. Although both positive and negative BOLD responses in the hemisphere contralateral to stimulation were identified in all 6 infants, activation at the level of the contralateral adult primary motor and sensory areas was only found in 3 infants, 1 of whom (37 wks PMA) exhibited bilateral positive response. Activation was instead predominantly localized in correspondence of what has been suggested to be the early supplementary motor area (SMA). Interestingly, the single subject studied at preterm age (34 wks PMA) also exhibited brainstem activation following stimulation of both hands.

In 2006 Erberich and colleagues successfully extended their experimental paradigm to a larger study population, which included 24 ex-preterm subjects with mild cerebral abnormalities imaged at around term equivalent age (38-49 wks), and a further 6 term-born control subjects imaged at 3-9 months PNA (Erberich et al., 2006). Statistical analysis was performed by fitting the data with a standard GLM in which stimulation blocks were convolved with the canonical adult HRF. In the control cohort, all responses were found to be positive and contralateral to the side of stimulation, with no negative BOLD response identified. Within the 24 neonatal subjects however both positive activation and negative deactivation patterns were observed at the level of the sensorimotor cortex, with a clear preponderance towards exclusively contralateral activation (over 50%), while between 36.4 and 43.8% of subjects exhibited bilateral responses, and ipsilateral responses were only identified in 2 infants (Erberich et al., 2006). These findings highlighted a potential age dependence of the magnitude and “lateralization” of somatosensory responses, and indicated that fMRI may be a suitable technique to characterize longitudinal patterns of early functional development.

Heep and colleagues performed a further somatosensory fMRI study in 2009 on a cohort of 5 (8 total, 3 excluded) ex-preterm infants imaged at term equivalent age (38-39 wks), all diagnosed with intraventricular haemorrhage (Heep et al., 2009). The experimenters implemented a block paradigm in which somatosensory stimulation was performed via passive unilateral arm flexion/extension in epochs of approximately 20s. The movement was manually induced by a nearby physician instructed

to pull on wristbands (Infant limb holder; J.T. Posey Company, Arcadia, CA) attached to the infants' wrists at a frequency of circa 1Hz. Contrarily to what has been reported by Erberich and colleagues, the authors reported exclusively bilateral patterns of functional response, with bilateral deactivation observed in 4 out of the 5 subjects included, and bilateral activation observed in the remaining neonate (Heep et al., 2009). A later neurologic evaluation of the infants' psychomotor development at the age of 4 to 6 revealed that only the subject who exhibited positive BOLD response suffered from mild motor delays, leading the authors to conclude that sensori-motor activation at term equivalent age may represent important biomarkers of later clinical outcome (Heep et al., 2009).

3.2.6 Resting-state fMRI studies

While task-dependent fMRI directly tests the reflexive nature of brain activity arising from transient environmental interactions or tasks, resting-state fMRI is based on the assumption that brain activity is predominantly intrinsic, and characterized by a constant processing, consolidation and elaboration of external and endogenous information (Tusor et al., 2014, Arichi, 2012). This gives rise to continuous low-frequency BOLD signal fluctuations that are coherent across networks of cerebral regions sharing the same function (Tusor et al., 2014, Llinás and Ribary, 2001, Brown, 1914, Biswal et al., 1995, Buckner and Vincent, 2007). Based on this theory, resting-state fMRI studies have utilized several analytical approaches, including seed-based mapping and blind source separation (i.e. ICA) (reviewed in (Lee et al., 2013)), to analyze patterns of temporal correlation found within fMRI datasets acquired with the subject simply resting in the scanner (Biswal et al., 1995, Arichi, 2012), and have successfully inferred spatially independent functional resting state networks (RSNs). While the exact role of high spontaneous fluctuations in the resting brain remains unclear, with theories suggesting these to be indicative of rehearsal, learning, consolidation, or future preparation (Smith et al., 2009, Buckner and Vincent, 2007), repertoires of 10 to 20 RSNs have reliably and consistently been reported in adult resting-state fMRI studies. These have been shown to spatially replicate patterns of cerebral activation exhibited in response to a range of functional tasks (Smith et al., 2009).

While task-dependent fMRI protocols allow for a direct probing of the functional network of interest in a controlled manner, resting-state fMRI features some intrinsically attractive properties that render it more easily amenable to a naturally uncooperative population such as that of newborn infants. In addition to its ability to segregate multiple functional networks following a single scanning session, resting-state fMRI does not require using stimulation protocols and is free from the restrictions imposed by traditional GLM analysis (Smyser et al., 2011). For these reasons, in recent years a growing number of resting-state fMRI studies have been conducted on young infants (Fransson et al., 2007b, Fransson et al., 2009b, Fransson et al., 2011, Lin et al., 2008, Gao et al., 2011, Gao et al., 2013, Gao et al., 2009, Doria et al., 2010, Smyser et al., 2010). Utilising both seed-based temporal correlation mapping and ICA, these have revealed that facsimiles of the adult resting state networks are already apparent at term age, with coherent and independent patterns of neural activities found at the level of the visual, somatosensory, motor, auditory, cerebellar and premotor areas (Fransson et al., 2009a, Fransson et al., 2007a, Doria et al., 2010, Smyser et al., 2010, Arichi, 2012). This apparent early establishment of large scale neural organisation (Fransson et al., 2009a, Fransson et al., 2007a) prompted further studies aimed at characterizing the longitudinal development of resting state networks that seemingly begins long before term. In 2010 two separate research groups (Doria et al., 2010, Smyser et al., 2010) observed a clear maturational trend across RSNs throughout the preterm period; these were found to appear scattered and fragmented at 30 weeks PMA, to then gradually develop an adult-like complex, long-range and bilateral morphology observed at around term equivalent age (Doria et al., 2010, Smyser et al., 2010). Interestingly, these findings support and further expand the notion that the third trimester of gestation represents a crucial juncture for human cerebral maturation, during which the rapid neural growth and synaptogenesis previously reported by ex-vivo histological studies (Kostovic and Jovanov-Milosevic, 2006) is ultimately paralleled by an equally dramatic development of functional connectivity (Arichi, 2012). RSNs do not however appear to mature synchronously, with the visual and auditory networks exhibiting an adult-like morphology before others. The somatosensory and motor RSNs are instead observed to follow a clear maturation pattern, as they progress from an initially unilateral to an increasingly bilateral configuration (Doria et al., 2010). It is curious to note

that, although these findings may partly explain the relatively consistent results obtained via auditory fMRI, they also further question the inconsistencies observed across fMRI studies using visual tasks.

As a final remark, as described in Chapter 1, it is accepted that both spontaneous and sensory induced brain activity are necessary to the early establishment of cerebral connectivity, and that both coexist in the subplate to possibly aid guiding thalamic projections towards their cortical target areas (Volpe, 2008, Doria et al., 2010, Arichi, 2012). Crucially, the subplate peaks at 33 wks PMA, which roughly corresponds to the early preterm period, and progressively regresses as intercortical and intracortical circuitry is established, and synaptogenesis dominates (Doria et al., 2010). With this in mind, Doria and colleagues observed that the visual, auditory, and motor networks progressively grow to incorporate portions of the thalamus throughout the preterm period; and therefore suggested that the maturing spatial distribution of RSNs may arise directly from the progressive establishment of the underlying thalamo-cortical connectivity (Doria et al., 2010).

3.3 fMRI of the newborn: challenges and considerations

3.3.1 Practical considerations

Successfully performing fMRI acquisitions in newborn infants is an inherently challenging process, which requires carefully implementing a series of practical steps to ensure the subjects' safety and immobility, while avoiding distress (Arichi, 2012). Careful implementation of physiological monitoring is required throughout acquisitions to prompt intervention in case of oxygen desaturation and bradycardia; and hearing protection is necessary to attenuate the high levels of noise produced by the scanner, especially during fast EPI sequences (Merchant et al., 2009, Arichi, 2012, Pennock, 2001). Minimizing head motion is also paramount in ensuring fMRI data quality; while larger movements can corrupt entire datasets, even smaller movements, particularly if in correspondence of epochs of stimulation, can introduce significant biasing of the data and potentially lead to false discovery of activation patterns (Hajnal et al., 1994, Power et al., 2012, Satterthwaite et al., 2012, Van Dijk et al., 2012b, Arichi, 2012). This issue is particularly relevant amongst preterm subjects due to their tendency to become distressed within the MRI environment and to their uncooperative nature (Arichi, 2012). Gross head motion is commonly restricted via foam padding or vacuum-evacuated bead-filled bags appropriately positioned within the head coil (Merchant et al., 2009, Arichi, 2012, Pennock, 2001). To further minimize motion and avoid distress, infants are normally imaged while in a state of sleep, which is naturally ("feed and wrap" technique) or pharmacologically induced (sedation) (Edwards and Arthurs, 2011, Arichi, 2012). It is however important to note that while a previous systematic review of the literature has demonstrated that infant sedation allows achieving consistently good MR image quality, there is no such evidence to support that the feed and wrap technique can produce equally robust results (Edwards and Arthurs, 2011, Arichi, 2012).

A potential downfall undermining the use of anaesthetic agents is their potential to affect the observed functional activity, with authors speculating that sedation may alter neural activity, neurovascular coupling, and baseline vascular parameters (Gaillard et al., 2001, Seghier et al., 2006, Harris et al., 2011, Arichi, 2012).

3.3.2 Study population

As the review of the literature indicates, the majority of task-dependent fMRI studies of the newborn brain have been conducted on small populations, with subjects grouped together regardless of their age or clinical status. This is partly due to limitations in the recruitment and experimental methods imposed by stringent ethical regulations (Arichi, 2012). However, age and pathology are both likely to profoundly affect the spatio-temporal characteristics and magnitude of the observed functional responses, with this high degree of variance markedly reducing the sensitivity of later group analysis (Arichi, 2012). These limitations are especially true for developmental studies, where multiple large samples of developmentally and clinically equivalent subjects need to be recruited if statistically significant results are to be obtained.

Interestingly, positive BOLD responses were identified in all of the (few) studies performed on large cohorts of healthy term-born infants (Dehaene-Lambertz et al., 2002, Perani et al., 2011b, Perani et al., 2010, Blasi et al., 2011a, Arichi, 2012), strengthening the notion that at least some of the previously reported heterogeneity of responses may have resulted from the inappropriate selection of study populations.

3.3.3 Stimulation paradigms

The robust implementation of an appropriate stimulation paradigm is a fundamental premise of all task-dependent fMRI studies, and represents one of the most challenging undertakings in transposing this technique to the newborn population. Since, ideally, infants should be asleep throughout scanning, an important requirement is for stimuli to be delivered in a manner that is sufficiently gentle not to unrest the subjects, but forceful enough to elicit a functional response (Arichi, 2012). For this to be achieved it is also crucial that the frequency and magnitude of stimulation are accurately gauged, ensuring that the stimulus can be easily processed by the maturing brain. It is also paramount that the stimulus is synchronised to image acquisitions and delivered in a robust manner, allowing for stimulation patterns to be highly repeatable both within and between

scanning sessions. Such fully automated approach also requires designing and developing custom stimulation systems and interfaces that adhere to strict safety standards, a process which is likely to require non-standard engineering solutions. While interfaces used in adult studies often feature non-ferromagnetic metals and ferromagnetic components are generally shielded and placed in the proximity of the scanner bore (Gassert et al., 2008, Gassert et al., 2006b, Tsekos et al., 2007), infant studies require a different approach since no metallic component can be placed within the MR scanner room.

3.3.4 Data Analysis

A number of fMRI data analysis packages are currently available, and feature standardized analytical pipelines optimized to assess functional responses in adult subjects. Crucially however, these pipelines need to be carefully re-evaluated before they can be transposed to the neonatal population (Arichi, 2012). For instance, the optimal values of several pre-processing parameters, including the size of the spatial and temporal smoothing filters, are still unknown for neonates, and are likely to differ greatly from those utilized in adult studies (Arichi, 2012).

A further important consideration is that statistical inference of activation is commonly performed by fitting fMRI data to a GLM, in which the expected BOLD response is modelled as the convolution between the stimulation paradigm and the canonical HRF (Friston et al., 1995b, Friston et al., 1994b). While the GLM approach allows generating statistical parametric brain maps by means of relatively simple univariate regressions, its reliability is highly dependent on the precision with which the time course of the stimulation paradigm can be measured (hence the importance of the experimental instrumentation), and on the accuracy with which the HRF approximates the actual time course of neural responses (Bandettini et al., 1992, Friston et al., 1995b, Friston et al., 1994b, Monti, 2011, Poline and Brett, 2012, Arichi, 2012). Adult fMRI studies have shown that even slight alterations of some key features of the “canonical” adult HRF, such smoothness and time to peak, can markedly affect statistical results (Aguirre et al., 1998b, Handwerker et al., 2004, Arichi, 2012), further emphasizing the importance of using an accurate HRF model in the GLM. With this in mind, although stark differences in neurovascular physiology (reviewed in (Volpe, 2008)) and animal

studies clearly indicate that the adult HRF is likely to differ significantly from that of infant subjects (Arichi, 2012), this uncertainty was not accounted for by the vast majority of neonatal task-dependent fMRI studies (Arichi, 2012). These have either implemented a canonical adult HRF (Dehaene-Lambertz et al., 2002, Erberich et al., 2003b, Erberich et al., 2006, Perani et al., 2011a, Perani et al., 2010), or have deviated from the classical GLM approach and used simple image subtraction instead (Born et al., 1996, Lee et al., 2012, Arichi, 2012). This seemingly critical methodological issue can be attributed to the fact that, previous to the work conducted by our group (Arichi, 2012, Arichi et al., 2012, Arichi et al., 2010), the infant-specific HRF morphology had not been systematically characterized, with only Seghier and colleagues attempting a preliminary description in 2004 (Seghier et al., 2004, Arichi, 2012).

Within and between-session fMRI data analyses require signal time courses to be extracted from spatially equivalent voxels. This is normally achieved by co-registering all image volumes to a standard template via multidimensional spatial transformations, such that homologous anatomical regions are superimposed (Jenkinson et al., 2002, Arichi, 2012). In the neonatal brain, co-registration is rendered markedly harder by several factors which include the larger variability of tissue contrast properties, the reduced amount of anatomical landmarks and the lower spatial resolution at which these are imaged (Aljabar et al., 2008, Aljabar et al., 2011, Ball et al., 2010, Arichi, 2012). Moreover, the dramatic maturation that the brain undergoes throughout the preterm and term period render difficult co-registering all images to a single template, with multiple age-specific standard templates likely to be required instead (Kuklisova-Murgasova et al., 2011, Serag et al., 2012, Arichi, 2012).

3.4 Task-dependent vs. resting-state fMRI

As previously mentioned, resting state fMRI is quickly gaining popularity in both the clinical and research fields due to some inherent advantages that this technique offers compared to standard task-dependent fMRI (Biswal et al., 2010). A key feature of resting state fMRI is its ability to delineate multiple RSNs following a single, relatively short, scanning session, without requiring stimulation to be induced or tasks to be performed. This has allowed determining large scale functional

connectivity in clinically unwell populations that are unable to perform specific tasks, while also eliminating the potential variance in the response introduced by task performance differences within and between trials (Cole et al., 2010, Biswal et al., 2010). Task-independence further suggests that RSN activity may also be a good measure of “baseline” brain function as it is not “contaminated” by task induced BOLD changes (Cole et al., 2010). These statistical and physiological considerations have led researchers to advocate the use of resting-state fMRI to characterize longitudinal brain development, and signatures of pathology. Recent studies have indeed begun to provide preliminary evidence that resting-state fMRI can be used to identify distorted sensorimotor areas in tumor and epileptic patients, and, pending further validation, potentially replace task fMRI in future presurgical evaluation of patients unable to initiate tasks (reviewed in (Lee et al., 2013)). Alterations in specific RSNs have also been found to be often associated with neurologic and psychiatric diseases; a fascinating hypothesis currently being investigated is that such resting state connectivity abnormalities are the result of a progressive degeneration, which can be detected early to provide diagnostic and prognostic clinical information (reviewed in (Lee et al., 2013)). It is however generally accepted that, before clinical implementation, rs-fMRI will at least require large proof of concept studies with significant statistical power (Biswal et al., 2010).

Despite these early promises, and undoubted methodological advantages, resting-state fMRI is presented with some fundamental pitfalls that suggest caution in interpreting the results; it is therefore unsurprising that this technique has so far primarily been utilized to complement and further validate the findings obtained from task-fMRI. Perhaps the primary concern relative to resting-state fMRI is derived from the evasive nature of the spontaneous BOLD low frequency (0.01–0.08 Hz) signal fluctuations used to localize RSNs (Cole et al., 2010). It is tempting to assume that these fluctuations reflect intrinsic metabolic activity arising from synchronous firing of neuronal populations that share the same function, and which are therefore subjected to strong synaptic co-interactions (intrinsic functional connectivity) presumably developed through plasticity (Saini et al., 2004, Lewis et al., 2009). However, as previously mentioned, despite a number of proposed theoretical explanations (including functional consolidation, preparation, re-elaboration), there is currently no agreement on the underlying causes of such low frequency neural firing activity

(reviewed in (Fox and Raichle, 2007)). This has led some authors to even propose that fluctuations may not be reflective of neural activity, but merely be due to time varying physiological changes linked to local cytoarchitecture and/or vasculature, which are not indicative of neural function (Beckmann et al., 2005). A recent study by Smith and colleagues has found that, in 36 healthy adult subjects, RSNs seemed, however, to spatially replicate patterns of functional connectivity identified via task-dependent experiments (Smith et al., 2009). While further studies have also revealed this qualitative activity approximation (Fox and Raichle, 2007, Fair et al., 2007, Greicius et al., 2003), recent findings in both healthy (Buckner et al., 2013, Hermundstad et al., 2013, Mennes et al., 2013) and pathological (Saleh et al., 2012, Rosazza et al., 2013) subjects have highlighted that RSNs and task dependent activation provide qualitatively different mappings of activity, suggesting rs-fMRI findings should be interpreted with care (Saleh et al., 2012, Rosazza et al., 2013) .

Another major challenge inherent to rs-fMRI lies in the limitations imposed by the statistical approaches used for RSNs parcellation (reviewed in (Cole et al., 2010)). In particular, seed-based correlation analysis techniques require an a-priori selection of an anatomical region thought to belong to the network of interest (Biswal et al., 1995, Greicius et al., 2003, Fox et al., 2005). ICA-based RSN segregation, instead, requires the number of spatially independent components to be selected arbitrarily, thus frequently over- or underestimating the level of depth at which networks are segmented (McKeown et al., 1998, Kiviniemi et al., 2003, Beckmann et al., 2005). This implies that assumptions need to be made concerning both the localization and the extent of functional responses, and these are often based on task-dependent fMRI experiments. It is therefore evident that using an rs-fMRI approach alone is particularly unfavourable in cases in which non-standard patterns of functional connectivity are expected. This is especially true in a highly dynamic and vulnerable population such as that of preterm babies where the spatial localization of response and their longitudinal development remain vastly ambiguous and unexplored.

In general, despite its promises and the potential ability to map large scale functional connectivity at rest, rs-fMRI has so far been hampered by major physiological and methodological concerns that will need to be resolved before findings can be appropriately interpreted and transposed to the clinical field. Despite potential sources of inconsistencies due to random stimulus-

dependent variance, the higher amplitude BOLD responses produced by task-dependent fMRI are attributable to a thoroughly documented circumstantial increase in neural metabolism, and are still widely considered as a more robust and reliable indicator of functional connectivity in both research and clinical fields (Rosazza et al., 2013).

Although conclusions could not be drawn regarding the ability of individual networks to process information, rs-fMRI has previously been used to characterize the spatial maturation of RSNs in the preterm population (Doria et al., 2010, Smyser et al., 2010). In the work of this thesis I will aim to complement these findings by performing a longitudinal characterization specific to the somatosensory network via task-dependent fMRI. It is hoped that a direct probing of “active” somatosensory functional connectivity, could provide more detailed, and physiologically significant information relative to the preterm brain’s ability to process a specific sensory input. As previously discussed, despite a great potential for neural plasticity, the preterm population is often specifically affected by motor deficits. This renders longitudinal characterization of somatosensory function particularly important, as identifying potential deviations from the standard pattern of functional somatosensory development could provide predictive and evaluative biomarkers of neuro-motor disorders.

It is also important to note however, that since underlying cerebral function is clearly an intricate circuitry of highly interconnected connectivity, it is highly plausible that appreciating specific pathological signatures will require gaining an insight on the overall, as well as task specific functional status of the brain. Moreover, in keeping with neurodevelopmental physiology, appropriate establishment of functional and structural connectivity requires both sensory-evoked and spontaneous cerebral activation. With this in mind, a future research direction therefore involves adopting an increasingly integrated approach; whereby dynamic neuro-evaluation could be performed via using rs-fMRI to assess large scale spontaneous RSNs, and utilizing task-dependent fMRI to probe network specific elicited responses. To further advocate this holistic approach, although they generally provide complementary information, RSNs and task dependent activations have also exhibited strong longitudinal correlation, with sensory elicited activity thought to shape RSNs, although the converse causative process has also been proposed. Furthermore, it has been

suggested that, even during task paradigms, the BOLD response results from the combination of both task-induced and resting state activity (Fox et al., 2005). In task-dependent fMRI, low frequency fluctuations are currently treated as noise; it has however been suggested that these signal changes could be explained by introducing the relevant RSN time course into the model, thus dramatically enhancing experimental SNR (Cole et al., 2010).

3.5 A novel approach to task-dependent fMRI of the newborn

The numerous methodological limitations reported in this section highlighted the necessity to develop a novel approach before longitudinal task-dependent fMRI studies of the preterm population could be performed. Before the work of this thesis was initiated, our group had begun to develop a preliminary systematic and robust method to study the somatosensory neural correlates in newborn subjects, which was adopted to characterize the neonatal HRF.

3.5.1 Stimulation system

The somatosensory stimulus interface was inspired by the work of Erberich and colleagues (Erberich et al., 2003b), and consisted of an inflatable balloon tailor made from a layer of nylon mesh enclosed between 2 layers of latex, and appropriately sized to fit within the infant's hand. Somatosensory stimulation was delivered in a fully automated manner; the balloon inflation/deflation was pneumatically driven at a constant amplitude and frequency to produce a robust pattern of passive extension/flexion of the fingers (Arichi, 2012, Arichi et al., 2010). All materials utilized within the scanner room were completely MR safe, with the balloon connected to a control box located outside of the scanner room by means of 7 m long plastic pneumatic tubing (Arichi, 2012, Arichi et al., 2010). Within the control room, a standard PC running custom software (LabView; National Instruments Corp., Austin, TX, USA) was used to control a proportional valve (MPPEs series, Festo Corp., Essling am Neckar, DE), to deliver a constant pressure profile to the balloon (Arichi, 2012, Arichi et al., 2010). A data acquisition card (DAQ) (USB-6211; National Instruments Corp., Austin, TX, USA), was used to collect pressure data in real time, and to synchronize actuation with the transistor to transistor logic (TTL) pulses produced by the MRI scanner with each TR (Arichi, 2012, Arichi et al., 2010). This method was initially used in conjunction with a block stimulation paradigm and produced statistically robust and reproducible BOLD responses contralateral to the side of stimulation in a cohort of 11 preterm infants, 18 ex-preterm infants studied at term equivalent age, and 6 control term infants (excluding subjects discarded) (Arichi, 2012, Arichi et al., 2010). Interestingly, 8 (44%) of ex-preterm infants studied at term exhibited a bilateral pattern of functional activity, while a negative contralateral BOLD

response was only observed in 2 (preterm) out of the 35 infants. Since an age-appropriate HRF was not yet available, in this initial study fMRI data analysis was performed by the convoluting the binary time course of the stimulation paradigm with an optimal basis set representing a dispersion range of possible HRF waveforms (Arichi, 2012, Arichi et al., 2010, Woolrich et al., 2004).

3.5.2 Neonatal HRF

Arichi and colleagues hypothesized that, as suggested by studies of the rodent brain, the HRF may vary significantly throughout subsequent stages of human neural development (Arichi et al., 2012). The authors therefore implemented an approach similar to the one previously described (Arichi, 2012, Arichi et al., 2010), to characterize the preterm and term specific HRFs (Arichi et al., 2012). To allow directly extrapolating the time course of the HRF morphology from the sampled BOLD signal, an event-related experiment consisting of 1s stimuli interspersed with 40.5 s of rest was conducted in a population of 10 preterm infants, 12 ex-preterm infants studied at term and 3 control term infants (excluding subjects discarded) (Arichi et al., 2012). For all infants, a region of interest (ROI) was firstly determined, as the most significant cluster of functional activation at the level of the contralateral somatosensory cortex, revealed by a GLM using an optimal basis set to span a temporal range of possible canonical HRF waveforms (Arichi, 2012, Arichi et al., 2010). The time courses of BOLD percent-signal change were then computed for each subject at each ROI. The obtained BOLD responses were subsequently subdivided into 3 age groups, and each fitted with a double gamma distribution function to estimate the group-specific average HRFs (Arichi, 2012, Arichi et al., 2010).

Although exhibiting a lower peak amplitude ($\approx 0.5\%$ vs. $\approx 1.6\%$), the term HRF was found to closely approximate the morphology of the adult response. The preterm HRF was found to reach a peak amplitude similar to that of the term population, but at a significantly increased delay. In fact median time-to-peaks in the adult, term and preterm population were found to be of approximately 5, 7 and 11 s respectively. In general, the time-to peak parameter was found to decrease exponentially with post-menstrual age (Arichi, 2012, Arichi et al., 2010). The preterm subjects' datasets from the previous study were then re-analyzed by using both the standard adult and preterm HRF in the GLM.

Using an age-appropriate HRF led to a statistically significant improvement in the sensitivity with which functional activity is detected (Arichi, 2012, Arichi et al., 2010).

3.5.3 Current limitations and improvements required

The novel experimental apparatus and protocol developed by Arichi and colleagues allowed, for the first time, to robustly and systematically characterize somatosensory responses in the preterm human brain using task-dependent BOLD fMRI (Arichi, 2012, Arichi et al., 2010). The encouraging results obtained combining the automated stimulation hardware, and the newly identified preterm HRF, suggest that at least some of the inconsistencies reported by previous studies (Erberich et al., 2003b, Erberich et al., 2006, Heep et al., 2009) may have been caused by methodological issues; and that these were largely resolved by this novel approach (Arichi, 2012, Arichi et al., 2010). However, practical and theoretical considerations suggest that further improvements are required before this fully automated approach can be utilized to reliably characterize the longitudinal development of functional responses in the preterm population. Moreover, new interfaces are needed before the entire somatosensory system, as well as the complete preterm sensory homunculus can be explored in an equally robust and reliable manner.

Practical considerations

An important practical issue that the experimenters were presented with was the difficulty of retaining the balloon stimulator within the infant's hand throughout scanning. This problem was especially evident during periods of balloon inflation/deflation. A solution adopted by Arichi and colleagues was to wrap an elastic adhesive tape around the infants' hand. While this method successfully retained the balloon in place, it also represented a potential safety hazard, as the infant's hand was at risk of becoming excessively compressed during balloon inflation. Furthermore, the increased pressure may also give rise to an un-modelled experimental confound (see later). Unfortunately, the nature of the interface renders solving this issue non-trivial, and it was therefore left unresolved.

Another problem and potential hazard was the tendency of the latex within the custom-made balloon to degenerate and lose its elasticity after a few months. This resulted in a stark decrease in the amplitude of the inflation, and thus of the stimulation, caused by a given input pressure. Since the volume increase of the balloon could not be monitored directly (see later), this might have caused un-modelled between- and within-trial inconsistencies in the amplitude of stimulation. In addition, merely attempting to achieve higher inflations could have pushed the balloon beyond its linear region, causing it to inflate un-controllably and, potentially, to burst. This posed obvious health and safety concerns. Finally, the gradual degeneration of the balloon could have caused a concurrent and parallel decrease in amplitude of inflation throughout the entirety of experiments, clearly limiting the benefits of the automated approach.

Proprioception and mechanoreception

The somatosensory system can be sub-categorized into several sensory modalities, which include proprioception and mechanoreception. Understanding the dichotomy between these is fundamental importance when designing the task-dependent fMRI interfaces, especially since it is often difficult to separately elicit proprioceptive and mechanoreceptive responses. Physiologically, mechanoreceptors are subcutaneous tactile receptors, which can be broadly categorized into rapidly and slowly adapting mechanoreceptors, and usually exhibit a preferential response to a given type of stimulation, such as: oriented stretching, oriented pressure and superficial brushing (Blanchard et al., 2011). In contrast, proprioception is associated with muscular spindles, which encode information concerning the position and orientation of the joints (and body) in space; this feedback is crucial to sensing movement (Blanchard et al., 2011).

Most studies in the task-dependent fMRI literature simply report investigating somatosensory responses. However, though interlinked (Blanchard et al., 2011) and both expressed in the somatosensory cortical substrate (Germann and Stanfield, 2004), tactile and proprioceptive stimulations are associated with spatially distinct cortical maps (reviewed in (Moore et al., 2000)) which are also apparent in adult fMRI studies (Moore et al., 2000). Furthermore, contingent proprio-tactile stimulation has also been shown to elicit patterns of activation that cannot be predicted by

either modality alone, possibly due to underlying processes of multi-sensory integration (Kavounoudias et al., 2008).

In view of these considerations, it is clear that an ideal task-dependent fMRI stimulator should be able to discriminatively elicit tactile or proprioceptive activation. The balloon interface has been developed with the aim of assessing proprioception. It should however be noted that, in addition to inducing finger flexion/extension, inflation of the balloon also contingently stimulates 2 types of tactile mechanoreceptors, by the effects of skin stretching and hand compression.

Monitoring stimulation

As previously suggested, a clear limitation of the interface used by Arichi and colleagues was the inability to directly monitor the amplitude of stimulation. This was primarily due to the clear limitations imposed by MR safety to the development and implementation of appropriate sensing solutions. However, since the amplitude, as well as the frequency of stimulus may represent important experimental confounds, both clearly need to be closely monitored if a systematic task-dependent fMRI study is to be performed.

Chapter 4: Novel robotic tools to systematically characterize the somatotopic mapping of the preterm brain via task-fMRI

Overview - I designed, developed and implemented novel robotic tools to systematically study the intracerebral correlates of sensorimotor functional activity in the preterm brain using task-fMRI. In keeping with previous neuroimaging studies, and to ensure the consistency and specificity of stimulation, robotic interfaces were designed to elicit and monitor flexion/extension of the wrist and ankle joints. Following preliminary validation experiments, which have demonstrated the ability of the devices to identify the intracerebral correlates of both spontaneous and passively induced movements, the systems were used to investigate the emergence of the human body's somatosensory "homunculus", the precise spatial configuration of which had not been previously described during the preterm period. In addition to complementing our current understanding on the processes that drive early functional specialization of cerebral substrates, characterizing the preterm homunculus could be of significant clinical value as it could improve the specificity with which structural injuries can be related to future functional outcome.

Acknowledgment of contributions

The consenting, preparation and scanning of infant subjects was performed in collaboration with the research, nursing and neuroradiologist team of the Centre for the Developing Brain, King's College, St Thomas' Hospital London; formerly part of the Weston group, Imperial College, Queen Charlotte and Chelsea Hospital London.

Probabilistic tractography of high-angular resolution diffusion-weighted imaging (HARDI) data presented in Section 4.3.2 was performed by Dr. Harriet Cullen. Analysis of fMRI data sets was performed in collaboration with Dr. Tomoki Arichi. The robotic systems were developed and operated by the author.

This Chapter contains excerpts and figures from the following publications:

Allievi, A. G., Arichi, T., Gordon, A. L., & Burdet, E. (2014). Technology-aided assessment of sensori-motor function in early infancy. *Frontiers in Neurology*, 5. doi:10.3389/fneur.2014.00197

Allievi, A. G., Melendez-Calderon, A., Arichi, T., Edwards, A. D., & Burdet, E. (2013). An fMRI compatible wrist robotic interface to study brain development in neonates. *Annals of Biomedical Engineering*, 41(6), 1181–92. doi:10.1007/s10439-013-0782-x

4.1 Somatosensory mapping of the preterm brain

4.1.1 Study hypothesis and aims

It is hypothesized that during the third trimester of gestation, in addition to exhibiting large scale sensory networks (Doria et al., 2010, Smyser et al., 2010), the human brain undergoes a progressive topographic organization of the primary somatosensory and primary motor cortices, and that this highly specific spatial organization can be investigated via task-fMRI.

Therefore, I aim to develop a range of robotic tools which can control task conditions and be used in conjunction with fMRI, in order to investigate the sensory and motor somatotopic mapping of the newborn brain in a robust and systematic manner. These robotic tools need to fulfil stringent MR and clinical safety requirements, and be able to induce specific passive joint movements, as well as to monitor active movements throughout the experiments. Additionally, all the tools are developed to be based on a simple, flexible architecture in order to allow ease of operation, maintenance and replication. It is hopeful that this will allow researchers across a wide range of clinical centres to safely perform task-fMRI on the preterm population in a standardized manner.

This chapter will begin by describing the clinical and scientific importance of characterizing the emerging functional topography of the preterm brain and report on the potential to expand on the current knowledge via task-fMRI studies. I will then describe the stringent safety criteria which need to be met when designing and developing stimulator systems for fMRI studies of the newborn population. Finally, the developed wrist and ankle stimulators will be presented together with the novel findings that these have provided so far, including the emergence of a spatially well-defined somatotopic arrangement of the preterm somatosensory cortex.

4.1.2 The emergence of the somatotopic arrangement

Within the adult human nervous system, all somatosensory information is transduced at the peripheral level via multiple sensory receptors (as described in Chapter 3 Section 3.5.3), with different types of qualitative, quantitative and often spatial information then being conveyed to

higher brain centres cortex via ascending sensory pathways. Amongst these, the dorsal-column system transmits vibratory, fine tactile and proprioceptive sensory information to the somatosensory cortex (Purves et al., 2001) via a three-neuron communication stream. The first order neuron is located in a dorsal root ganglion, and its axonal projections extend directly from peripheral subcutaneous mechanoreceptors and muscle spindles, through the dorsal horn of the spinal cord, and up to the dorsal column nuclei of the medulla. Within the medulla primary neuron axons synapse with the cellular body of a second order neuron, the axonal projections of which immediately decussate contralaterally, before reaching the ventral posterior aspect of the thalamus (the lateral spinothalamic tract). A third order neuron then forms the final ipsilateral pathway which extends from the thalamus, through the internal capsule into the somatosensory cortex (the thalamocortical tract) (Purves et al., 2001). In its classical representation known as the “homunculus”, the adult somatosensory cortex is somatotopically divided into sub-regions which are dedicated to the processing of sensory information arising from specific body parts (Purves et al., 2001).

While an early primary sensory area has been observed to differentiate within the foetal cortical plate between gestation week 18 and 23 (Tawia, 1992), and cortical somatosensory evoked responses have been reported in preterm infants from gestation week 25 (Hrbek et al., 1973, Taylor et al., 1996, Vanhatalo and Lauronen, 2006) (discussed further in Chapter 6), the exact timing, mechanisms and dynamics underlying the early establishment of the cortical somatotopic arrangement into functional areas are largely unknown (Tawia, 1992, Rees et al., 2010). An hypothesis derived from animal models and human developmental physiology posits that somatotopic mapping of the somatosensory cortex arises throughout gestation and early postnatal life in 3 phases (Vanderhaeghen et al., 2000, Seelke et al., 2012, Krubitzer and Huffman, 2000): (i) a first phase during which activity-independent, likely genetic, factors lead to the formation of a first, rough aeralization of the cortex; (ii) a second phase in which the rough somatotopic map is remodelled through activity-dependent processes, with some thalamocortical and corticocortical projections strengthened (or weakened) via Hebbian plasticity; (iii) a third phase in which activity-dependent processes lead to a further refinement of the topographic map, by guiding the selective pruning of exuberant connectivity. Importantly, recent findings in rodents suggest that while adult-

like anatomical substrates of the primary somatosensory cortex are already apparent during the first few days of postnatal life, these are not associated with their mature functional specificity. Instead functional and structural congruence is progressively acquired at different rates by all regions over the course of the first 2 weeks postnatally (approximately equivalent to the late third trimester of human gestation), with anatomical and functional specificity of the barrel cortex becoming apparent before others (Seelke et al., 2012). The authors concluded that since the early establishment of functional maps cannot be directly inferred from anatomy, and cortical receptive fields mature at different times, characterizing the early establishment of somatotopic organization requires studying the development of functionality across the entire primary somatosensory cortex (Seelke et al., 2012). This notion can be extended to the primary motor cortex, where animal studies have demonstrated that early somatosensory input is crucial in the differentiation and maintenance of later motor representation (Chakrabarty and Martin, 2005).

A true understanding of the development of the somatotopic organization of the somatosensory cortex (and potentially of the motor cortex) during human preterm development is therefore likely to only be gained via techniques capable of directly probing the cortical representation of individual body parts with a high degree of spatial specificity. Providing new insights on the emergence, dynamics and potential plasticity of the functional somatotopic arrangement could add further clinical value to structural imaging techniques such as DTI and MRI. Functional abnormalities could be directly linked with structural injuries in order to highly enhance the specificity, sensitivity and precision with which neurodevelopmental outcome can be predicted, and also to guide and evaluate the effectiveness of therapeutic interventions.

4.1.3 Functional studies of the early somatotopic arrangement

Electrophysiological techniques have long been used to assess somatosensory responses to both upper and lower limb stimulation, suggesting that a broad somatotopic map may already be present during the first stages of preterm development. However, these findings have been constrained by the relatively poor spatial resolution offered by multichannel techniques, which prevent precisely mapping function to the underlying cerebral structures (e.g. (Karniski, 1992, Karniski et al., 1992)).

Electroencephalography (EEG) studies of the preterm population have revealed that somatosensory evoked potentials (SEPs) consistently emerge contralaterally to the side of stimulation. Stimuli have predominantly been presented via tactile or electrical cues at the level of the finger (Hrbek et al., 1973), median nerve (wrist) (e.g.(Tombini et al., 2009, Taylor et al., 1996)) or posterior tibial nerve (leg) (e.g (White and Cooke, 1994)), with resulting SEPs grossly localized within the lateral and medial contralateral somatosensory cortex. Magnetoencephalography (MEG) studies of term aged infants (Pihko and Lauronen, 2004, Nevalainen et al., 2008, Pihko et al., 2009) have reported that somatosensory evoked magnetic fields (SEFs) in response to tactile hand stimulation, firstly occur contralaterally within the lateral primary somatosensory cortex, and then propagate to the secondary and ipsilateral somatosensory cortices (Nevalainen et al., 2008). Multichannel Near Infrared Spectroscopy (NIRS) studies of preterm infants (24 to 38 wks PMA) have further revealed that passive knee and elbow movements induce oxy- and deoxy-Haemoglobin concentration changes approximately at the level of the contralateral (and less prominently, ipsilateral) somatosensory cortex (Kusaka et al., 2011). However potentially due to the limited spatial resolution inherent to this imaging modality, and to the proximity of the knee and elbow topographic representation, responses to the two types of stimuli appeared to largely overlap (Isobe et al., 2001, Kusaka et al., 2011). Finally, as discussed in Chapter 3, despite some inconsistencies, to date somatosensory task-fMRI studies of the preterm population have all utilized wrist or hand stimulation and identified well localized clusters of both contralateral and bilateral BOLD responses at the level of the lateral primary somatosensory cortices (Erberich et al., 2003b, Erberich et al., 2006, Heep et al., 2009, Arichi et al., 2010).

These findings have highlighted that fMRI could be used to provide a spatially accurate characterization of the early somatotopic arrangement of the somatosensory cortex. However, tools able to precisely and accurately elicit somatosensory stimulations need to be developed before longitudinal studies can be performed.

4.1.4 Design of MR safe robotic stimulators for newborns

In adults, several groups have used fMRI and robots to investigate the brain mechanisms related to active and passive movements, motor planning, and execution (reviewed in (Gassert et al., 2008, Gassert et al., 2006b)). However, the development of such fMRI compatible robotic interfaces for preterm infants requires further particular technical specifications and safety standards, and non-traditional mechatronics. The robotic system should be safe, appropriately sized, and able to elicit patterns of motor stimulation of controlled amplitude and frequency which are synchronized with MR image acquisition. Another important feature is the system's ability to monitor operation of the interface and provide experimenters with feedback on the safe, correct and consistent delivery of stimulation, as well as on potential spontaneous activity on behalf of the subject. Additionally, the movement elicited by the robotic interface should not cause head motion as fMRI is particularly sensitive to motion artefacts, leading to false positive activation and correlations (Van Dijk et al., 2012a, Power et al., 2012). The interface also needs to be suitable for a range of infants born at different gestational ages, and thus to allow for significant differences in size while remaining biomechanically safe. Hence it is important to choose an appropriate design, materials, and actuation mechanism, and to avoid fixed structures which could restrict the infant's natural movements and cause distress. Non-ferromagnetic metals deemed MR safe and often used to build or actuate adult robotic interfaces may yet cause thermal and mechanical hazards and cannot be employed with infants (Kuehn, 2009). These challenges are more significant for young infants, as the entire body is inside the head coil where the static and switching magnetic fields are strongest (Figure 4.1 right). The robotic interface thus needs to be MR Safe, a term defined by the American Society of Testing and Material and recognized by the U.S. Food and Drug Administration agency (<http://enterprise.astm.org/>), meaning an item that poses no physical risks in all MR environments. Additionally, the device should also be MR-compatible, i.e. should not induce image artifacts. Meeting these criteria requires an attentive evaluation of the bidirectional interactions that may arise between the scanner and the device. A list of requirements for a robotic stimulator for fMRI studies of newborns is presented in Table 4.1.

Table 4.1: List of requirements for a robotic system for task-dependent fMRI studies of the newborn.

Interface requirements
Contain no ferrous materials and be fully MRI/fMRI compatible.
Be mechanically safe to avoid distress or possible harm to the infant.
Be able to provide stimulation synchronised with fMRI acquisition.
Be light, small and flexible enough to avoid the infant suffering movement restriction or discomfort.
Be easily cleanable to prevent infection spreading from one infant to another.
System requirements
Be able to induce stimulation patterns of constant amplitude and frequency, which are sufficient to elicit robust functional responses.
Be capable of presenting a stimulation type and pattern which is appropriate for the neuro-developmental stage of the study population.
Allow monitoring the operation of the stimulus remotely to ensure stimulation is presented in a consistent and safe manner.
Not additionally induce head movements and so avoid resulting image artifacts.

As explained in Chapter 2, MRI utilizes a strong static magnetic field B_0 to polarize atomic nuclei, rapid switching gradients to encode their spins, and fast radiofrequency pulses to deliver selective excitations. The strong magnetic fields exert attractive forces on objects containing ferrous and ferromagnetic materials, which can turn them into high-speed projectiles potentially causing severe injuries to the patients and/or damage the equipment (Tsekos et al., 2007, Schenck, 2000, Schenck, 1998, Shellock and Crues, 2004). Furthermore, when placed inside the scanner, ferrous and ferromagnetic materials can disrupt the homogeneity of the magnetic field resulting in severe image artefacts such as complete signal loss (known as signal “drop-out”) (Tsekos et al., 2007). Additionally, the rapid switching gradients used by the imaging sequences can also induce Eddy currents within conductive materials, potentially leading to overheating and causing the patients to suffer thermal burns (Dempsey et al., 2001, Tsekos et al., 2007, Shellock, 2000). Eddy currents can also give rise to local magnetic field inhomogeneities and severely impact image quality (Shellock and Crues, 2004, Tsekos et al., 2007, Shellock, 2000, Gassert et al., 2006b); and image artifacts can also be generated by interfaces between paramagnetic materials with different NMR susceptibilities (Tsekos et al., 2007). In general, MRI is also extremely sensitive to electromagnetic noise, which may propagate from any electronic equipment or wiring acting as radiating antennas. When developing a device that is MR safe, it is therefore fundamental to utilize suitable materials,

actuators and sensors, as well as to appropriately shield and position electronic circuits and potentially hazardous components (Gassert et al., 2006b, Tsekos et al., 2007).

Materials

MR safe robots should incorporate only minimally conductive, non-magnetic materials such as plastic, ceramic, fiberglass, carbon fiber, and other composite materials. With the recent advent of 3D printing, plastic interfaces are quickly gaining popularity as complex and custom structures can be easily manufactured. This solution is ideal for producing non-standard devices tailored to preterm subjects.

Actuation

When designing robotic interfaces for fMRI studies of the infant population, it is necessary to consider that the subject is contained almost fully within the scanner's head coil and is therefore in close proximity to the magnet's iso-center. Electric actuators such as ultrasonic motors and piezoelectric elements have been widely utilized in adult studies as they can be placed directly at the end-effector thus highly simplifying control and experimental set up (e.g. (Tsekos et al., 2007, Masamune et al., 1995, Kaiser et al., 2000, Koseki et al., 2002, Chinzei and Miller, 2001)). They are unsuitable for infant studies as they generally need to be shielded and placed at a minimum distance from the scanner bore, away from the imaging region (Gassert et al., 2006b). Remote drive offers intrinsically MR safe solutions, and is usually performed by implementing a master slave configuration, in which a master actuator located outside of the scanner room is used to drive a slave actuator which is coupled to the interface (Gassert et al., 2006b). Common master/slave transmissions include mechanical, pneumatic and hydrostatic drive systems. While mechanical transmissions such as cable and belts offer several advantages such as minimal backlash, high bandwidth and low inertia, they need to be kept under constant tension which requires mounting fixed structures (Gassert et al., 2006b, Tsekos et al., 2007). The robots or interfaces thus driven also need to be fixed within the scanner limiting the subject's mobility, and are therefore unsuitable for infant applications. Hydrostatic transmission removes the need for fixed structures and can produce large forces at the expense of bandwidth, which is limited by the compressibility, friction and viscosity of the driving fluid (Tsekos et al., 2007, Gassert et al., 2006b). Complications associated

with hydraulic drive arise from the need to keep the system pressurized, for which it is necessary to avoid fluid leakage and formation of air bubbles. Oil leakage, even in small quantities is clearly not acceptable in studies of the newborn population (Tsekos et al., 2007). Pneumatic transmission does not present this issue, and offers enhanced flexibility, simplicity and efficiency. Although it produces lower forces than hydraulic transmission, it is suitable to generate binary movements and has been used in several fMRI studies focusing on somatotopic mapping of the sensorimotor cortex (Gassert et al., 2006b, Briggs et al., 2004, Stippich et al., 1998, Golaszewski et al., 2002). While accurate position control is difficult to achieve in view of the high compressibility of air, which limits the transmission bandwidth and imposes large delays, highly controllable pneumatic stepper motors have been custom developed by either utilizing multiple pistons or sprocket wheels (Gassert et al., 2006b). Exemplary of these is the PneuStep (Tsekos et al., 2007, Stoianovici et al., 2007, Muntener et al., 2006) where a mechanism composed of 3 triple-parallel cranks and 3 pneumatically actuated diaphragms is utilized to induce an eccentric stepping motion of an external toothed gear ring. Rotation is then transferred to an internal drive gear fixed on a central rotational axis. However these motors are generally too large and bulky to be used in interfaces for infant subjects.

In summary, hydraulic and pneumatic remote drive systems are the only intrinsically MR safe solutions that also allow the interface not to be fixed into position, and to be safely introduced within the isocenter of the scanner's magnet. While hydraulic actuation is able to generate higher forces, and can be used to more easily achieve accurate position control via simple master/slave piston systems, pneumatic actuation is inherently cleaner due to no risk of leakage, and is also faster and more efficient. The restrictions imposed by the infant population require the interface to be flexible, compact and to operate within the bore of the MR scanner. Remote drive by means of small pneumatic actuators represents an ideal solution for this application.

Sensing

Like actuators, many standard sensors contain ferromagnetic materials, and/or require electrical wiring, and therefore cannot be used within the MR scanner. Fibre optic technology allows flexible and inherently MR safe sensing solutions as only the encoder is attached to the interface, while the light source (i.e. LED) signal conditioning (i.e. photodiodes) and amplification equipment can all be

placed outside of the scanner room. Simple embodiments of fibre optic position sensors are custom linear or rotational incremental encoders, in which a grated film intermittently interrupts a light beam transmission between an emitting and a receiving fibre optic cable. The resolution of these encoders is however limited by the diameter of the light beam. Fibre optic sensors able to infer force and torque by directly measuring light intensity have also been developed. In one embodiment of a torque sensor developed by Gassert and colleagues, the (minimal) physical deformation of a rigid structure attached to a reflective surface was used to modulate the intensity of the light which was projected onto such surface by the emitter fibre, and reflected back to the receiver fibre (Gassert et al., 2006a). In addition, companies such as Opsens (www.opsens.com; Quebec, Canada) offer a range of sensors which use multimode optical fibres and light interferometry to measure temperature and pressure.

4.1.5 Robotic tools for somatosensory fMRI studies of the preterm brain

As described in Chapter 3, prior to the approach presented in this thesis, the experimental setups adopted in the somatosensory study of premature and ex-premature infants have suffered from several limitations including the inability to time-lock stimuli to TR acquisitions, and the inaccurate control over the frequency and amplitude of stimulation. In 2003 and 2006, Erberich and colleagues used a manually controlled master-slave rubber bulb system to passively close and spread a premature infant's hand (Erberich et al., 2003b, Erberich et al., 2006). During activation periods one of the investigators in the control room squeezed and released the master bulb to inflate and deflate the slave bulb placed in the infant's palm at approximately 1 Hz. In 2009, Heep and colleagues induced passive sensorimotor stimulation via unilateral forearm flexion/extension manually performed at approximately 1 Hz by a physiotherapist standing in the scanner room (Heep et al., 2009). In 2010, our group solved some of these problems by using a custom made pneumatically actuated balloon placed in the infant's hand to induce somatosensory activation (Arichi et al., 2010). The computerized pneumatic control of the system allowed the frequency and amplitude of balloon inflation/deflation to be both precisely controlled, and synchronized with the MR image acquisition.

This method produced statistically robust and reproducible results in premature babies, with well-localized BOLD responses contralateral to the side of stimulation (Arichi et al., 2010). However, the system presented the experimenters with some practical and theoretical challenges which had to be resolved before the approach could be utilized for systematic and longitudinal fMRI studies. The most salient practical issues (described in Chapter 3) concerned the inability of the system to monitor and/or provide feedback on balloon actuation, and the tendency of the balloon to lose its elasticity over time thus not guaranteeing a consistent inter-subject pattern of stimulation. The balloon also had to be taped within the infants' hand which, combined with a lack of pressure monitoring, represented a safety hazard. Additionally, the balloon inherently caused stretching of the skin, as well as finger flexion/extension, thus presenting a potentially important experimental confound as both proprioception and mechanoreception were elicited.

Therefore, I aimed to improve upon the previous system, and to develop a range of interfaces that meet all the criteria reported in Table 4.1 and that would overcome current limitations. I aimed to develop devices that not only are suitable for systematic developmental studies, but also capable of inducing and recording movement of body parts whose cortical correlates are spatially distinguishable within the adult homunculus. In keeping with previous neuroimaging studies (reviewed in (Vanhatalo and Lauronen, 2006, Milh et al., 2007)), and owing to their extensive and distinct cortical representation, I identified hand and foot movements as ideal substrates. More specifically, I aimed at presenting a very specific pattern of proprioceptive stimulation by performing and monitoring flexion/extension of the wrist and ankle joints. Here I first present the design and validation of the wrist stimulator, followed by that of the ankle stimulator, to subsequently conclude by reporting the mapping study of the preterm homunculus.

4.2 Wrist Robotic System

I have developed a novel MR compatible robotic system (Figure 4.1left), specifically tailored to both monitor and elicit specific motor tasks in neonates. I used rapid prototyping together with fibre optic sensors to develop the first wrist robotic system that meets the technical and safety requirements to detect the cortical patterns of fMRI activity in premature infants associated with both active (i.e., initiated by the infant) and passive (i.e., generated by the device) wrist movements. A summary of the technical specifications of the wrist device is presented in Table 4.2.

Here the technical aspects of this development will first be reported, and the results of fMRI validation experiments in a healthy adult and two preterm infants will be subsequently presented.

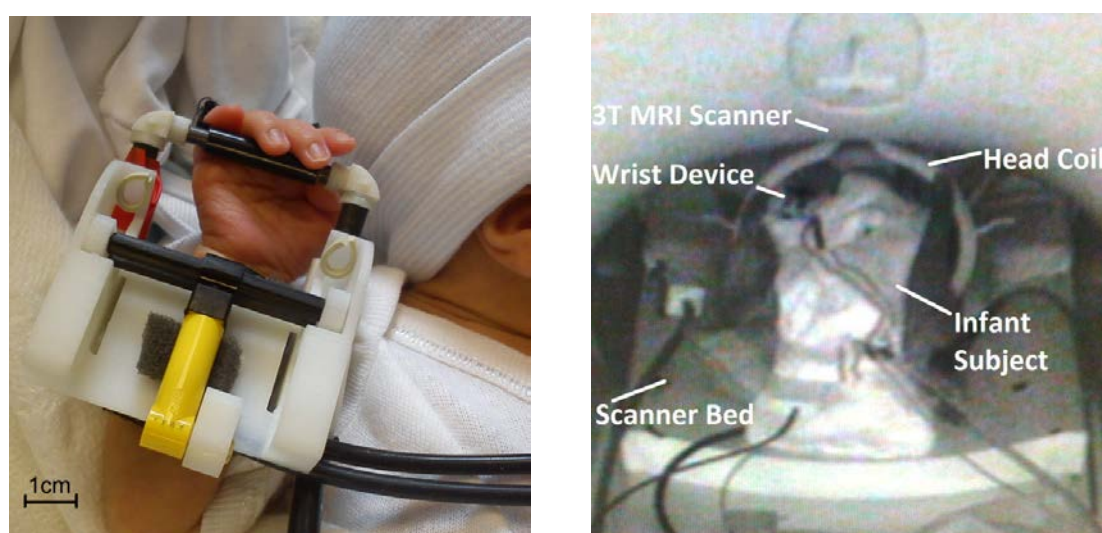


Figure 4.1: A preterm infant equipped with the wrist interface (left); the same infant within the MRI scanner (right).

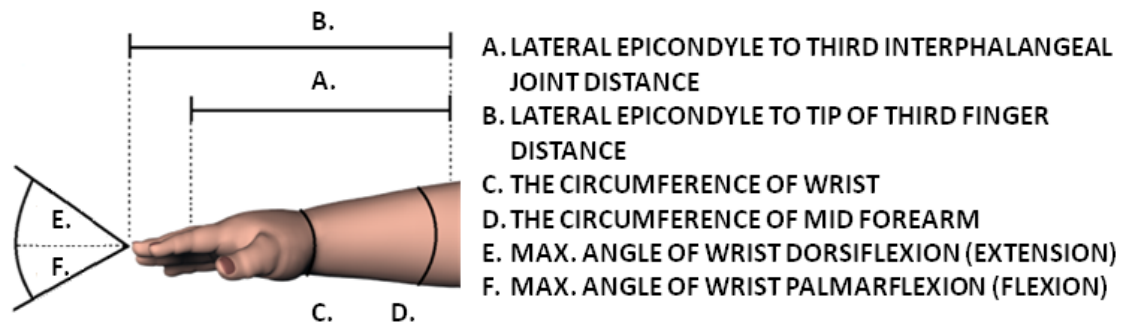
Table 4.2: Wrist interface technical specifications.

Technical Specifications	
Rotation angle range	-15° to 20°
Extension torque at 0.7 atm	44.3–50.2 N mm
Flexion torque at 0.7 atm	31.4–35.5 N mm
Position sensor resolution	±1.1°
Weight	55 g
Size (w x h x d)	(54 x 39 x 45) mm
Software	Windows 7/Labview RT

Biomechanical Considerations

The robotic wrist interface was designed to accommodate a preterm infant's forearm on a central platform and provide a guided wrist flexion/extension within safe limits. Measurements of wrist and hand dimensions were performed on 6 preterm and term infants aged between 29+2 to 40+3 wks PMA (reported in Table 4.3), and utilized to appropriately dimension the interface such that it would fit infants within a similar age range. All infants assessed could achieve a wrist dorsiflexion (extension) of at least 30° and a wrist palmar flexion (flexion) of at least 80°. The largest forearm circumference was measured at 90 mm (40+2 weeks PMA infant), with a 15 mm height and 30 mm width. Based on these parameters the interface's central platform was tailored to house forearms up to 32 mm in width. The platform was fitted with an adjustable Velcro® strap to account for the variance in forearm height across infants. Wrist flexion/extension was restricted to 20° and 10° respectively, a range that was considered both safe and able to produce a significant somatosensory response by expert clinicians.

Table 4.3: Measurements of six infants' forearm and wrist dimensions.



Corrected age (wks)	Weight (g)	A (mm)	B (mm)	C (mm)	D (mm)	E (o)	F(o)
29 + 2	895	71	89	50	53	70	100
32 + 6	1450	77	106	61	69	60	80
34 + 0	1790	96	117	61	74	30	90
40 + 2	3200	113	148	77	90	30	90
40 + 3	4184	111	146	98	109	30	90

4.2.1 Interface

Mechanical Design

The final robotic wrist interface was designed to meet the requirements reported in Table 4.1, and dimensioned in accordance with the restrictions posed by the biomechanics of the infants' wrists reported in Table 4.2. The robot consists of a rapid prototyped main body, a pneumatic piston actuator, and a fibre optic position sensor. The main body of the wrist interface was custom designed using CAD software (SolidWorks, Dassault systèmes SA, Waltham MA, USA) and manufactured via fused deposition of acrylonitrile butadiene styrene (ABS) (3D printer, Objet30pro, Stratasys Corp., Eden Prairie MN, USA). ABS was selected due to its light weight, hardness, rigidity, and low friction coefficient; moreover it is MR safe, making this material ideal for my application. A commercially available two-inlet pneumatic piston (LEGO Pneumatic Cylinder with 2 Inlets Small (32mm) 6005292, The Lego Group, Billund, DN) was used as the actuator due to its material, stroke, and size properties. The piston was used to drive the lower extremity of two L-shaped arms (Figure 4.2) with the infant's hand is secured to the handle contained between the arms' upper extremities via a Velcro[®] strap. In this configuration, the L-shaped arms are caused to pivot around the approximate natural axis of rotation of the infants' wrist thus inducing wrist flexion/extension. The interface was designed to work in two modes: (i) a passive mode, in which the device drives the motion of the infant's wrist, and (ii) an active mode, in which the device is driven by the infants' spontaneous movements. During passive mode, the torque that the device needs to exert to induce full wrist extension depends on the weight of the infant's hand and on the intrinsic compliance of the infants' wrist. In practice, I estimated the minimum torque required to drive a passive infants' wrist to full flexion/extension to be approximately 30 Nmm. Given the very low forces that preterm infants can produce, it is critical that during the active mode the interface does not exert significant resistive forces if spontaneous movements are to be measured. To achieve this two measures were implemented; firstly low friction bearings (iglidur J series, igus GmbH, Köln, DE) and acrylic rods were used as pivots for the rotating L-shaped arms; secondly the axle driven by the piston was designed to be easily accessible such that it could be quickly removed prior to the active motor task; this disengaged the piston starkly reducing the drag and inertia of the device. A custom ABS rod was also implemented in parallel to the driving axle, to ensure that the rotation of the arms is still coupled

upon disengagement of the piston. Finally, the handle was designed such that it could be optionally instrumented with a balloon similar to that used in a previous interface (described in (Arichi et al., 2010)), thus enabling the concurrent investigation and contrasting of wrist and hand correlates.

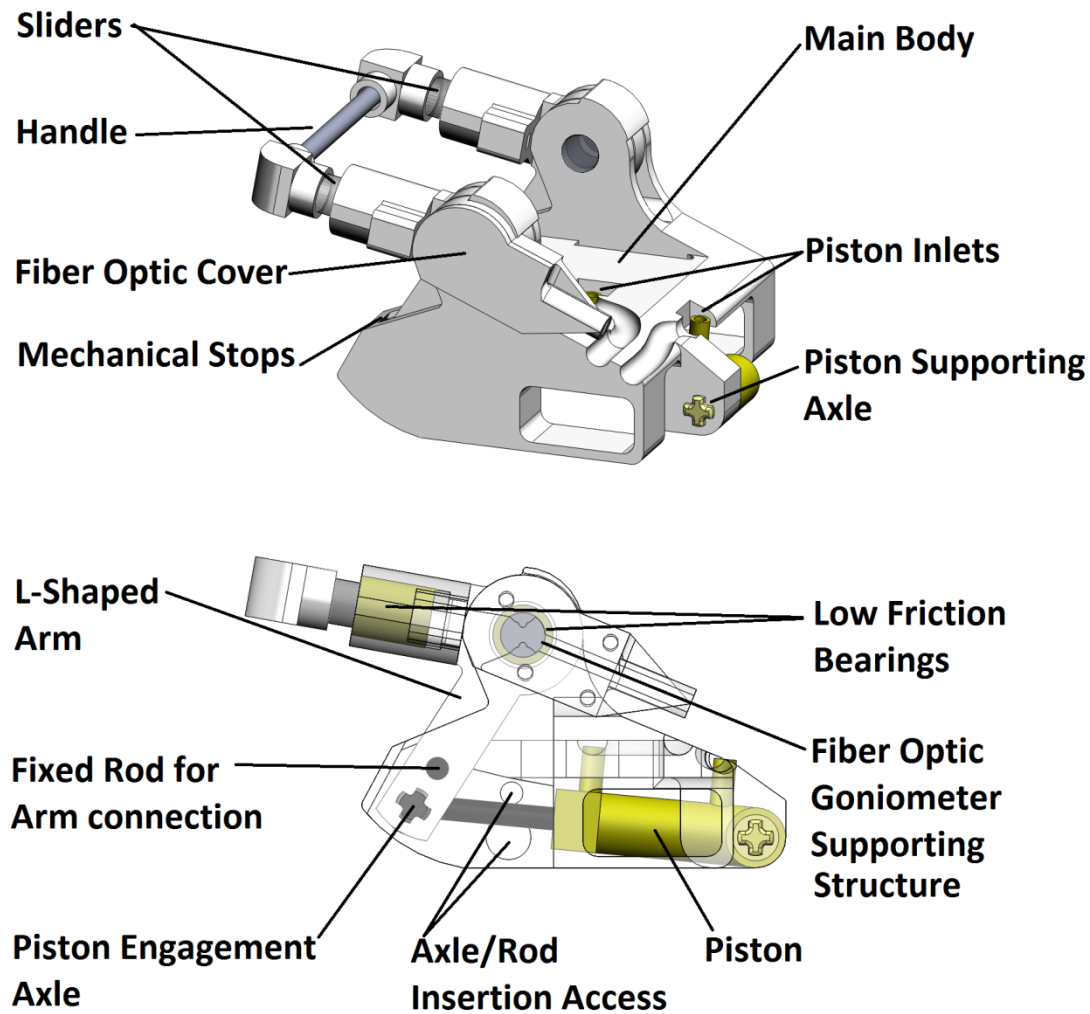


Figure 4.2: Dimetric view and side view of 3D solid models (SolidWorks, Dassault systèmes SA, Waltham MA, USA) of the wrist interface.

Mechanical Safety Measures

The handle is allowed to slide longitudinally with respect to the L-shaped arms (Figure 4.3). This degree of freedom prevents the creation of strain forces at the wrist level by compensating for

misalignments between the mechanical rotation axis of the interface and the natural rotation axis of the wrist. Further, wrist hyper- flexion/extension is prevented by the geometry of the design as the piston has a maximum stroke of 15 mm acting at a distance of 23.4 mm from the L-shaped bar pivot, which can induce a maximum total rotation of 30° . In case of piston failure, two mechanical stops were also integrated to restrict the piston's maximum stroke (Figure 4.3b).

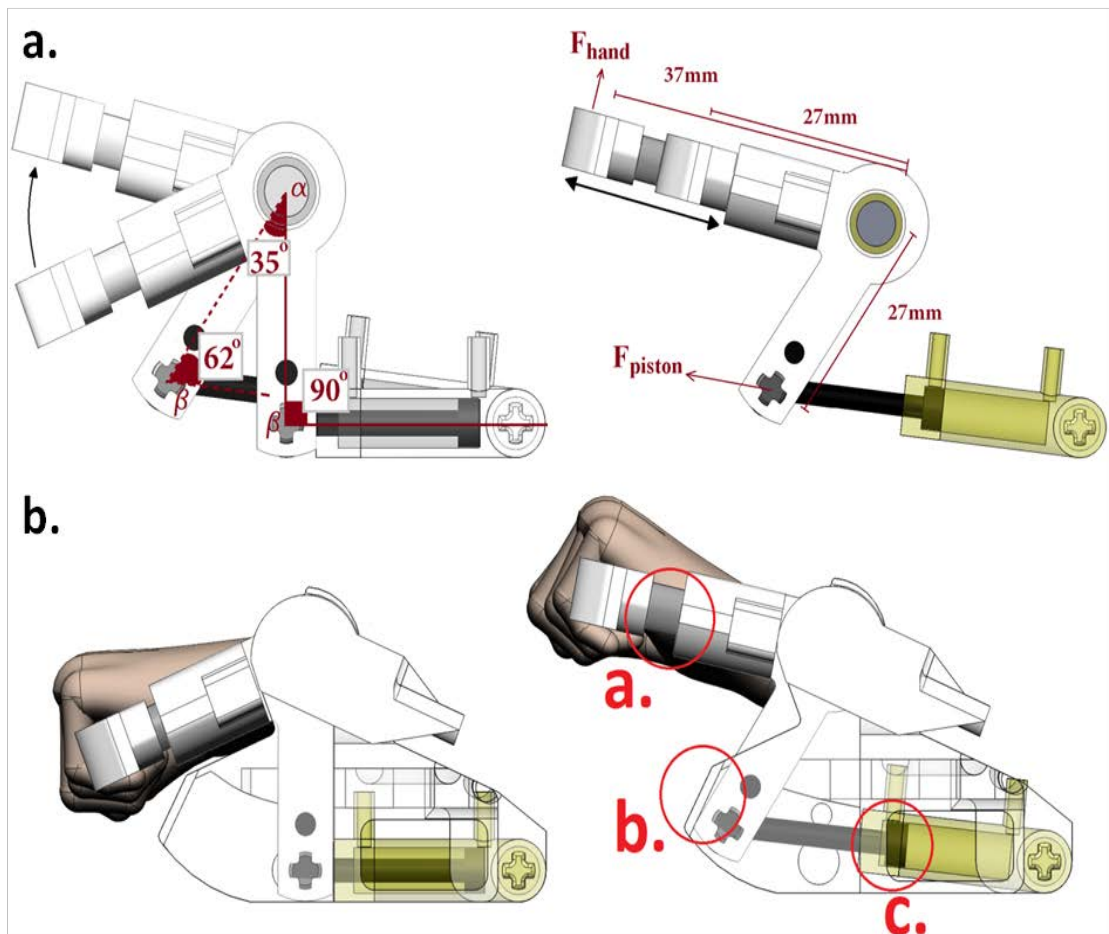


Figure 4.3: (a) Forces acting on the interface. The interface has been dimensioned such that the minimum force transmitted (at full extension) would be sufficient to lift the infant's hand. At 0.7 atm the piston exerts a force F_{piston} of 1.86 N, resulting in a minimum torque T of 44.3 N mm ($T = F_{piston} (\sin(62^\circ)) \times 27 \text{ mm}$). Practically, this provided a force of 1.2–1.6 N which is more than sufficient to lift the average infant's wrist. (b) The mechanical safety measures: a. sliding handle, b. mechanical piston stop, c. piston stroke limit.

Position Sensing

In order to monitor motion during passive mode and track spontaneous wrist movements during active mode I developed a robust and affordable position sensor by exploiting the principle proposed by (Kuang et al., 2002). A segmented mono-core plastic optic fibre was fitted to the wrist interface by running it to the exterior of its left L-shaped arm. The segmented region of the fibre was carefully aligned to the rotation axis of the device. As wrist motion causes the L-shaped arm to rotate, the fibre optic is forced to bend around its segmented region causing an increasing amount of light to escape from the fibre, thus allowing inferring joint position from the attenuation of the measured fibre optic signal intensity. Mechanical inserts were incorporated into the main body of the device to impose a pivot point, and restrict any motion of the optic fibre other than rotation around the central axis of the L-shaped arm joint. A low diameter (500 μm core $\varnothing$) (FU-38, Keyence Corp., Osaka, JP) plastic optic fibre was chosen as the sensing element due to its high flexural compliance, allowing the fibre to comfortably achieve 30° flexion and, crucially, avoiding the creation of a resistive force that would restrict the baby's spontaneous wrist movements.

The fibre optic goniometer was calibrated using a rotary encoder (HEDS-5701, 360 PPR, 0.25° resolution in X4 mode, Avago Technologies Corp., San Jose CA, USA) connected in parallel with the interface and aligned with its rotation axis; the calibration setup is shown in Figure 4.4a. Two 50 s long calibration sessions at frequencies of 0.1 and 0.5 Hz and 30° amplitude were performed by actively controlling the piston. A third calibration session was performed by disengaging the piston and manually operating the device at varying frequencies and amplitudes. Results show that the sensor is linearly able to detect flexion with a maximum standard deviation of 0.0198 V corresponding to approximately 2.3% of total signal change. Linear regression of signal intensity and angular position presented a coefficient of determination R^2 of 0.996 and a maximal error of $\pm 1.1^\circ$. Throughout the development of the sensor, several positions and configurations were tested. It was found that placing the sensing, segmented region of the sensor in line with the axis of rotation of the device greatly diminished the incidence of hysteresis. The sensor was only marginally affected by hysteresis at around 27° as shown in Figure 4.4b.

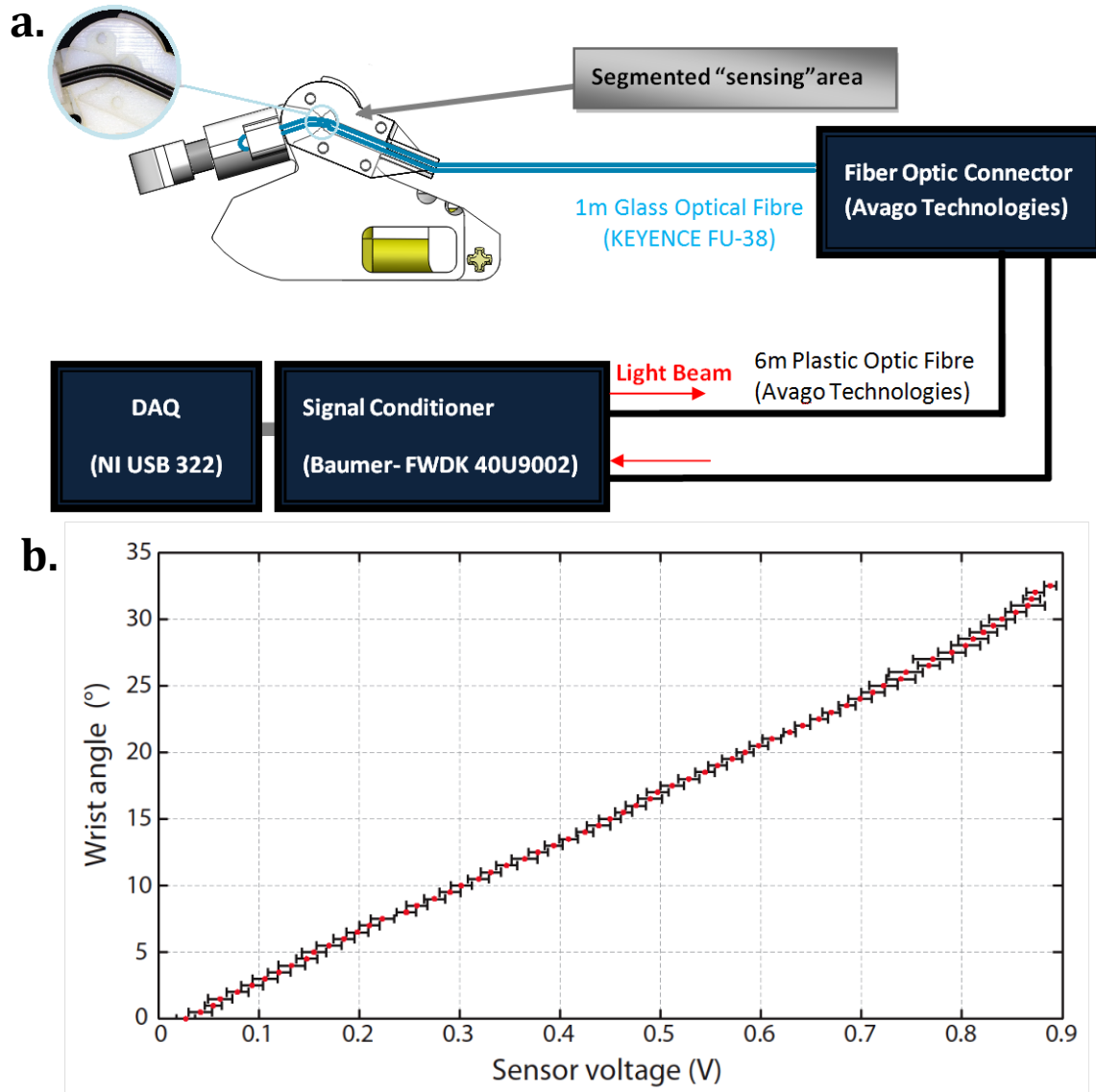


Figure 4.4: (a) Schematic drawing of the fibre optic sensor set up. The segmented portion of a (500 μm core $\emptyset$) plastic optical fibre (POF) is allowed to rotate around the interface's axis of rotation. 1 m length of highly flexible POF is connected to 6 m of less sensitive (1 mm core $\emptyset$) POF to reduce the risk of measurement errors due to handling of the fibres. A signal conditioner containing a photodiode and an amplifier measures signal attenuation and provides an analogue signal to the DAQ. Sensitivity of the signal conditioner can be manually adjusted. (b) Calibration curve of custom fibre optic sensor with AVAGO HEDS-5701 rotary encoder (0.25° resolution) graphed at a 0.5° resolution. A maximum standard deviation of 0.0198 was found at 27°, where hysteresis peaks.

4.2.2 Controller Architecture and Software

The robotic system consists of two main components: the MRI compatible wrist interface equipped with the fibre optic position sensor, and actuated via a pneumatic 2 inlet piston; and a control unit. The interface is located within the scanner room, and connected to the control unit

located within the control room via two 6 m long pneumatic tubing for actuation, and a 6 m long fibre optic extension lead for position monitoring. The control unit houses a Data Acquisition Card (DAQ) (NI USB-6211, National Instruments Corp., Austin TX, USA) operating at a sampling frequency of 100 Hz. The DAQ analogically sets and monitors the air pressure delivered to the piston via two pneumatic proportional valves (MPPES series, Festo Corp., Essling am Neckar, DE). Although the valves use an integrated pressure sensor and PID controller to maintain the air pressure required by the user, a second PID loop is implemented to improve the valves' time response (Figure 4.5) thus ensuring a controlled wrist actuation at a frequency of 0.3 Hz. This rate of stimulation was chosen empirically based on stimulation frequencies from previous work (Arichi et al., 2010, Erberich et al., 2006, Erberich et al., 2003a).

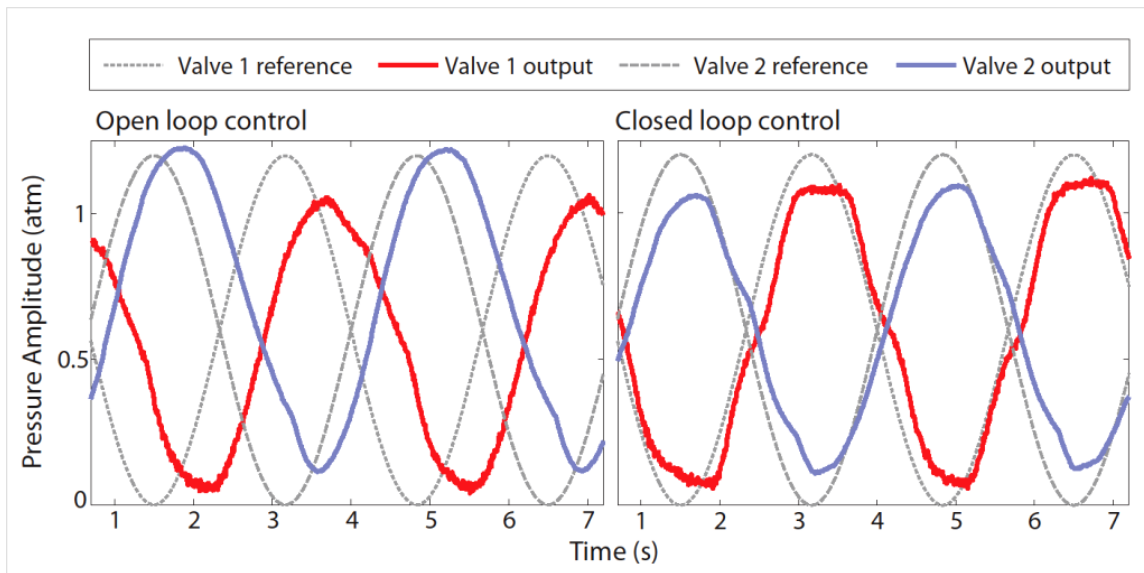


Figure 4.5: Time response of the FESTO MPPES proportional valves to a sinusoidal input pressure profile. A second PID controller iteration (right) was used to reduce the time delay and improve the amplitude response of the original system (left).

The DAQ receives analogue and digital inputs from a fibre optic signal transducer and the MRI scanner, respectively. The digital signal produced by the scanner with each time to repeat (TR), is used to achieve synchronization between wrist stimulation and fMRI image acquisition. The program uses the scanner's TTL pulses as a clock to trigger the reading of the digital matrix and perform epochs of passive wrist actuation (1 s) or rest (0 s) that are thus synchronized with each

volume of fMRI image acquisition (Figure 4.6a,b). In practice a constant delay in actuation was recorded for each subject, and this was modelled directly into the fMRI data analysis. The DAQ is interfaced with a customizable graphical user interface (GUI) developed in the LabVIEW software environment (National Instruments Corp., Austin, TX, USA) that allows the investigator to set up the experimental protocol and monitor the experimental parameters. The GUI allows the investigator to alter the frequency of stimulation and maximum pressure driving the piston; and also to provide real-time feedback through constantly monitoring the proportional valves' pressure values and wrist position. The software architecture is presented in Figure 4.7.

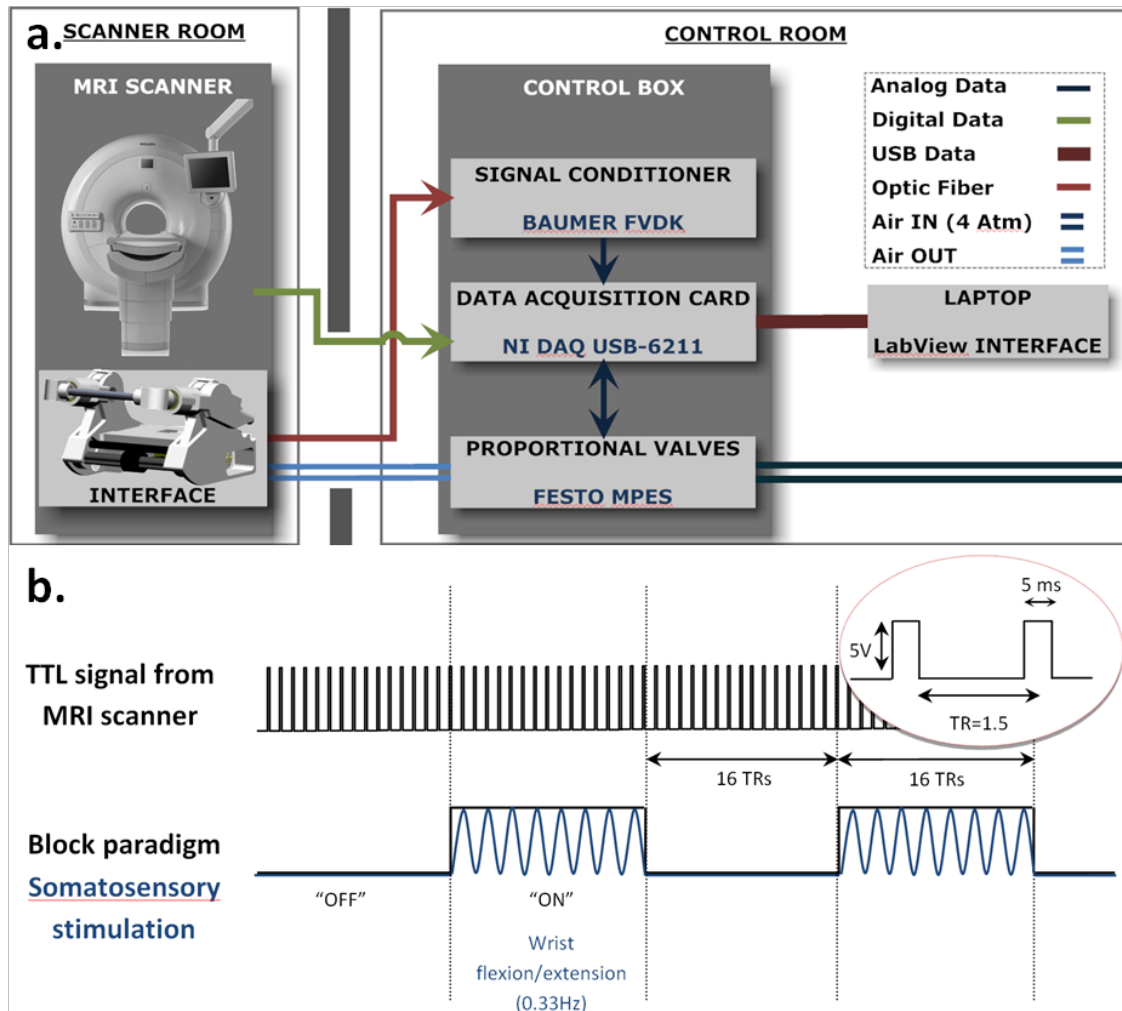


Figure 4.6: (a) Architecture of the robotic system and diagram of the synchronization modality between somatosensory stimulation (passive mode). (b) MR image acquisitions using the TTL pulses and the user defined block sequence.

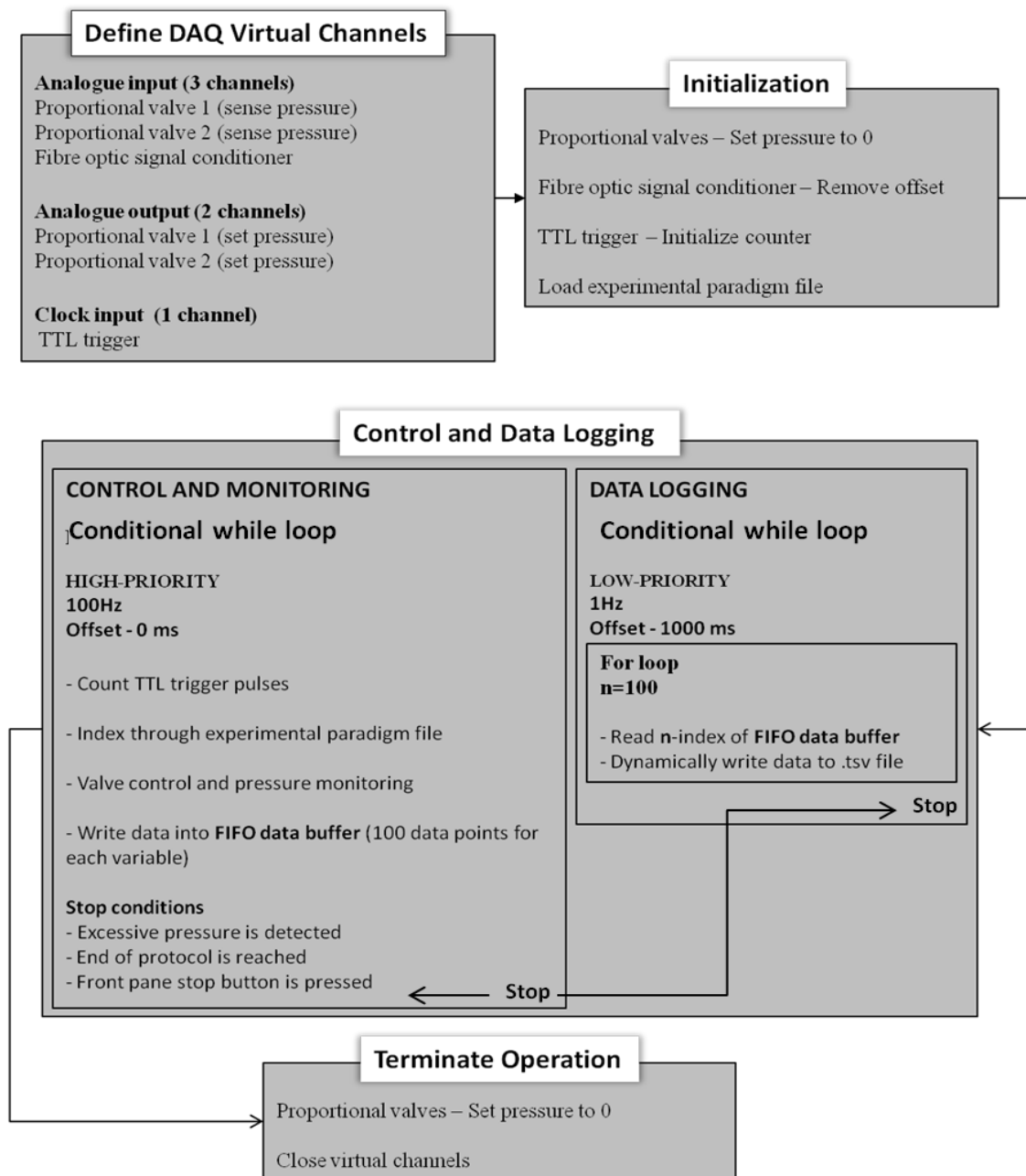


Figure 4.7: Architecture of the control software, Firstly the DAQ communication is initialized, and software virtual channels are assigned to the device’s physical channels. The proportional valves’ output pressure and the sensor signal are reset. The control and data logging then proceeds via two parallel loops, where a high-priority loop running at 100Hz monitors TTL pulses, controls the valves, monitors and displays pressure and position, and writes all data into a dynamic FIFO buffer. A low-priority data logging loop runs in parallel, dynamically reading data from the FIFO buffer and saving it into a .tsv file. Several stop conditions are implemented within the high-priority loop. When a stop condition is met, the proportional valves are closed and communication with the DAQ is terminated.

Additional Safety Measures

In addition to intrinsic safety from the mechanical design (described in Section 4.2.1), the software is designed to constantly monitor and limit the air pressure supplied by the valves. It is also possible for the investigator to cut the power to the pneumatic valves via an emergency stop button incorporated into the top of the control box, thus halting air flow and pneumatic actuation if all other safety measures fail. To detect whether infants are experiencing excessive distress during scanning, their vital parameters such as temperature, echocardiogram (ECG), and oxygen saturation are constantly monitored (detailed in (Merchant et al., 2009)). Hearing protection is employed in babies (silicon dental putty (President Putty, Coltene Whaledent Inc., Mahwah NJ, USA) and adhesive ear muffs (Minimuffs, Natus Medical Inc., San Carlos, CA, USA)). Furthermore the MRI scanner room is also instrumented with a video camera and microphone, providing the investigators with constant video and audio feedback in order to detect if the infant is moving excessively or crying.

4.2.3 Experimental Validation

The work was approved by the NHS research ethics committee (ref: 07/H0707/101), and written subject (or parental in the case of neonatal subjects) consent was obtained prior to all sessions of data acquisition.

Experiments were carried out to investigate whether the wrist stimulation device system can be used to objectively elicit and monitor cortical responses to both passive and active wrist movements. A first test was performed on a healthy adult volunteer in order to verify the system and the protocol's ability to elicit activation in the characteristic cerebral areas associated with sensorimotor stimulation. The system was subsequently tested on two premature infants to assess its ability to qualitatively study the responses of the premature brain to active and passive wrist movements. Both infants were scanned during natural sleep, induced via the feed and wrap technique. MR imaging was performed on a Philips Achieva 3-Tesla system (Best, Netherlands) and the imaging sequence parameters and acquisition methods were set as in (Arichi et al., 2010). fMRI volumes were acquired using an EPI sequence with: TR 1500 ms, TE 45 ms, flip angle 90°, 22 slices, voxel size, 2.5 mm², slice thickness 3.25 mm, and total 256 volumes (Arichi et al., 2010).

Subjects and Protocol

The first test of the wrist interface was performed on a healthy right-handed, 23 year old, adult male volunteer. The subject was told he would undergo a session of passive finger actuation followed by a second session where he would have to alternate 10 s of finger tapping to 10 s of finger rest. The volunteer was then placed in the scanner with device strapped to left index finger (Fig. 7a, right) and the scanning TR time was set to 1.5 s. The passive finger stimulation paradigm lasted 256 TRs (384 s) and consisted of 8 blocks of 32TRs (48 s) in which 16TRs (24 s) of rest were followed by 16 TRs of passive stimulation. During passive stimulation the index finger was flexed/extended by 30° at a frequency of 0.3 Hz. The device actuation was then halted and the subject spontaneously moved the finger as instructed for a second session of 256 TRs. Finger position was recorded throughout the task.

Two additional experiments were carried out on two healthy preterm infants, Infant A born at 34 weeks gestation (and scanned at 34 weeks +3 days) and Infant B born at 33 weeks gestation (and scanned at 33 weeks +3 days). Both infants underwent a passive session (Figure 4.9a) consisting of the wrist being actuated at 0.3 Hz and allowed to flex/extend by 30° (Figure 4.9b) for 256 TRs; and as in the previous experiment, 16 TRs of rest interspersed with 16 TRs of passive stimulation. The delay in at the onset of actuation was constant for each infant, and was recorded and implemented into the fMRI analysis. In practice the delay was constant across blocks of stimulation, and subjects, averaging a total mean of 630 ms and standard deviation of 20 ms; a constant delay of 0.6 s from TR onset was therefore implemented in the model design. Infant B underwent an additional active session in which the piston was disengaged, and the sampled fMRI data was then analyzed with respect to the recorded spontaneous wrist activity. This session lasted 512 TRs (13 minutes) and four spontaneous movements of different amplitudes were recorded (Figure 4.10).

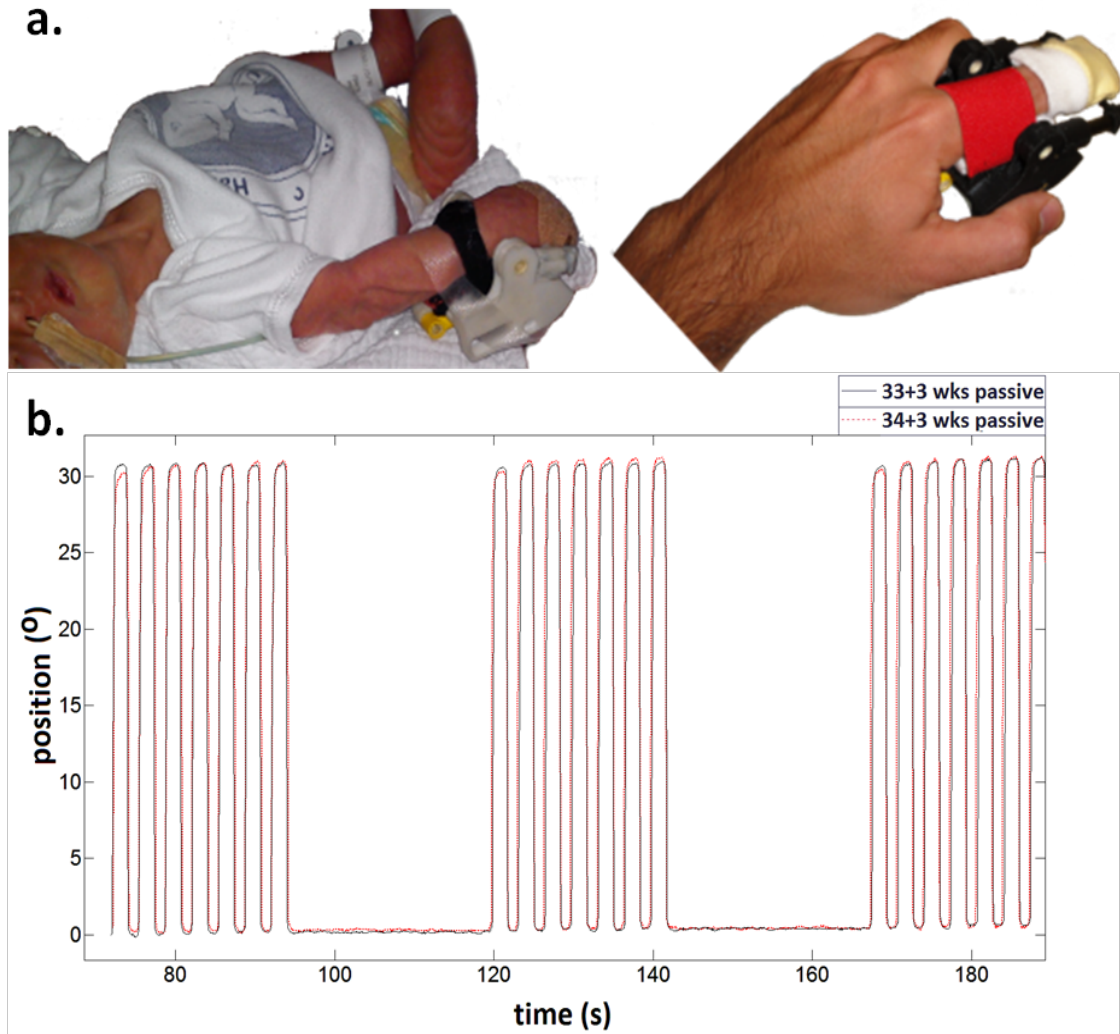


Figure 4.9: (a) The wrist interface (v3) attached to the 34 + 3 week post-menstrual age premature infant's right wrist (left) and a previous version of the interface (v2) attached to the 23 years old healthy adult volunteer's left index finger (right). (b) Position sensor feedback during passive stimulation paradigm. Homogeneity in amplitude and frequency of stimulation is achieved across epochs and patients.

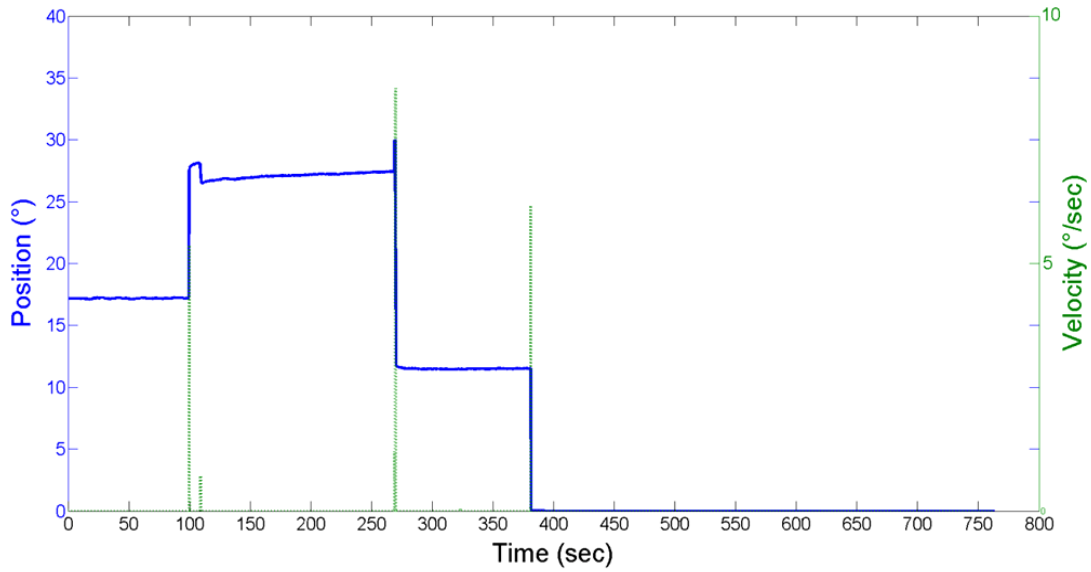


Figure 4.10: Wrist position over time (top blue) was differentiated to obtain wrist velocity. Absolute value of the latter was taken (top red) and averaged across the TRs (bottom) resulting in 4 peaks of different amplitudes. This correlation between wrist velocity and TRs is fundamental for our event related fMRI analysis.

Data analysis

fMRI analysis was carried out by utilizing a General Linear Model (GLM) as implemented in FEAT (fMRI Expert Analysis Tool v5.98, part of the FSL image processing package, www.fmrib.ox.ac.uk/fsl). Data was pre-processed using standard steps comprising of motion correction, slice-timing correction, non-brain tissue removal, spatial smoothing, global intensity normalization, and high-pass temporal filtering (see Chapter 2). For passive wrist actuation, the design model was specified by the convolution of the block design (the binary matrix with 1's representing an event and 0's representing rest, appropriately delayed to reflect the time of stimulus onset) and an age-appropriate linear basis set as defined in (Arichi et al., 2012). For spontaneous movements, the measured finger/wrist displacement was converted in the absolute average finger/wrist velocity in each TR. These values were then used as regressors in the GLM to perform a weighted convolution between the brain acquisition and the HRF to infer cortical activation. A qualitative assessment of the structural connectivity underlying the seed region of activated voxels was performed using DTI probabilistic tractography. DTI data was analyzed FMRIB diffusion toolbox (FDT, version 2.0) implemented in FSL (Smith et al., 2004a) as specified in (Arichi, 2012).

4.2.4 Results

Adult subjects

The validation test carried out on a healthy male volunteer showed well localized contralateral areas of functional activity during passive and active movement of the left index finger. Passive finger movement resulted in patterns of cortical activation in the contralateral right primary somatosensory cortex and supplementary motor area (Figure 4.11, blue), whereas active finger motion resulted in activation of the contralateral primary motor cortex (Figure 4.11, red). Responses of the passive and active conditions were found to lie adjacent to each other at a central-to-superior level of the lateral primary somatosensory and motor cortices respectively. T2 weighted structural MR images of the volunteer showed no evidence of intra-cerebral damage or abnormalities.

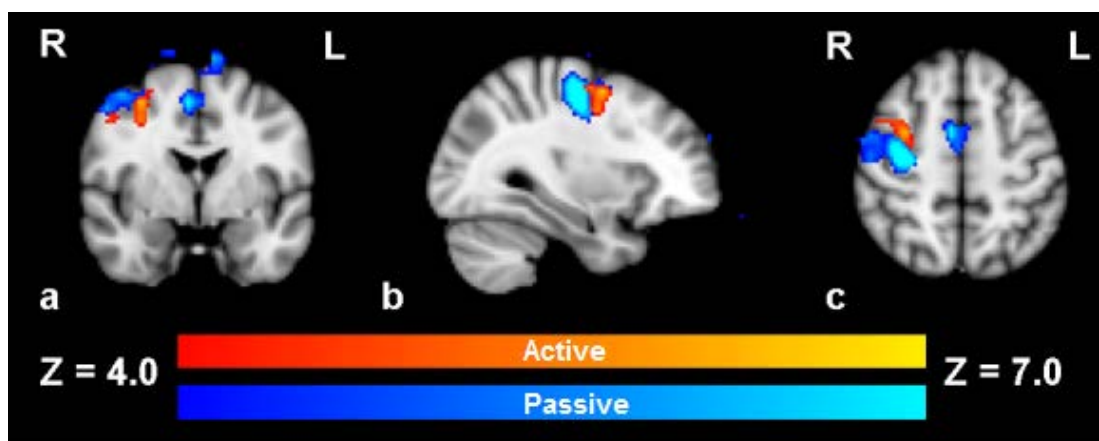


Figure 4.11: fMRI correlates of passive and active movement of the left index in a healthy 23 year old male subject. Thresholded statistical maps with a corrected cluster significance of $p < 0.05$ have been overlaid onto the standard MNI brain space. Functional activity can be seen in the primary somatosensory cortex and supplementary motor area during the passive condition (blue) and in the primary motor cortex during the active condition (red).

Infant Subjects

In both infant subjects, no evidence of any intracerebral damage or abnormalities was identified, following detailed review of their high-resolution structural MR images by an expert Neuroradiologist.

The brain activation of Infant A during the passive session revealed a well localized cluster of positive BOLD activation in the contralateral hemisphere in close proximity to the mature somatotopic representation of the wrist and hand. Passive wrist stimulation of Infant B was found to elicit a similarly localized contralateral cluster of positive BOLD response (Figure 4.12, blue). However, the infant's spontaneous wrist movements were associated with a cortical response adjacent, but more anterior than that elicited by passive stimulation (Figure 4.12, red).

Diffusion tensor imaging (DTI) and probabilistic tractography analysis was then applied to reveal the neural pathways associated with both cortical regions of activation (Figure 4.12c). The activation areas associated with active and passive wrist motion were used as seed areas for the tractography. Both tracts were found to run through the posterior limb of the internal capsule (often called the PLIC), the major axon fibre bundle which carries movement information. Further, the active tract was found to descend to the cerebral peduncles, which are known to then connect down to the spine and carry the active movement signals out to the contralateral arm via the lateral corticospinal tracts.

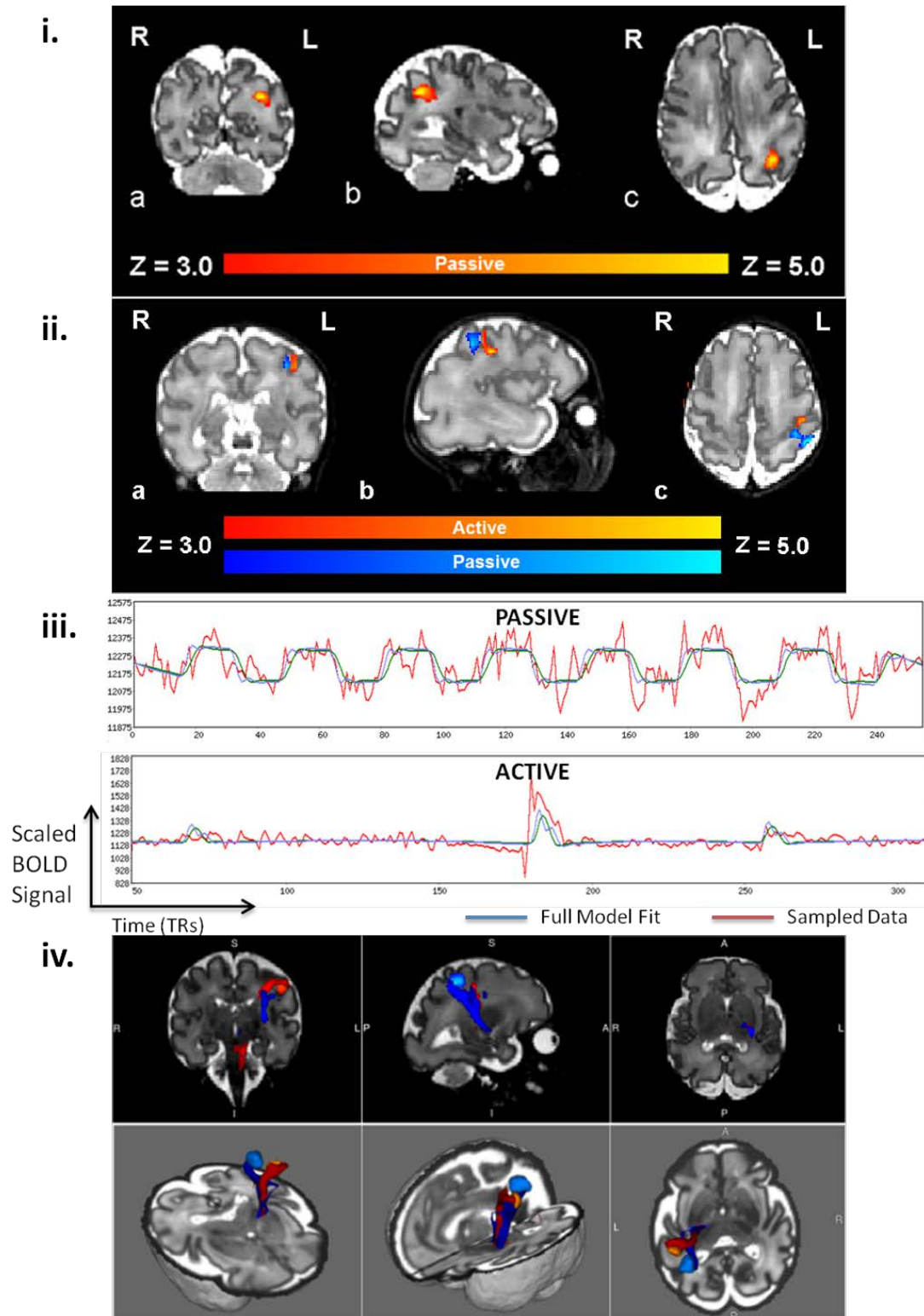


Figure 4.12: (i) T2-weighted images with an overlaid thresholded statistical map (corrected cluster significance of $p < 0.05$) showing functional response to passive right wrist stimulation in a preterm infant (infant A) scanned at 34 + 3 weeks PMA. A well-localized cluster of positive BOLD contrast can be seen at the level of the contralateral primary somatosensory cortex. (ii) Correlates of active and passive wrist movement in a healthy 33 + 3 weeks old premature infant (Infant B). Functional activity is localized to the contralateral primary somatosensory cortex (blue) and contralateral primary motor cortex (red). (iii) Time series within the clusters of active and passive activation, the

sampled Blood oxygen level dependent (BOLD) signal (red) can be seen to closely fit the predicted response as modelled by the pattern of stimulation and an age-appropriate Hemodynamic response function (HRF) model (blue).
(iv) Axonal tracts as estimated via probabilistic tractography stemming from the seed regions of functional activation in response to active (red) and passive (blue) wrist movements of Infant B.

4.2.5 Discussion

I have described the development and testing of an MR safe wrist stimulating device for investigating the neural correlates of passive wrist movement in premature babies using a robust and repeatable stimulation paradigm similar to that described in (Arichi et al., 2010). The device was developed in accordance to strict safety standards, with specific hardware and software measures being implemented to avoid wrist actuation from being harmful or uncomfortable for the baby. The interface's minimal weight and flexibility avoids infant distress, and is fundamental to allow spontaneous wrist movements. The torque of the driving pneumatic piston is more than sufficient for the interface to perform up to 20° wrist flexion and 10° extension on premature babies at a controlled frequency of 0.3 Hz. Position sensing is accurate to $\pm 1.1^\circ$ throughout the range of motion.

When developing the wrist interface, it was found that the high stick-slip effect in the LEGO piston combined with the compressibility of air in the 6 m long pipes and the low volume of air contained in the piston chambers are limiting factors for the device's bandwidth and ability to deliver smooth patterns of stimulation. However the valves' response time was improved, and by controlling the pressure differential applied to the two inlets a smoother piston extension/retraction was provided, partly compensating for the stick-slip effect. The device was therefore able to deliver a gentle wrist motion at a frequency of 0.3 Hz. Although a more sophisticated, faster controller could be developed, the current computerized control of the wrist interface is sufficient to ensure a passive wrist actuation of constant amplitude and frequency. This is important, since frequency (Berns et al., 1999) and amplitude (Zhang et al., 2007) of somatosensory stimulation may be correlated with the extent of elicited functional responses and ensuring they are constant throughout experiments may be fundamental when performing robust group statistical analysis. Attaining image/stimulus synchronization is fundamental to ensuring a reliable time course response when MR images are convolved with the HRF. Due to the system's time response and varying wrist stiffness across

different infants, blocks of stimulation were presented with a slight delay from the TR onset; however, since this delay was constant for each subject, it was easily modelled into the analysis. This could not be done with the previous balloon device, as patterns of inflation could not be monitored.

The interface developed also offers the ability to study the neural correlates associated with spontaneous movements in neonates. This is achieved by monitoring active wrist movements using the dedicated position sensor I have developed, which costs approximately one-tenth of the price and exhibits an accuracy almost four times superior to the best alternative commercial solution found at the time of this study (Measurand s720, Measurand Inc., Fredricton NB, CA) over the same range of motion. Quantitatively measuring spontaneous wrist movement may be essential in since correlation may exist between the velocity of movement and neural activity (Boynton et al., 1996).

Preliminary validation experiments revealed that the wrist interface is able to elicit a well localized positive BOLD fMRI response at the level of the contralateral central somatosensory cortex in premature infants. These findings are in line with my passive hand stimulation study, and indicate that a broad somatotopic organization may already be present in the preterm somatosensory cortex. Cortical correlates corresponding to a 33 +3 week PMA premature infants' spontaneous wrist movements were found to lie adjacent but anterior to the passive activation area. This observation is in agreement with what is observed in adults, where the primary motor cortex lies anterior to the primary somatosensory cortex and follows a similar topography. To the best of the authors' knowledge, this is the first time that the intra-cerebral correlates of spontaneous motor activity have been visualized in human neonates using fMRI. The neural correlates of active movements will be more extensively probed in Chapter 6, however I propose two potential interpretations of these early findings: (i) efferent motor pathways from the primary motor cortex, although still largely unmyelinated during preterm age, may initiate or modulate spontaneous movements. Animal (Chakrabarty and Martin, 2005) and human (Eyre et al., 2000) studies have shown that corticospinal synapses do indeed exhibit early firing patterns, which are however proposed to serve guiding the establishment of early motor connectivity rather than induce spontaneous limb movements (ii) spontaneous movements generated by spinal reflexes, may produce afferent information which is

transmitted via somatosensory pathways to the sensorimotor cortical region; this is in keeping with animal studies which have ascribed spontaneous movements to an endogenous peripheral reflex which serves to guide the early establishment of afferent somatosensory connectivity through activity-dependent plasticity (Khazipov et al., 2004).

In conclusion here I have presented a novel robotic system that is suitable to conduct robust and systematic studies of cortical activity related to both passive and active motor tasks in premature infants. Such study will be presented in Chapter 6.

4.3 Ankle robotic interface

With the aim of further exploring the emerging somatotopic arrangement in preterm infants I further developed a robotic ankle interface (shown in Figure 4.13) by integrating a new design with actuation, sensing, and control principles identical to those utilized for the previously described wrist robot. The idea behind this approach is that the same experimental setup (i.e. the pneumatic pipes, the fiber optic extension, and the control box/software) can be used to perform wrist and ankle stimulation via a simple plug-&-play system, where the interfaces can be conveniently switched.

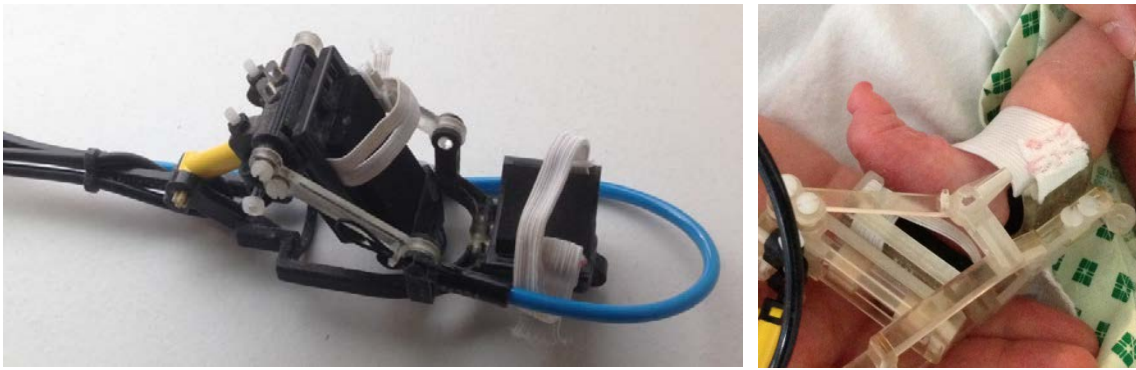


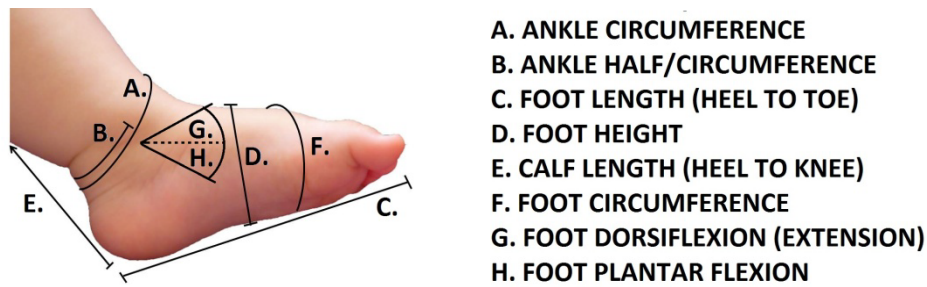
Figure 4.13: (left) The final version (v5) of the ankle interface. (right) A preterm infant equipped with the penultimate version (v4) of the ankle interface.

Biomechanical considerations

As for the previously presented wrist robot, designing and dimensioning the interface required careful consideration of the biomechanical properties of the infants' ankle as well as of the requirements reported in Table 4.1. Measurements of ankle dimensions were acquired across 6 preterm infants between 34+5 wks and 40+6 wks PMA (measures reported in Table 4.3), and it was found that all infants could comfortably achieve an open-chain (with the foot off the ground) ankle dorsiflexion/plantar flexion of 15° and 30° respectively. Angular measurements were taken just below the malleolus, an anatomical landmark which is in line with the neutral axis of ankle pronation/supination. The foot width was measured to range between ca. 20mm and 30mm (at 43+1wks PMA), and the foot length was measured to range from ca. 40mm (at 34+5wks PMA) to 70mm (at 40+6wks PMA). The interface was manufactured to accommodate the infants' foot based

on these parameters, and tailored to fit a maximum foot width of 35mm, and a maximum foot length of ca. 70 mm. No dorsiflexion was performed, and the maximum induced ankle plantar flexion was set to 20°, a range which was considered safe and sufficient to induce proprioceptive stimulation by expert clinicians.

Table 4.3: Foot and ankle measurements from 6 infants of different gestation age and weight



PMA (wks)	A. (mm)		B. (mm)		C. (mm)		D. (mm)		E. (mm)		F. (mm)		G. (°)		H. (°)	
	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R
34+5	78	75	38	36	40	40	N/A	10	78	78	72	70	25	25	55	50
37+3	83	81	36	31	50	51	13	14	86	81	N/A	N/A	20	25	30	40
43+1	100	102	48	53	65	55	16	17	105	94	96	95	15	20	50	40
40+6	105	106	37	49	70	71	20	18	106	94	95	98	20	20	60	40
41+2	82	84	44	48	65	55	16	17	104	96	96	95	15	20	50	40
34+5	79	75	35	40	44	51	15	15	63	73	75	74	30	30	30	30

4.3.1 Interface

Mechanical Design

As done for the wrist robot, the main body of the ankle interface was rapid prototyped via fused deposition of acrylonitrile butadiene styrene (ABS) (3D printer, Objet3Dpro). The body consists of a supporting structure which features a calf rest, and a pedal connected via 2 arm links. The piston was used to drive the bottom surface of the pedal, while allowed to rotate around its tail attached to the main supporting structure (Figure 4.14). With the infant's calf and foot respectively secured to the rest and pedal, plantar flexion/dorsiflexion is induced as the arm links pivot around the approximate

neutral axis of ankle. To replicate the dynamics of foot rotation and minimize ankle strain, the link and pedal system was designed to replicate the anatomical “L-shape” that links the neutral axis of the ankle and the distal plantar region of the infant’s foot (to which the pedal is fixed). The heel is left unconstrained (Figure 4.15). As for the wrist device, the interface was designed to operate in both active and passive modes. During passive mode, although the device does not need to lift the entire weight of the foot, the ankle joint was estimated to be stiffer than the wrist and to act at a ca. 6mm longer lever arm from the mechanical axis of rotation. I therefore envisaged that the actuation torque required by the ankle would closely approximate that of the wrist (30 Nmm) (Figure 4.15). The low friction required for the active mode of operation was attained by implementing the previously described arm link bearings, and by designing the attachment mechanism such that the piston could be easily unclipped and rotated in an unobtrusive position before active experiments. Further the device was equipped with a position sensor identical to that previously described.

Safety Measures

In addition to the previously described safety measures implemented at the level of the software and control box, mechanical safety was guaranteed by the geometry of the design. The pedal and the calf-rest were both allowed to slide, thus compensating for misalignments between the rotation axis of the interface and that of the ankle. Excessive ankle flexion/extension was also prevented, by geometrically defining the maximum angle of rotation that could be induced by the piston’s stroke (Figure 4.14).

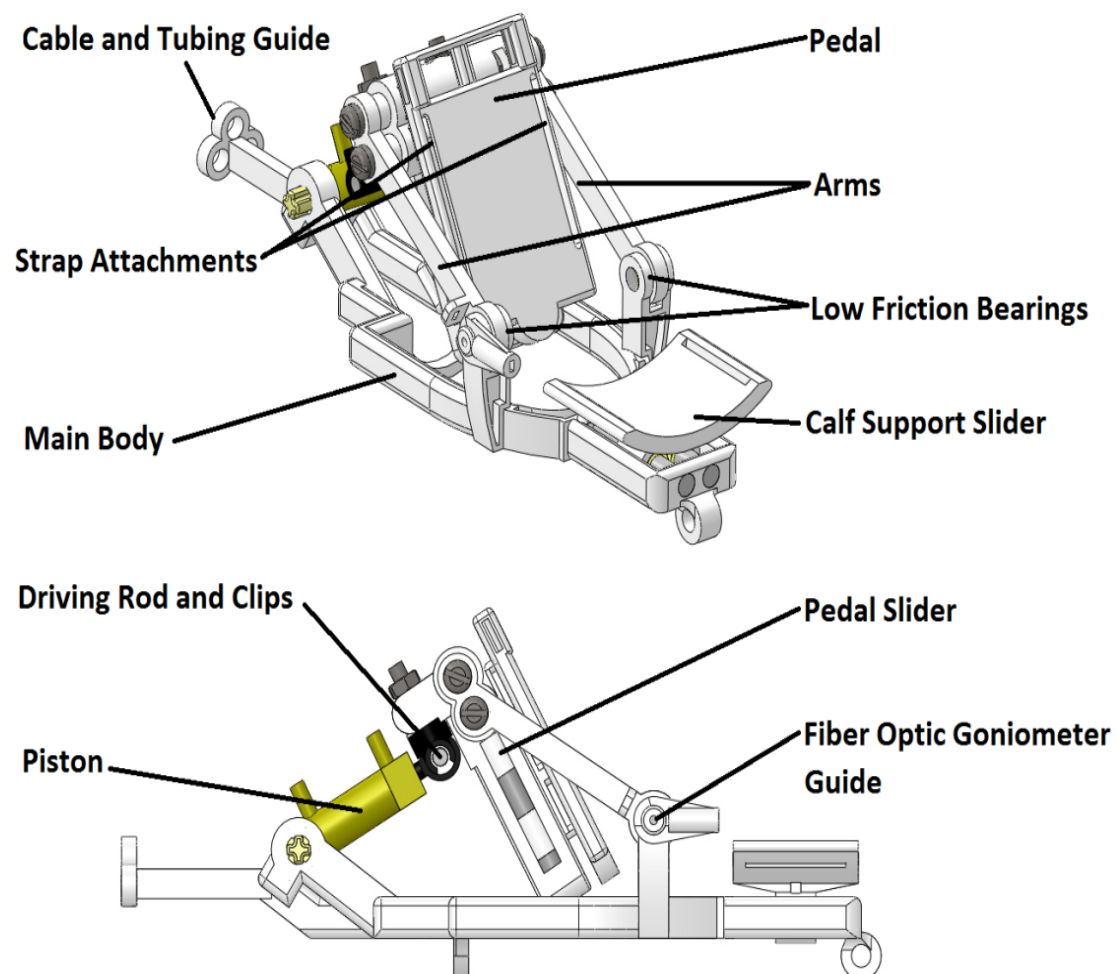


Figure 4.14: Dimetric view and side view of 3D solid models (SolidWorks, Dassault systèmes SA, Waltham MA, USA) of the ankle interface and labelled components.

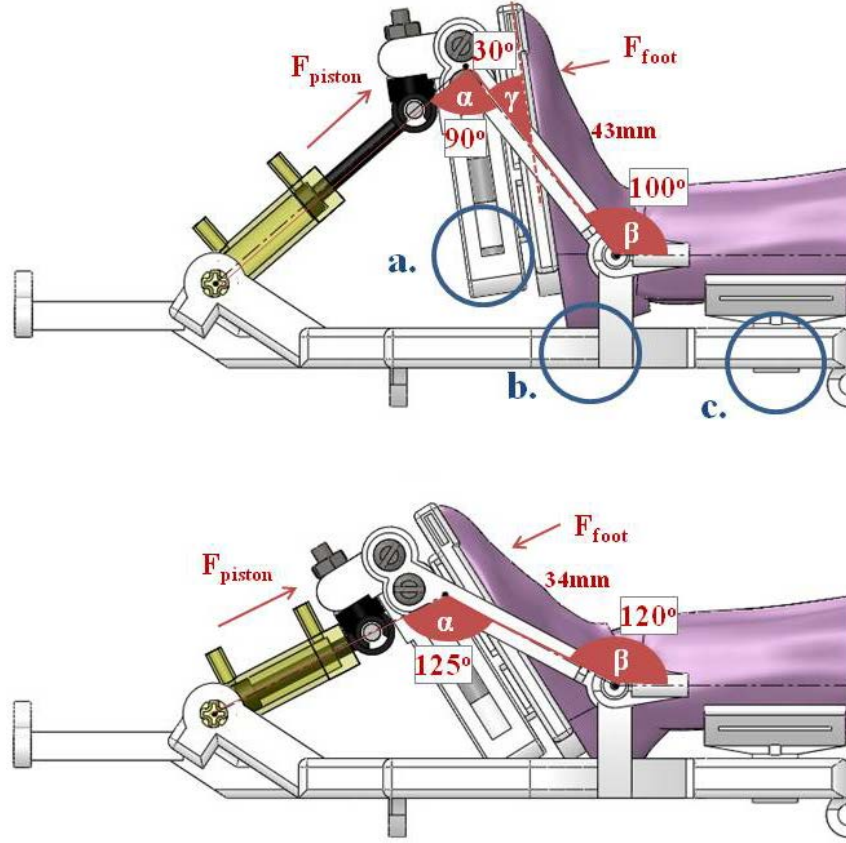


Figure 4.15: The interface has been dimensioned such that the minimum force transmitted (at full extension) would be sufficient to lift the infant's foot. At full plantar flexion (bottom), the foot exerts a maximum torque $T_{\text{foot}} = (F_{\text{foot}} \cos(\gamma) \times 43\text{mm})$ of approximately 30 Nmm, while at 0.7 atm the piston exerts a torque of approximately 38 Nmm ($F_{\text{piston}} \sin(\alpha) \times 34\text{mm}$) which is sufficient to induce ankle dorsiflexion. Mechanical safety measures: **a.** Sliding pedal, **b.** Unconstrained heel, **c.** Sliding calf support. Additionally, the design geometry limits the maximum ankle joint movement that can be induced by the piston stroke to 20° plantar flexion.

4.3.2 Experimental Validation

Images for validation were acquired on a 3-Tesla Philips Achieva MRI scanner (Best, Netherlands) located within the Neonatal Intensive Care Unit at St Thomas' Hospital, London using a 32 channel phased-array head coil. All work was approved by the NHS UK research ethics committee (ref: 12/LO/1247), and signed parental consent was taken for all subjects prior to data collection. Infant preparation, physiological monitoring and image acquisitions were performed as described in Section 4.2.3.

Subjects and Protocol

Experiments were conducted on 1 preterm subject (Infant A) born at 26 weeks PMA and scanned at 35+6 weeks PMA; and 1 ex-preterm subject (Infant B) born at 29+1 weeks PMA, and scanned at 40+1 weeks PMA. Both infants exhibited no abnormalities upon clinical examination, the preterm subject was scanned during natural sleep induced via the “feed and wrap” technique; while oral sedation (chloral hydrate 30-50 mg/kg) was administered to the term infant approximately 20 minutes prior to the beginning of the MRI scan.

To allow directly identifying, comparing and contrasting the potential somatotopic distribution of neural responses, stimulation of wrist and ankle was performed on both subjects. Infant A was equipped with a left ankle, and a left wrist stimulation robot. Infant B was equipped with a left wrist, right wrist and right ankle stimulator. Initially, only a single interface was remotely connected to the control system, however all stimulators were equipped with easily accessible connections to both the sensor and the pistons inlets. The fMRI paradigms consisted of individual 256 TRs sessions (2 sessions for infant A, 3 sessions for infant B) where 16TRs of rest were interspersed with 16TR epochs of either ankle or wrist stimulation. As in Section 4.2, wrist stimulation consisted of a 30° joint flexion/extension, while ankle stimulation consisted of a 20° plantar flexion; both were induced at a frequency of 0.3 Hz. The interface to be actuated at each session was manually selected by simply plugging the pneumatic pipes and the fibre optic extension lead into the device required.

Individual lower-level statistical analysis of fMRI data sets was performed as described in Section 6.2.4 of Chapter 6. Probabilistic tractography was carried out on diffusion MRI data acquired in 64 non-colinear directions, by generating orientations obtained via constrained spherical deconvolution (CSD) as implemented in the MRtrix software package (<http://www.brain.org.au/software/mrtrix/>), and utilizing clusters of functional activity identified via fMRI as seed regions.

4.3.3 Results

In both infant subjects, no evidence of any intracerebral damage or abnormalities was identified, following detailed review of their high-resolution structural MR images by an expert neuroradiologist. Infants did not exhibit signs of distress throughout experimental sessions.

In infant A, two distinct and well localized clusters of positive BOLD response were identified at the level of the primary somatosensory cortex contralateral to the side of stimulation in response to both wrist and ankle stimulation (Figure 4.16i). More specifically, wrist and ankle activity was respectively observed at the level of the lateral and medial post-central gyrus, which is in keeping with the mature adult somatosensory representation. Infant B also exhibited somatotopically organized clusters of positive BOLD response contralateral to the side of stimulation (Figure 4.16ii).

In both subjects probabilistic tractography from the seed areas defined as the maximally activated voxels following wrist and ankle stimulation, revealed a significant and well localised pattern of structural connectivity descending periventricularly and adjoining at the level of the PLIC, before reaching the ipsilateral cerebral peduncle. This is in agreement with the upper motor neuron axonal pathways commonly observed in adults, conferring further anatomical validation to the areas of functional connectivity identified using task-fMRI.

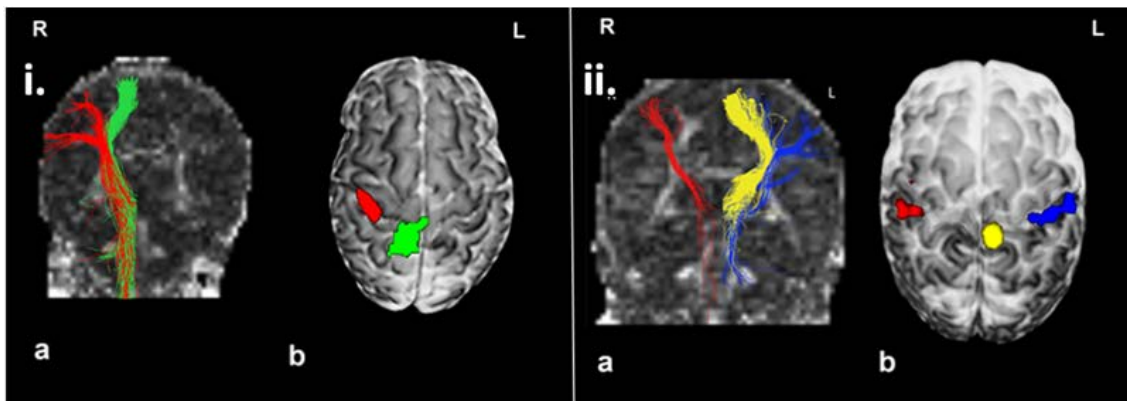


Figure 4.16: (i) Axonal pathways (a) and functional responses (b) following left wrist (red) and left ankle (green) stimulation in infant A (35+6 weeks PMA). (ii) Axonal pathways (a) and functional responses (b) following left wrist (red), right wrist (blue), and right ankle (yellow) stimulation in preterm infant B (40+1 weeks PMA).

4.4 The preterm homunculus

With the aim of robustly characterizing the preterm homunculus, the developed study protocol was extended to a wider population of preterm infants of 34 weeks median PMA at scan; population details are reported in Table 4.4. All infants exhibited no clinical abnormalities, and were scanned during natural sleep induced via the “feed and wrap” technique. Individual lower-level and higher-level group analysis of fMRI data sets were performed as described in Section 6.2.4 of Chapter 6.

Table 4.4: Preterm subjects included in the functional mapping study (the population also includes the 13 age matched group of infants in whom right wrist stimulation was performed and reported in Chapter 6)

Subject number	GA birth	PMA scan	R ankle	L ankle	R wrist	L wrist
1	33+2	34+3	X			
2	33+5	35+5	X			
3	33+3	34+4	X			X
4	31+1	32+3	X			
5	32	33	X			X
6	28+5	31+6	X			X
7	32+2	34+1	X			X
8	26	35+6		X		X

The group-level localization of functional responses elicited by passive movement of the right wrist (13 subjects, median PMA 34+1 weeks); right ankle (4 subjects, median PMA 33+4 weeks); left wrist (4 subjects, median PMA 34+2 weeks); and the left ankle (1 subject, 35+6 weeks PMA) are shown overlaid on an age-specific brain template in Figure 4.17. Stimulation of each joint was associated with well localized clusters of positive BOLD contrast contralateral to the side of stimulation. Functional responses associated with ankle and wrist movements appeared spatially distinct and respectively localized to the supra-medial and lateral perirolandic regions, suggesting that an adult-like somatotopic arrangement of the primary somatosensory cortex emerges early during the preterm period.

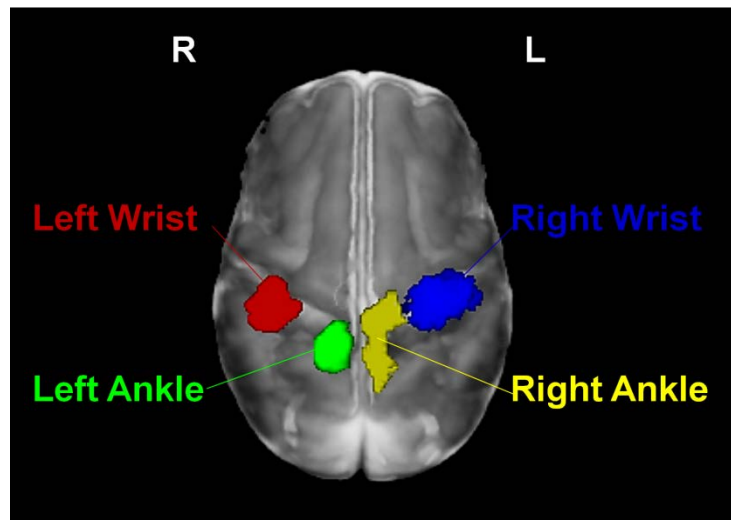


Figure 4.17: Functional mapping of the somatosensory “homunculus” in the preterm brain. Group-level functional responses induced by passive movement of the right wrist (13 subjects, median PMA 34+1 weeks); right ankle (4 subjects, median PMA 33+4 weeks); left wrist (4 subjects, median PMA 34+2 weeks); and the left ankle (1 subject, 35+6 weeks PMA) are shown overlaid on an age-specific brain template. Results indicate the emergence at preterm age (median PMA 34 wks) of an adult-like somatotopic representation within the contralateral primary somatosensory cortex, with lower limbs represented supero-medially in comparison to the upper limbs.

Contralateral, somatotopically localized functional responses were already apparent in the youngest subject studied (infant 6, 31+6 wks PMA). The cluster of positive BOLD contrast induced by right ankle stimulation can be observed in Figure 4.18.

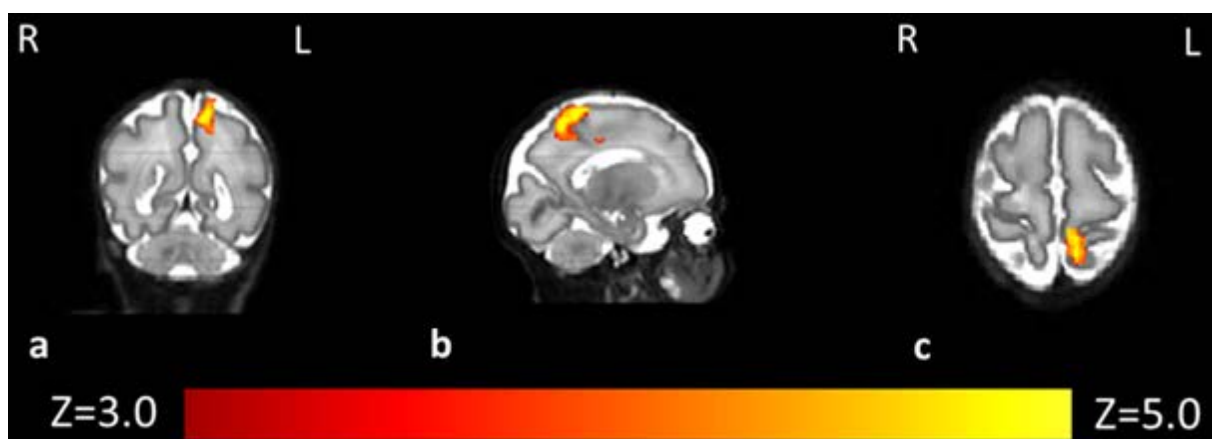


Figure 4.18: Functional response to passive right ankle stimulation is localized to the contralateral medial peri-rolandic cortical region in the infant studied at 31+6 weeks PMA.

These results are consistent with the broad spatial localization of somatosensory evoked responses previously reported by electrophysiological studies, and further highlight the specificity with which the somatotopic mapping of the wrist and ankle joints is established early in brain development. Seelke and colleagues have proposed that in rodents functional and structural specificity of the main effector organs (vibrissae) is achieved before that of other body parts (Seelke et al., 2012). Similarly, in human infants, the hands and feet are important early sensorimotor exploratory organs, which have further been proposed to play crucial role in providing the early sensory feedback necessary for the activity-dependent growth of afferent and efferent neural motor pathways (Chakrabarty and Martin, 2005, Khazipov et al., 2004). It is thus possible that the receptive fields of distal limbs achieve a structural and functional specificity early during gestation, which precedes that of other less “developmentally important” body parts. This notion could be further probed by exploring the early cortical representation of the lips and mouth, which are clearly important effector organs during the early postnatal development (attempted in Chapter 5).

4.4.1 Conclusion

In conclusion I have developed a control system and a range of robotic tools that allow objectively and systematically investigating the neural correlates of sensorimotor function in the preterm and newborn brain. The system developed in this chapter (i) helps defining a set of important performance criteria that need to be met by future neonatal fMRI stimulus systems (ii) holds the potential to elucidate some important neuroscientific notions with potential clinical implications, through population studies of preterm infants,

A new technical benchmark

In addition to the system specifications highlighted in Chapter 4, fMRI-compatible stimulation systems based on the approach here presented need to meet specific performance requirements. These can be subcategorized into actuation and sensing requirements, where actuation requirements entail the amplitude, bandwidth and delay of operation, and sensing requirements entail the accuracy, response time and range of the sensor utilized.

Stimulation Amplitude: Although, ideally, each of these parameters needs to be kept constant, the amplitude of motion represents perhaps the most important experimental confound. In the system presented, the amplitude of wrist and ankle movement is consistently repeated through the implementation of mechanical stops, geometrical considerations and an open loop control which allows the piston to overcome joint stiffness and always perform a full stroke.

Stimulation Bandwidth and Delay: The systems presented operate at a set frequency of 0.3Hz, therefore actuation bandwidth is of no concern. At this frequency of operation, the actuation delay was measured to be 630ms with a standard deviation of ± 20 ms. Considering that the HRF response model used in the analysis was sampled at a temporal resolution of 500ms (2Hz), it is recommended that the delay's standard deviation is kept below 250ms. However this tolerance varies according to the HRF model used, and these considerations may not apply to non-GLM based fMRI analysis.

Sensing Requirements: The system presented features a fiber optic sensor with a response time in the order of microseconds sampled 100 Hz. While a sampling frequency of only 8Hz (to ascertain the is accurate to 250ms) may be sufficient to ensure that passive stimulation is appropriately delivered, higher sampling frequencies can be used to improve the sensor's SNR. This is particularly useful during spontaneous joint movement analysis, as the detected motion is converted into a scalar value through averaging across each TR. A sampling frequency of at least 1kHz is required if the user were to implement a real-time closed-loop control. The sensor's range should encompass the entire range of motion of the device, and its accuracy should be sufficient to detect the minimum movement of interest. As infants usually perform high-amplitude jerky movements, a resolution of $\pm 1.1^\circ$ over a 30° range of motion was considered suitable for this application.

Neuroscientific and clinical potential

So far I have used wrist and ankle stimulators to reveal, for the first time, that a spatially well-defined somatotopic arrangement of the somatosensory cortex is already apparent during the mid preterm period, and that spontaneous movements are also associated with cortical functional activity. It is hopeful that in the future, my approach can be used to further characterize the maturation of the

body's functional representation throughout the early preterm period, thus providing an anatomical template which can be used to guide functional studies, such as resting-state fMRI, where prior assumptions on functional seed regions need to be made. Furthermore, I envisage that the ability to monitor the functional development of both the somatosensory and primary motor cortex could be crucial in understanding the pathophysiology underlying the development of neurological disorders such as CP. A dynamic assessment of cerebral function with robotic tools and fMRI could integrate and complement other imaging techniques such as diffusion imaging to provide a comprehensive understanding of the macroscopic consequences of early brain injury. Such crucial data may also provide a means with which to accurately identify which infants will develop later adverse clinical outcome; thereby providing families with much needed prognostic information, and allowing clinicians to initiate the necessary rehabilitative therapies in an early and timely manner.

Chapter 5: Novel robotic tools to systematically characterize functional processing of sensory input in the preterm brain via task-fMRI

Overview – A major aim of this thesis is to develop a standard approach and a set of tools to conduct multisensory task-dependent fMRI studies of the preterm and neonatal populations in a robust and systematic manner. In this Chapter I aimed at expanding the range of sensory stimulations that can be presented by our system, by developing (i) an orocutaneous stimulator (ii) an olfactory stimulator (olfactometer). The orocutaneous and olfactory stimuli were selected to probe the proposed theory (reported in Chapter 4) that the functional representation of developmentally important effectors may already be well established by the time of birth. Furthermore, the pacifier may allow to further investigate the early differentiation of the somatosensory homunculus, and to reveal intracerebral correlates of spontaneous sucking activity. On the other hand, the emergence of the olfactory function, despite its developmental importance, has never been studied in newborns. Finally, both stimulators may be suitable for future experiments aimed at studying the effects of conditioning.

Acknowledgment of contributions

The consenting, preparation and scanning of infant subjects was performed in collaboration with the research, nursing and neuroradiologist team of the Centre for the Developing Brain, King's College, St Thomas' Hospital London; formerly part of the Weston group, Imperial College, Queen Charlotte and Chelsea Hospital London.

The fMRI data collection and analysis presented in Section 5.1 (the pacifier study) was performed in collaboration with Dr. Tomoki Arichi. The pacifier system was developed and operated by the author.

The fMRI data collection and analysis presented in Section 5.2 (the olfactory study) was performed in collaboration with Dr. Tomoki Arichi and Dr. Richard Gordon-Williams. The olfactometer system was developed and operated by the author. The medical-grade, odour delivery components were sourced by Dr. Richard Gordon-Williams.

Section 5.2 of this Chapter contains excerpts and figures that were published in:

Arichi, T., R. Gordon-Williams, A. Allievi, A.M. Groves, E. Burdet and A. D. Edwards (2013). "Computer-controlled stimulation for functional magnetic resonance imaging studies of the neonatal olfactory system." *Acta Paediatr* 102(9): 868-875.

5.1 An instrumented pacifier to investigate the neural correlates of non-nutritive sucking and orocutaneous stimulation

The sucking motor skill, perhaps due to its evolutionary importance, emerges in utero between the 15th and 18th weeks of gestation (Miller et al., 2003), with a patterned oro-rhythmic behaviour subsequently developing between gestation weeks 28 and 34 (Hack et al., 1985). The first, primitive sucking motor behaviour has been termed non-nutritive sucking (NNS), a repetitive activity of the mouth on a blind nipple (Wolff, 1968). NNS has been observed in preterm infants as young as 26 weeks PMA (Hafstrom and Kjellmer, 2001), and typically consists of short bursts of up to 6 sucks occurring at approximately 2 Hz, interspersed by rest periods to accommodate breathing (Barlow et al., 2010). Throughout the late gestation period, this somatic motor rhythm is controlled via a suck central pattern generator (sCPG), a neural network extending bilaterally within structures of the brainstem (Barlow et al., 2010, Tanaka et al., 1999). Crucially, behavioural studies indicate that the sCPG is highly responsive to direct external stimulations such as tactile touch, with newborns having been shown to synchronize their sucking patterns to externally induced orocutaneous stimulation. This concept is being exploited in order to “train” sucking through actuated pacifiers (Finan and Barlow, 1998). Animal studies have revealed the existence of feedback connectivity modulating the oro-rhythmic sCPG output, which entails bidirectional projections from the cerebellum and descending pathways originating from the sensorimotor cortex (Barlow et al., 2012, Byrant et al., 2007, Boughter and Bachmanov, 2007). Barlow and colleagues have proposed that delivering stimulations to the mechanoreceptors within the newborn’s lips, tongue, and jaw, creates a flow of synchronous sensory information which triggers a feedback loop between the cranial nerves and orofacial regions of the sensorimotor cortex (connected via thalamocortical projections) that modulates sucking activity (Barlow, 2009a, Barlow, 2009b).

To the best of the author’s knowledge, only two studies have attempted to validate some of the afore-described hypotheses based on animal studies. These studies have directly investigated brain function at the level of the parietal lobes via electroencephalography (EEG), both after (Song et al., 2014, Barlow et al., 2014) and during (Song et al., 2014) periods of orocutaneous stimulation. Their results are consistent with the notion of an early cortical involvement in the processing and

adaptation to external stimuli, further suggesting the potential for orocutaneous therapy to enhance brain reorganization and development through activity-driven plasticity (Song et al., 2014, Barlow et al., 2014).

With mounting evidence suggesting that sucking is the result of a complex sensorimotor process (Barlow et al., 2008), and in line with one of the major goals of this thesis, I aimed to develop a robust and systematic tool to study oro-cutaneous as well as oro-motor neural correlates in preterm infants and neonates using task-dependent fMRI. This would allow the verification of the involvement of early cerebral areas responsible for the processing and modulation of sucking activity, and establishing their somatotopic mapping. Furthermore, in view of the correlation that exists between early sucking competences and overall cerebral maturation and condition, as well as the later neurodevelopmental outcome (Mizuno and Ueda, 2005, Medoff-Cooper et al., 2009), this approach may be crucial for devising early clinical biomarkers and assess therapeutic interventions.

State of the art

The concept of instrumenting a pacifier to quantitatively assess NNS has been previously explored. Since 1996, Finan and Barlow have developed various embodiments of the “actifier”, a pneumatically actuated pacifier which aims to “abilitate” infant sucking by reproducing the “optimal NNS pattern” and to also provide an objective analogue measure of the intraluminal pressure of a pacifier (Barlow et al., 2008, Finan and Barlow, 1996). The most recent version of the actifier can be coupled to commercial Philips (<http://www.healthcare.philips.com>; Eindhoven, Netherlands) pacifiers and induces pacifier vibration by creating a vacuum within the pacifier lumen utilizing a position-controlled pneumatic linear servo motor. The device (depicted in Figure 5.1) features a receiver spherical head instrumented with a stainless steel cannula, which connects to the piston tubing, and is coupled in parallel to a pressure transducer via a Lure fitting. As reported in the design development section (Section 5.2.3), such a configuration can, however, lead to major pressure reading inaccuracies (especially for sustained pressure), as the air within the pacifier is allowed to backflow through the length of the piston tubing. To the best of the author’s knowledge the actifier system does not allow the pressure to be accurately monitored while stimulation is being delivered.

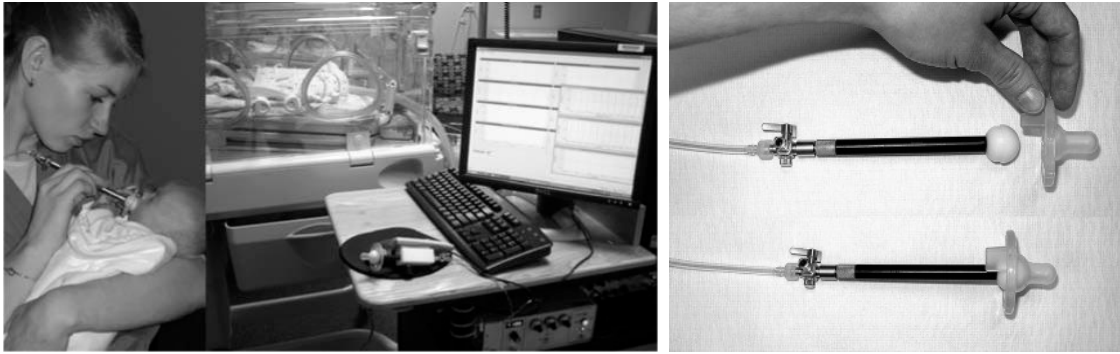


Figure 5.1: The actifier interface (left), used to “train” infants who exhibit uncoordinated patterns of non-nutritive sucking (right). Images reproduced from (Poore et al., 2008)

While achieving pacifier actuation poses several challenges, a range of instrumented pacifiers have been developed to test conditioning of the sCPG. For instance, in 2003 Bingham and colleagues fitted a pressure sensor and milk-impregnated gauze within a perforated pacifier. This led the authors to suggest that milk odour cues may encourage NNS in premature infants (Bingham et al., 2003). However, pressure amplitudes were not reported and readings are likely to have been biased by pacifier perforation (Bingham et al., 2003). The pacifier activated lullaby (PAL) system envisaged by Standley is instrumented with a pressure transducer and used to assess music as a clinical tool in the neonatal intensive care unit (NICU) setting with preterm infants (Standley, 2003). The pacifier provides a reinforcement cue in the form of a nursing song upon detection of a correct sucking pattern. However pressure recordings are not explicitly reported.

The challenge of correctly quantifying non-nutritive sucking pressure is apparent in the literature, with readings being highly dependent on the methodology used for sensing. For instance, in 2007 Boiron and colleagues used collected data from preterm infants via a catheter of unspecified length whose head was fitted within the pacifier nipple and the tail was connected to a pressure transducer. They indicated that the typical amplitude of the pressure recorded was of ca. 1 mmHg (Boiron et al., 2007). The actifier can record intraluminal pacifier pressures of between 14 and 36 mmHg across preterm infants of similar gestation age (GA) (Estep et al., 2008, Stumm et al., 2008). In 1996, Prieto and colleagues used a water filled catheter to measure nutritive-sucking pressures in newborns around the mother’s nipple. They reported pressures ranging from 50 to 197 mmHg (Prieto et al., 1996).

With these challenges in mind, I aimed to develop an MR-safe, instrumented, actuated pacifier able to objectively and reliably monitor NNS pressure in preterm infants. As this device is to be used in conjunction with fMRI in order to infer neural correlates of both oro-motor and oro-cutaneous cues, pressure reading should also be carried out throughout epochs of stimulation as to reveal potential oro-motor activity.

5.1.1 Stimulus system design and operation

Overview and specifications

The oro-cutaneous somatosensory stimulator comprises a neonatal pacifier instrumented with a commercial fibre optic pressure sensor and a custom inflatable balloon. The system (features and specifications reported in Table 5.1) is able to detect spontaneous patterns of sucking, and induce sustained or vibratory oro-cutaneous stimulation to the infant’s lips. Data acquisition and pneumatic control are fully automated and synchronized with fMRI image acquisitions.

The interface was engineered to satisfy the stringent safety and ethical requirements inherent to the studied population. In addition to meeting the MR safety criteria (Gassert et al., 2008), the instrumentation is entirely contained within a removable “plug” that can be interchangeably fitted within commercial pacifiers designed for the preterm and term population. This allows utilizing a new, sterile pacifier for each subject.

Table 5.1: Features and specifications of the pacifier.

Features	Specifications
MR safe	Pressure accuracy ± 1 mmHg
Sterile	Pressure range -50 mmHg to 300 mmHg
TR synchronized actuation and monitoring	Synchronous data sampling rate 100 Hz
Fits standard Philips neonatal pacifiers	

Interface

In addition to being specifically tailored to the preterm and term populations, the Philips pacifier features a back aperture into which experimental instrumentation can be inserted. The aperture was found to measure 19 mm across the range of pacifiers designed for infants from 30 wks post-menstrual age (PMA) to 3 months post-natal age (PNA): including the Wee Soothie[®] (30–34 weeks PMA), the NICU Soothie[®] (34 weeks – term) and the Soothie[®] (0-3 months PNA).

In view of these considerations, and owing to the MR safety requirements, a plug measuring 20 mm in diameter was rapid-prototyped via fused deposition modelling of ABS such that it could simply be press-fitted within the Philips pacifiers to form an airtight seal (Figure 5.2c). The plug was designed to be a cylinder, hollowed and tapered to reduce its weight and to facilitate pacifier insertion. Additionally, 2 holes (1 threaded, M5 and 1 non-threaded 4mm outside diameter (od)) were designed with the specific aim of hosting the pressure sensor and the pneumatic tube stemming from the balloon (Figure 5.2a). The holes were centrally positioned within the plug, and the convergence angle of the balloon hole was set to 87 degrees, such that: i) the balloon could easily slide into place upon insertion of the pacifier, and ii) the sensor tip could be located as close to the centre as possible, therefore providing reliable measurements of the overall pressure change. Since obtaining accurate pressure readings required the pacifier chamber to be maintained fully airtight (see Section 5.2.3), the balloon tubing was epoxy glued within its plug hole. The pressure sensor was instead fitted epoxy glued within a nylon M5 screw, which was drilled to accommodate the sensor tip (1.06 mm od); this was done by Opsens (www.opsens.com; Quebec, Canada), as per my instructions. The sensor tip was then screwed into the corresponding plug hole (Figure 5.2b). In addition to keeping the pacifier airtight, this configuration also allows easy insertion and removal of the sensor from the plug.

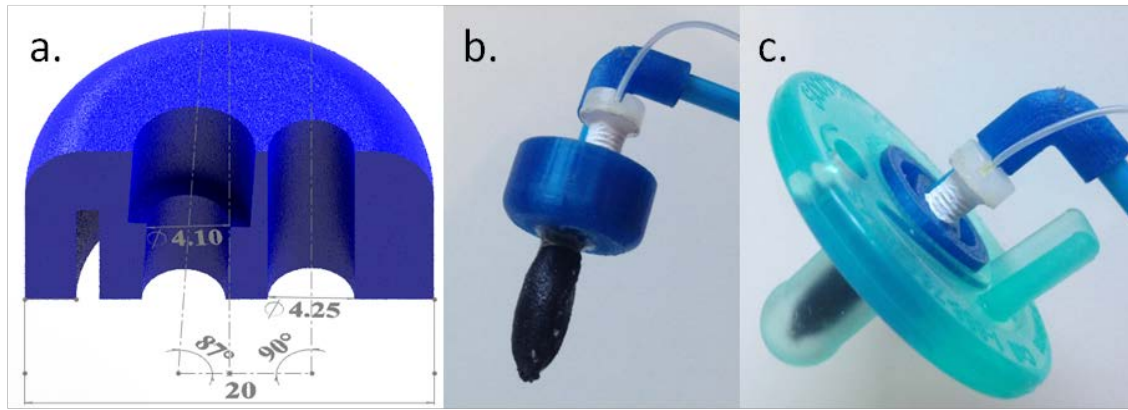


Figure 5.2 The interface consists of a “plug” (a) designed to accommodate a balloon and a pressure sensor (b), and dimensioned to fit within the commercial range of Philips neonatal pacifiers (c).

Balloon

The balloon was manufactured in accordance with the methodology reported by Arichi and colleagues in 2010 (Arichi et al., 2010), and is composed of a nylon mesh contained within 2 layers of latex rubber. It is shaped as an elongated ellipsoid with cross sectional width measuring approximately 5 mm, such that it can easily slip/rest within the pacifier’s lumen (Figure 5.2b,c). Melted silicone glue was used to fit the balloon onto a 4 mm od pneumatic pipe. Due to the space restrictions imposed by the proximity of the head coil to the infant's mouth, the pneumatic tubing was cut to a length of 10 mm past the plug, and fitted with a rapid-prototyped elbow junction.

In the absence of a robust method to accurately establish the balloon’s volumetric change with pressure, the optimal pressure of actuation was judged empirically. The pressure was manually adjusted and fixed via the air flow regulator (LRMA series, Festo Corp., Essling am Neckar, DE), and the balloon was allowed to expand until touching the inner surface of the pacifier’s chamber. This was achieved at an intraluminal pressure of ca. 80mmHg. It was found that this amplitude, combined with 1 Hz stimulation, could clearly be felt by an adult subject within the scanner. In practice, the balloon was tested until inducing up to 160mmHg of positive intraluminal pressure, and no safety issues became apparent.

Pressure sensor

The pressure sensor Opsens (OPP-M40) was selected due to it being fully MR safe, and commonly used for various medical applications. Furthermore, its measurement range (-50 mmHg to 300 mmHg) encompasses (in fact exceeds) the range of sucking pressures reported in previous studies of the neonatal population (between 50 and 197mmHg) (Prieto et al., 1996). The reduced size of the sensor (1.06mm) allowed for it to be easily fitted within a (relatively small) M4 nylon screw (Figure 5.3a); this was crucial considering the limited space available on the cap's surface. The sensor system is based on a proprietary technology which has been termed "White-Light Polarization Interferometry" (WLPI); a version of standard fibre optic interferometry (i.e. the measured quantity is inferred from the phase modulation of the light radiation) that utilizes a broadband (white) light source. The sensor tip (the sensing interferometer) contains a diaphragm and a dielectric mirror separated by a hollow chamber. The light is emitted and received from below the mirror such that it is projected onto the membrane before travelling back through the mirror to the signal conditioner (the readout interferometer). In keeping with Fabry-Perot's principle, pressure changes cause displacements of the diaphragm, which in turn causes the peaks in the measured light spectrum to move closer together and further away in a sinusoidal pattern that gradually decreases in amplitude as the distance between the membrane and the mirror increases. This modulation is monitored in real time by the measuring interferometer to assess the membrane position, and thus to infer pressure.

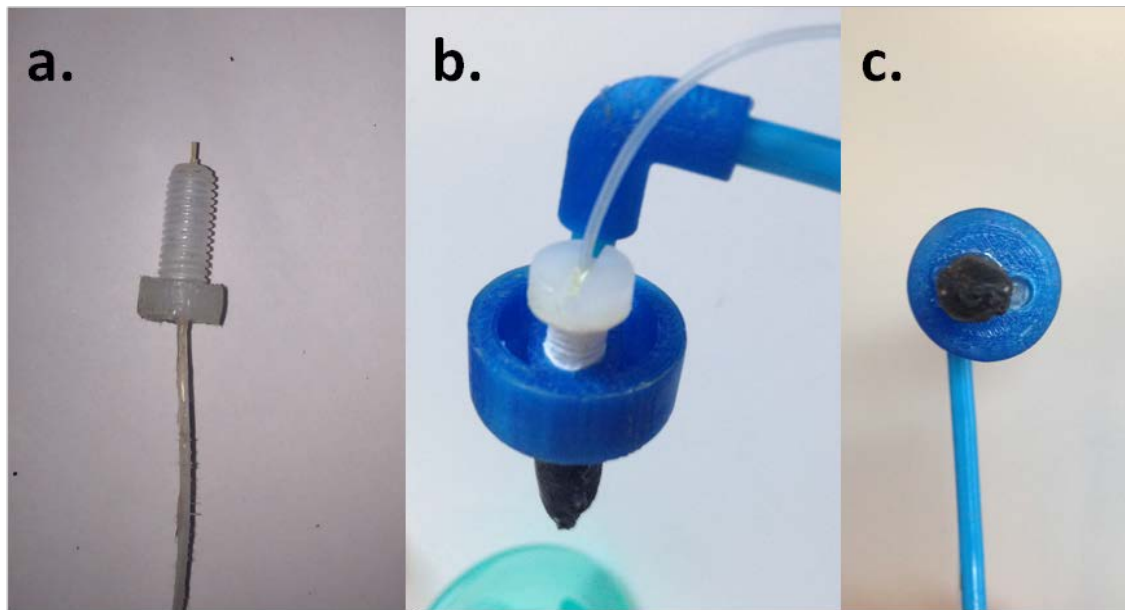


Figure 5.3: The OPP-M40 pressure sensor is epoxy glued within an M4 nylon screw (a) such that it can be screwed into the plug forming an airtight seal (b); the sensor tip is levelled with the inner surface of the plug (c).

Control box architecture

To ensure full MR safety of the experimental set-up, the interface was connected to a control box located outside the scanner room by means of 6 m pneumatic tubing and a 10 m fiber optic extension cable. Although a custom control unit was developed for the pacifier and is presented in the Section 5.2.3 below, because of technical issues the control box utilized for the validation experiments in neonatal subjects described in this section is that used in Chapter 4 (Section 4.2.2), with the analogue input of the pressure sensor conditioning unit replacing that of my custom position sensor. The DAQ (USB-6008, National Instruments Corp., Austin, TX, USA) operating at a sampling rate of 100 Hz, was used to read the TTL pulse produced by the scanner with each TR to ensure pacifier actuation and pressure data acquisition was synchronized with fMRI image acquisitions. Vibratory pneumatic actuation was delivered via a single proportional valve (MPPES series, Festo Corp., Essling am Neckar, DE). The signal from the pressure sensor was transduced, amplified and sampled via a conditioning unit (OEM-MNP, Opsens Inc., Quebec, CA) operating at a sampling rate of 250 Hz. The pressure data was then converted and output to the DAQ via an analogue port. The system was controlled at 100 Hz via a standard PC running custom LabView software (National Instruments Corp., Austin, TX, USA) based on that presented in Chapter 4 (Section 4.2.2).

Software

With the pressure sensor positioned within the pacifier, the signal conditioner was reset and calibrated via SoftSens (Opsens proprietary software, www.opsens.com) through serial communication (port RS-232). Except for the different declaration of virtual channels (due to the different equipment used), the LabView software implemented throughout the studies conducted in neonates is similar to that previously described in Chapter 4 (section 4.2.2). A total of only 3 virtual channels were defined: a digital output channel to control the proportional valve via an analogue square wave of 1 Hz frequency and 30% duty cycle, a digital input channel to acquire the scanner TTL pulses, and an analogue input channel to sample the pressure sensor data. As in my previous studies, both valve control and data logging were performed at a sampling rate of 100 Hz by the DAQ; pressure data was sampled in parallel directly from the interferometer via the SoftSens software at a sampling rate of 250 Hz.

Safety measures

Multiple safety measures were implemented and were mainly aimed at limiting the air pressure delivered to the balloon. Firstly, the compressed air supplied to the system was limited via the manual air flow regulator; as described in Section 5.1.1 (balloon); secondly, a software interrupt was implemented to halt valve operation upon detection of intraluminal pressure higher than 120mmHg to account for both the pressure change induced by the balloon (80mmHg), and by additional potential spontaneous sucking activity, while maintaining the device within its tested safety range (up to 160 mmHg, as reported in Section 5.1.1(balloon)). Additionally, on-screen live pressure monitoring, as well as physical and software emergency switches were provided to the experimenters, such that the paradigm could be manually halted at any time.

5.1.2 Experimental Validation

Images were acquired on a 3-Tesla Philips Achieva MRI scanner (Best, Netherlands) located within the Neonatal Intensive Care Unit at St Thomas' Hospital, London using a 16 channel phased-array head coil. All work was approved by the NHS UK research ethics committee, and signed parental consent was taken from all subjects prior to data collection. Infant preparation,

physiological monitoring and image acquisition were performed as described in Chapter 4 (Section 4.2.3).

Study population and experimental design

Experiments were carried out on 2 preterm twins born at 27+5 weeks of gestation and scanned at 32+5 weeks of gestation. The infants exhibited no abnormalities upon clinical examination performed by a paediatrician. Both infants were scanned during natural sleep induced via the feed and wrap technique.

The experimental protocol consisted in a block paradigm, in which 16 TR (24 s) epochs of rest were interspersed with 16 TR epochs during which oro-cutaneous somatosensory stimulation was induced by 1 Hz vibration of the pacifier. Pressure within the pacifier chamber was monitored and recorded throughout the experiment to ascertain correct pacifier actuation as well as to identify patterns of spontaneous sucking. An experimenter was required to stand near the scanner throughout acquisition and hold the pacifier to avoid it from falling out of the infant's mouth.

Results

Both subjects exhibited extreme patterns of motion. The fMRI datasets were therefore severely corrupted, and had to be discarded. Furthermore, due to poor visibility, the experimenter was unable to hold the pacifier within the mouth of infant A throughout the entire scan. The issue of visibility was partially resolved with the addition of a mirror above the head coil, prior to scanning infant B.

While the fMRI dataset of infant B had to be discarded, patterns of passive stimulation as well as occurrences of spontaneous non-nutritive sucking were clearly visible within the pressure data collected throughout the experiment; these are shown in Figure 5.4. The pattern of spontaneous non-nutritive sucking occurred with a frequency of approximately 1.3Hz, with a peak-to-peak amplitude of ca. 40 mmHg. Moreover, the recorded pressure profile also suggests the presence of a transient period in which the infant was sucking during oro-cutaneous stimulation. Although a slow drift in baseline intraluminal pressure was noted throughout blocks of stimulation, with the peak-to-peak amplitude of the stimulus also increasing slightly, the frequency of stimulation was consistent throughout the session.

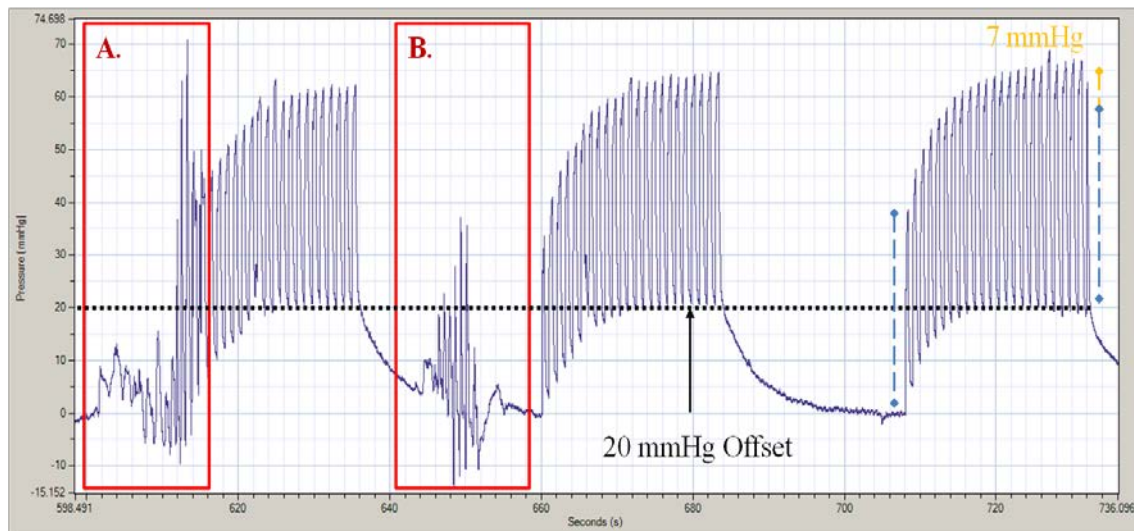


Figure 5.4: Intraluminal pressure trace during infant B's fMRI session. Spontaneous transients of non-nutritive sucking can be distinguished both during epochs of stimulation (box A) and rest (box B). Pacifier actuation induces a drift in baseline intraluminal pressure that reaches a steady state at 20mmHg. The peak-to-peak amplitude of pressure pulses increases by a total of ca.17% throughout the 24s blocks of stimulation.

Discussion

While the cortical correlates of oro-cutaneous stimulation could not be studied at this time, there is evidence described below that the device is suitable for future investigations. In addition to its ability to produce and monitor robust patterns of stimulation, the instrumented pacifier system can also be used to simultaneously record spontaneous patterns of non-nutritive sucking. Much like the previously reported proprioceptive stimulator, this device could therefore potentially be used not only to characterize the oro-cutaneous cortical correlates, but also to reveal potential cortical areas involved in the modulation, planning and execution of oro-motor tasks. In contrast to the wrist stimulator, however, this approach has the further advantage of being able to determine active and passive oral tasks during the same scanning session. These readings can be input as separate confounds within the GLM representation of fMRI data, potentially enhancing the statistical power with which patterns of functional activations in response to the separate task conditions can be contrasted and/or discriminated.

The large head movements exhibited by both subjects were the result of vigorous whole body motion during the experiment. This is attributed to the long 1 hour scanning session that both infants had already undergone by the time they were presented with the oro-cutaneous stimulation paradigm. This likely resulted in the gradual awakening of the subjects. It is possible that large-scale head motion may be avoided by ensuring that the experiment is conducted with the infants are asleep or sedated. However, a primary concern is that spontaneous sucking could still induce minor head “nodding”.

Ensuring that the pacifier is kept within the infant’s mouth throughout the scanning session is particularly challenging. Presently, this is done manually by an experimenter standing near the infant, however locating the mouth of the infant or even ascertaining that the pacifier is still within it is rendered difficult by the poor visibility. A potential means of solving this issue (pending ethical approval) is to equip the device with a weak elastic strap similar to that of standard continuous positive airway pressure (CPAP) masks.

Finally, in the study described, the control architecture originally developed to control the pacifier system (see Section 5.1.3 below) could not be used due to an instrumentation issue on the day of the experiment. The 3 on/off valve system (VX series, SMC Corp., Tokyo, JP) able to alternate phases of vacuum, positive pressure and pressure relief was essentially replaced with a single proportional valve. Compared to on/off valve system, the proportional valve has the inherent advantage of being able to provide both positive pressure and pressure relief. Additionally, it provides an intrinsic redundant safety measure, as input pressure is controlled both via the manual flow regulator, and by the proportional valve itself. However, despite the implemented PID controller architecture described in Chapter 4 (Section 4.2.2), it still presents a higher dynamic response delay, which resulted in a slow drift in baseline pressure within the pacifier lumen as the balloon was unable to fully deflate between pulses. While this effect was consistent throughout the experiment and does not therefore represent a serious confound, it is suggested that future studies utilize the original control architecture to enhance the bandwidth of stimulation frequencies and avoid luminal pressure drifts.

5.1.3 Concept development and initial pilot studies

The final oro-cutaneous somatosensory stimulator is the result of several iterations as well as continuous observations and improvements carried out in collaboration with two Bioengineering MsC students at Imperial College. The first version of the device was based on a markedly different operating principle; instead of providing a simple vibratory stimulus, the pacifier was designed to fully deflate and re-inflate within the subject's mouth. It was proposed that stimulation could be induced via epochs of quick inflation/deflation, while prolonged epochs of deflation would discourage infants from sucking thus inducing a "rest" state. Throughout development, it was noted that maintaining the pacifier chamber airtight and maintaining the inner surface of the plug (made from a rod of nylon 6-6) fully flat was fundamental in achieving sustained accurate pressure readings, instead of simple pressure spikes (Figure 5.5a,b). In this respect, a configuration similar to that utilized by Barlow and colleagues in which a pneumatic tube is connected directly to the plug could not be implemented. When the pacifier is compressed, the air within it escapes through the piping and only a "spike" of pressure is recorded. Moreover, the diameter and dimension of the pipe are also likely to affect the amplitude of peaks observed at faster compression rates. Achieving deflation/inflation, while maintaining accurate sensing therefore ultimately required fitting the interface with two inlets, equipped with 1-way valves oriented in opposing directions (Figure 5.5c). A vacuum generator, as well as a system of 3 on-off valves had to then be implemented within the control box to produce the required pattern of actuation, while also being able to monitor air pressure (Figure 5.6).

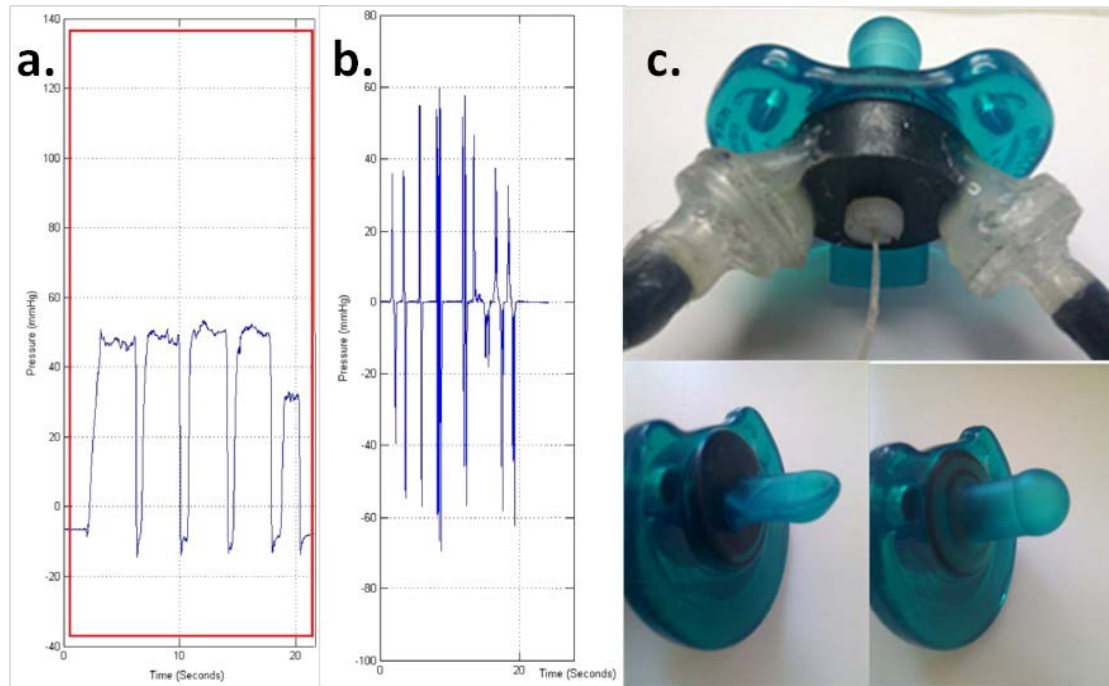


Figure 5.5: Patterns of sustained pressure can be detected by an airtight device (a), while non-airtight configurations only reveal rapid pressure spikes (b). The first airtight pacifier, designed to provide full nipple deflation/inflation (c).

The device was tested on a sample of 5 healthy adult volunteers. The experimental paradigm was consistent with that used throughout the studies reported in this thesis, and involved 16 TRs (TR=1.5s) of rest during which the pacifier remained deflated, interspersed with 16 TR epochs during which the pacifier was allowed to re-inflate and the subject instructed to perform spontaneous sucking. The subject was also required to manually hold the pacifier within his or her mouth throughout the protocol.

Although the study was able to identify areas of functional activity within the primary motor cortex, the device still suffered from several limitations. The plastic 1-way valves utilized (no return check valves designed for aquariums) were large and required using copious amounts of epoxy glue to be secured within the plug. Furthermore, they suffered from leakage such that applying positive pressure to seal the channel previously used to deflate the lumen via a vacuum at times resulted in over-inflation with the pacifier being projected from the plug.

The device was therefore redesigned in accordance with the final concept. A quasi-final version of the interface was developed by complementing a nylon 6-6 plug with a balloon. The final balloon shape was defined after several iterations aimed at qualitatively assessing their practicality. The final

control software was designed, in which the balloon vibration is controlled via a simple 3 state-machine. During epochs of stimulation, the controller utilizes 2 de-phased square waves to alternately switch between 2 configurations of on-off solenoid valves, respectively delivering vacuum and positive pressure pulses. This allows the driving of both inflation and deflation phases of the balloon vibration. During epochs of rest, a 3rd configuration of the solenoid valves then releases all pressure from the balloon and equalises it with that of the surroundings.

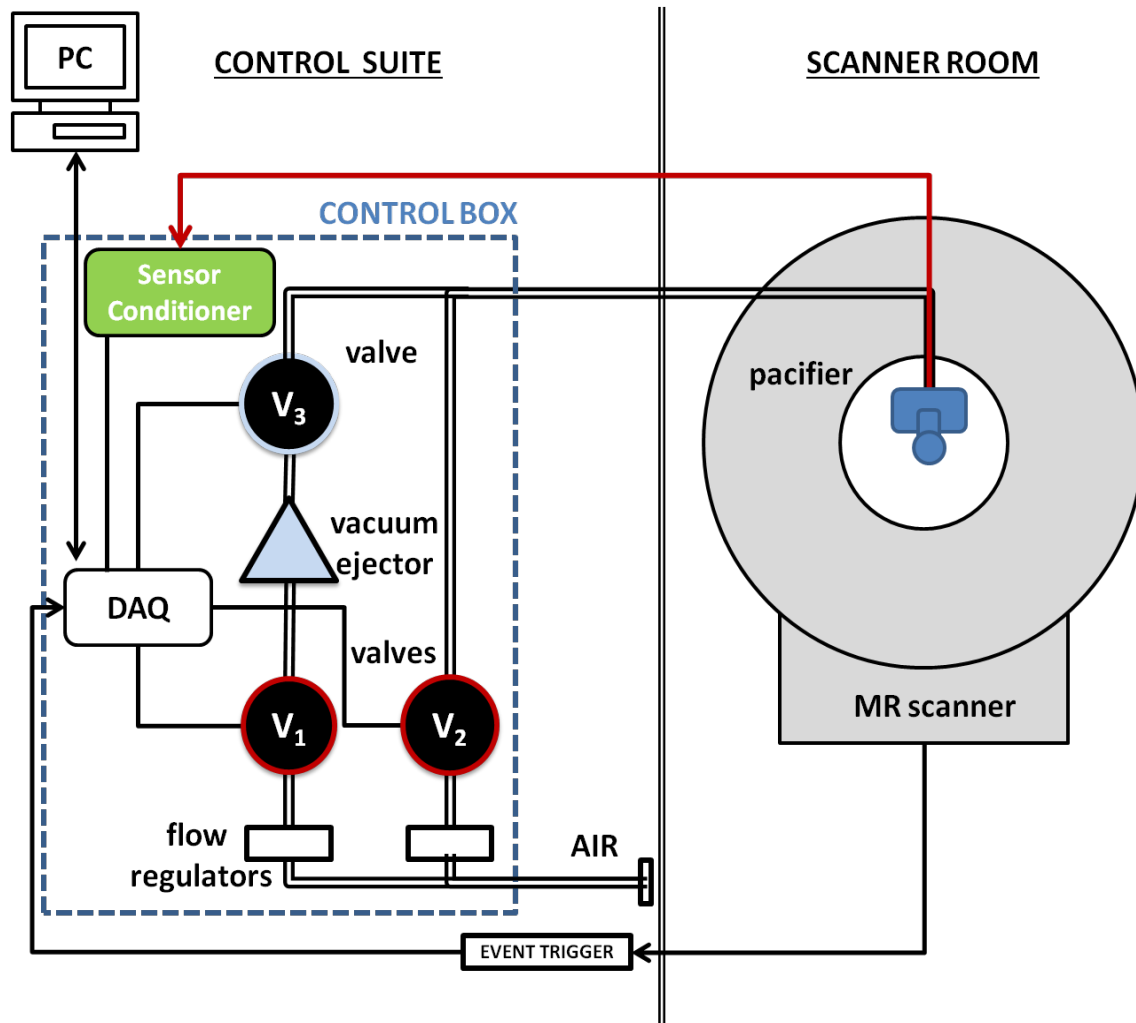


Figure 5.6: The final system architecture. The control box contains 3 on-off solenoid valves (SMC, VX series), a vacuum ejector (SMC, ZH series), 2 flow regulators (SMC, ARM series), a sensor conditioning unit (Opsens, OEM-MNP), and a DAQ (National Instruments, usb-6008). Three valve states can be used to achieve vacuum (Valve 1 ON, Valve 2 OFF, Valve 3 ON); positive pressure (Valve 1 ON, Valve 2 ON, Valve 3 OFF); and pressure compensation (Valve 1 OFF, Valve 2 OFF, Valve 3 ON).

A validation study was then conducted on 1 adult volunteer undergoing 3 different stimulation paradigms, and the results were compared against the known adult somatotopic arrangement. The experimental sessions comprised:

-A “passive” event-related paradigm, in which the subject was simply presented with random short sequences of passive stimulation.

This study revealed a bilateral region of functional activation in a region of the primary somatosensory area which roughly corresponds to that associated with the processing of sensory information gums, tongue and lips (Figure 5.7).

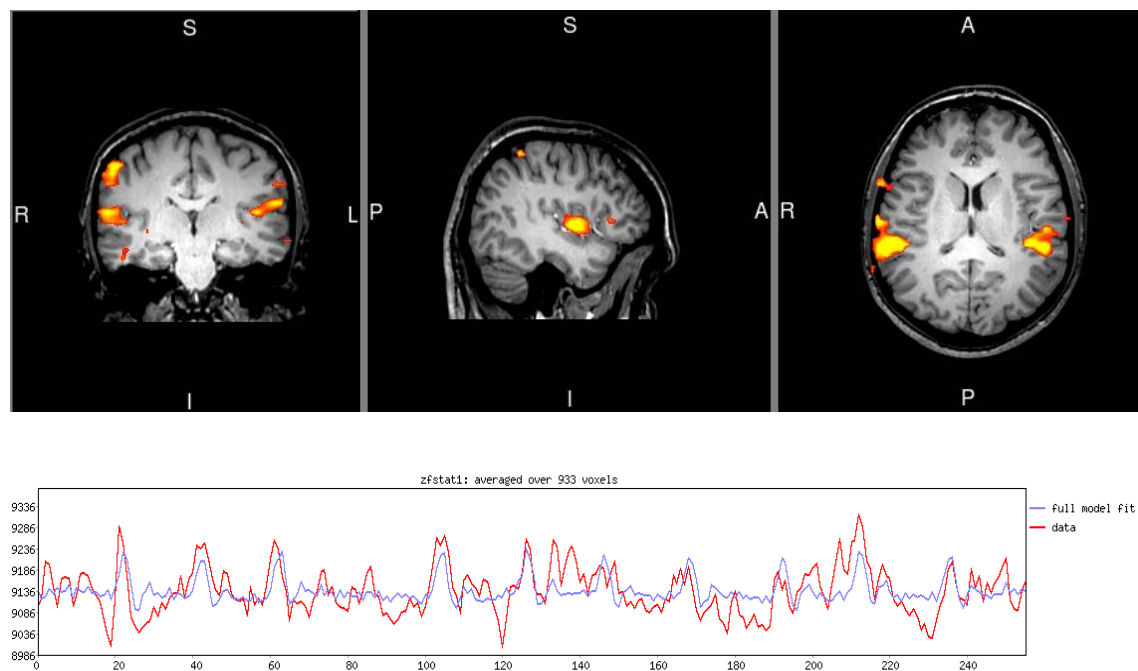


Figure 5.7: Areas of functional activity identified via fMRI in a healthy adult male following an event-related paradigm of passive stimulation. Cortical activation is well localized to the primary somatosensory cortex, in the region corresponding the lips and tongue on the homunculus (top). The timeseries of the cluster of activity (red) can be seen to rise during epochs of stimulation (model fit shown in blue).

-An “active” paradigm, where the subject was simply instructed to intermittently produce active sucking of the pacifier

This study revealed a bilateral region of functional activation in a region of the primary motor cortex, which roughly corresponds to that associated with the movement of the jaw, tongue and lips (Figure 5.8).

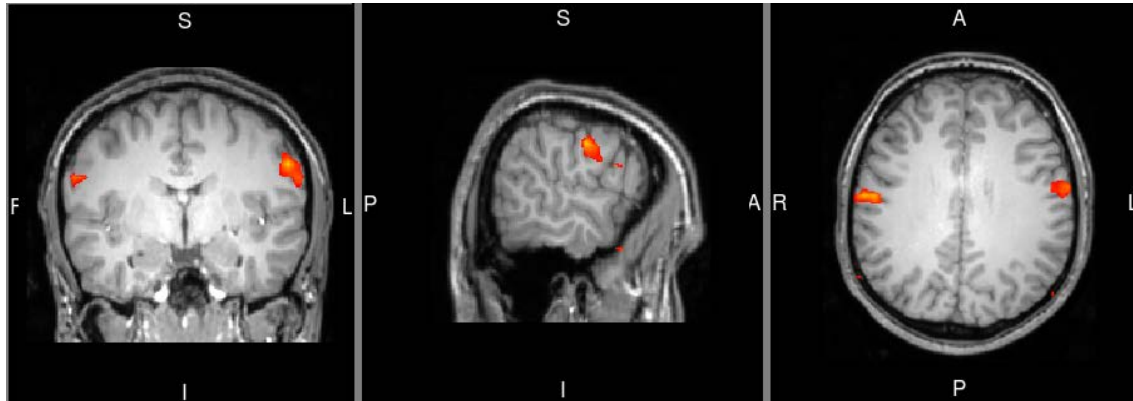


Figure 5.8: Areas of functional activity identified via fMRI in a healthy adult male following an active sucking paradigm. Cortical activation is well localized to the peri-rolandic region, with involvement of both the primary motor cortex and somatosensory cortex.

-A “mixed-cognitive” paradigm, in which the subject was presented with the block paradigm consistently used throughout the studies of this thesis, and additionally instructed to synchronize active sucking with the pattern of passive stimulation.

In addition to a pattern of functional activation at the level of the primary motor cortex similar to that observed in response to the “active” paradigm, this study also revealed activation of the cerebellum. Additionally, functional deactivation (negative BOLD) was identified at the level of the medial prefrontal, posterior cingulate and inferior-lateral parietal cortices (Figure 5.9). This pattern of spatial functional deactivation is consistent with the default mode network (DMN). The DMN has been described as “consistent regions of the brain which are active at rest but decreased during cognitive tasks” (Lee et al., 2013), which is in line with the subject needing to synchronize the sucking to the vibratory stimulus.

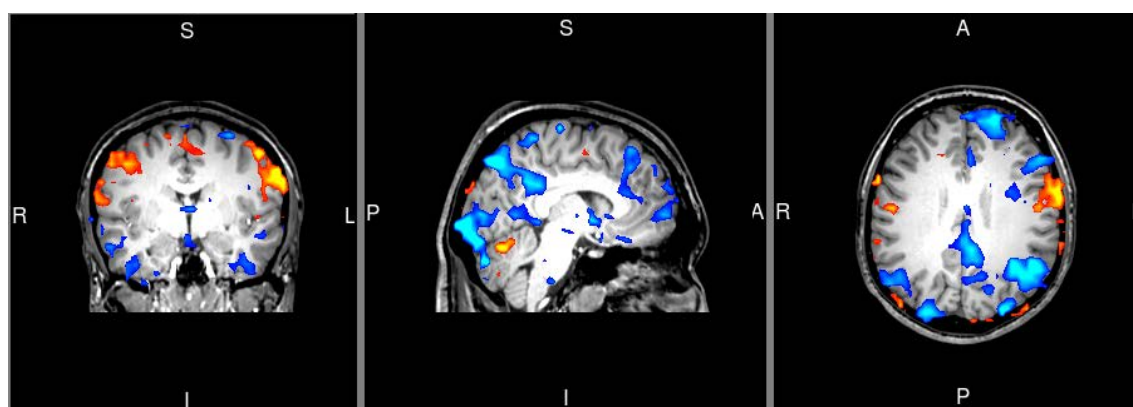


Figure 5.9: Areas of functional activity identified via fMRI in a healthy adult male following a mix-cognitive paradigm in which he was required to coordinate active sucking with the pattern of passive stimulation. Cerebral activation is observed at the level of the cerebellum and primary motor cortex (red), and is accompanied by cerebral deactivation consistent with the default mode network (blue).

5.2 A computer controlled olfactometer to investigate the neural correlates of the olfactory system

In the mature human olfactory system, primary olfactory neurons project directly from the olfactory epithelium into the olfactory bulb. Secondary neuron projections from the olfactory bulb then innervate a multitude of cortical regions, collectively known as the olfactory cortex (Wilson et al., 2006). At present the exact dynamics underlying processing and functionality of the olfactory cortex are unknown, with evidence suggesting that this large network of highly interconnected cortical subregions may be interpreted as a “bidirectional interface”, able to induce odour-guided behaviour by relaying peripheral sensory information with higher order synthetic processes (Wilson et al., 2006). Despite its apparent intricacy, functional imaging studies have suggested that the olfactory network may be organized in a hierarchical and parallel manner, which reflects the type and complexity of the odour task. In this model, a set of core regions including the amygdale and the piriform cortices are always engaged in the passive smelling of odours; and an increasing number of task-specific regions are then recruited according to the complexity of the task (Figure 5.10) (Savic et al., 2000, Savic, 2002b).

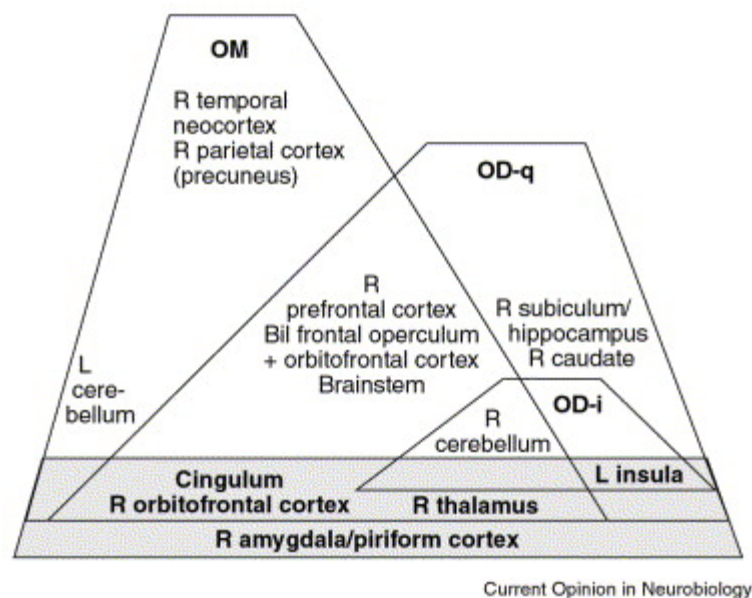


Figure 5.10: The parallel and hierarchical structure of olfactory processing proposed by Savic and colleagues. Each pyramid represents the cerebral structures involved in a specific task, the higher the pyramid the more complex the task. At the base of the pyramids are the olfactory core regions activated by the passive smelling of odors.

Abbreviations: monorhinal smelling of odorless air (AS), single odors (OS), discrimination of odor intensity (OD-i), discrimination of odor quality (OD-q), and odor recognition memory (OM). Image reproduced from (Savic et al., 2000).

While in-vivo neuroimaging techniques are quickly advancing our understanding of the adult olfactory network, there is a paucity of studies describing its ontogeny and functional development throughout the prenatal and perinatal period (Schneider and Floemer, 2009). Mammal studies have revealed the importance of the early establishment of olfaction, with cubs found to utilize smell cues they have learnt in-utero to reach their mother's nipple (Schneider and Floemer, 2009, Illig, 2007, Stickrod et al., 1982). Furthermore, experiments in rats have confirmed the importance of a sensory-driven early maturation of olfaction, with perinatal deprivation of odours leading to long-term dysfunction of the olfactory system (Best and Wilson, 2003). In human newborns, histological and macroscopic findings have revealed that olfactory bulbs and tracts are already apparent at 12 weeks gestation, although these can only be visualized in-vivo via MRI from approximately gestation week 30 (Azoulay et al., 2006), with olfactory bulbs then observed to quickly mature in a predefined and organized manner throughout the first year of postnatal life (Schneider and Floemer, 2009). The notion that the sense of smell is established early in human life is well documented by neonatal behavioural studies, which not only have demonstrated that neonates recognize, discriminate and are attracted to the mother's breast by means of olfactory cues, but are also responsive to the odour of amniotic fluid suggesting that chemosensory input during the foetal stage may play a crucial role in the development of the early olfactory sensitivity (Schaal and Marlier, 1998, Cernoch and Porter, 1985, Varendi et al., 1996). Furthermore, contrarily to other sensory systems, physiological and behavioural responses to new and aversive (trigeminal) odors have also been reported in preterm infants from 29 weeks post-menstrual age (Browne, 2008).

This large volume of behavioural evidence hints that a highly functional olfactory network may already be present within the newborn infant's brain. However methodological limitations have so far limited the researchers' ability to directly test this hypothesis (Winberg and Porter, 1998). Studies utilizing functional nearinfrared spectroscopy (fNIRS) have revealed that various types of olfactory stimulations can induce changes in cerebral haemoglobin concentration at the level of the

lateral and anterior orbito-frontal cortex in neonates (Aoyama et al., 2010, Bartocci et al., 2000, Bartocci et al., 2001). Even in its multichannel configuration, the spatial resolution offered by this technique, especially at deeper cerebral layers, is severely limited by the optical scattering properties of biological tissue. An alternative approach has involved utilizing electrophysiological techniques. However, these have been found to suffer from large EMG artefacts induced by facial movements (such as frowning) and to be highly susceptible to secondary effects of the odours such as lacrimation, eye movements and changes in respiratory patterns (Winberg and Porter, 1998). Despite an inherently lower temporal resolution, fMRI has long been used in adult olfactory studies as it offers higher spatial resolution, is free from several methodological limitations imposed by electrode-based measurements, and can detect function across the whole brain (e.g. (Weismann et al., 2001)). Transposing this technique to neonates would therefore allow simultaneous study of functional activity areas of the olfactory cortex engaged in both low- and high-level processing of odour information (Poellinger et al., 2001, Savic, 2002a), while avoiding or directly studying important effects in the olfactory system such as habituation and desensitization (Poellinger et al., 2001).

Throughout this thesis, a major aim is to develop robust and systematic tools for task-dependent fMRI studies of the preterm and neonatal populations. In this study, my approach was further expanded by developing and validating a safe and effective fMRI compatible tool for presenting olfactory stimuli at 3.0 Tesla (3T), specifically for newborn infants. The system was ultimately used to study the pattern of functional activation induced by a single odorant (formula milk) in a group of infants at term equivalent post-menstrual age (PMA).

State of the art

Although not as demanding in terms of temporal resolution, thermal stabilization, and flow rate magnitude as equipment used to assess neurophysiological activity-evoked potentials, an ideal fMRI compatible olfactometer still needs to be able to provide stimulus in a controlled, stable and repeatable manner (Lundstrom et al., 2010). This entails delivering odours at consistent onsets following TR pulses and ensuring that olfactory stimuli are not accompanied by tactile cues such as

“air-puffs” (Lundstrom et al., 2010). Several MR compatible/MR safe olfactometers meeting these requirements and associated validation studies in the adult population have been reported in the literature (Sobel et al., 1997, Lorig et al., 1999, Popp et al., 2004, Vigouroux et al., 2005, Lowen and Lukas, 2006, Borromeo et al., 2010, Lundstrom et al., 2010, Sommer et al., 2012, Sezille et al., 2013). Despite some implementation differences, the general construct of these (and all) olfactometers can be divided into three principal sub-systems (Johnson and Sobel, 2007, Sommer et al., 2012):

- (i) the airflow preparation and control apparatus, which entails the pneumatic control architecture, generally comprising solenoid electro-valves and airflow sensors, that allow automating canalization and delivery of pressurized air through the olfactometer system;
- (ii) the odour-sourcing apparatus, which may be placed after (e.g. (Vigouroux et al., 2005, Lowen and Lukas, 2006, Popp et al., 2004, Sommer et al., 2012)), or between (e.g. (Lorig et al., 1999, Borromeo et al., 2010, Sezille et al., 2013)) stages of airflow preparation, and refers to the hardware necessary to inject the odoring agent into the airflow. This is usually done by implementing “odour canisters” along the path of the airflow, containing liquid odour-releasing substrates, although solid substrates have also been reported (Sezille et al., 2013);
- (iii) the delivery apparatus, which normally consists of the tubing, manifolds and interface (such as nasal cannulae or masks) used to carry air towards the subject.

In 1997, Sobel and colleagues developed a sophisticated fMRI compatible olfactometer system able to present olfactory stimuli without imposing visual, auditory, tactile or thermal cues (Sobel et al., 1997). The stimulation was induced by supplying a manifold at a distance of approximately 20 cm from the subject’s head with: (i) one channel delivering a constant flow of air passing through a warmed “odorant canister”; (ii) one channel delivering a constant flow of air passing through a warmed “odourless canister”; (iii) two vacuum channels remotely controlled via a directional valve to selectively counteract the effects of either the odour or the odourless airflow channel; (iv) 1

channel independently and constantly supplying diluting fresh air. In 1999, Lorig offered a simplified solution, in which the stimulus was directly driven by a set of on-off solenoid valves, each leading to a separate odour vessels (Lorig et al., 1999). These were located close to the nose manifold within the scanner, and consisted of small capsules containing odorant-impregnated filter paper. With the exception of the device developed by Vigoroux and colleagues in 2005, in which the odour was dispensed semi-automatically via a custom injection system (Vigouroux et al., 2005), more recent studies have consistently adopted a simplified solution which integrates the approaches proposed by Lorig and Sobel (Lorig et al., 1999, Sobel et al., 1997). These olfactometers utilize a combination of solenoid valves similar to that adopted by Lorig and colleagues to directly control the airflow through an odorant canister similar to that described by Sobel and colleagues (e.g. (Popp et al., 2004, Lowen and Lukas, 2006, Sommer et al., 2012, Sezille et al., 2013)). The latter also demonstrated that at low flow rates, it is not necessary to moisturize the air via thermal bath. While in recent years this simplified principle of operation has become increasingly standard when designing MRI compatible olfactometers, to the best of the author's knowledge, no such device has been developed with the neonatal population in mind. In this study, I aimed to develop an olfactometer that would transpose current methodology into an embodiment that meets the enhanced safety requirements of this vulnerable population.

5.2.1 Stimulus system design and operation

As previously stated, the operating principle underlying the olfactometer I developed is similar to that adopted by recent adult fMRI studies, where computer controlled solenoid valves are used to direct medical grade air through an odorant canister, and to then deliver the resultant odorized gas to the subjects' nose (Benignus and Prah, 1980, Lorig et al., 1999, Johnson and Sobel, 2007). The system can therefore be subcategorized and described in terms of the aforementioned subsystems (airflow preparation and control, the odour-sourcing, and delivery), each of which was custom designed, but assembled in its entirety from off-the-shelf components. Since the olfactometer was developed specifically for the neonatal population, several measures were taken to ensure its safety and sterility. In particular, infective risks were minimized by integrating single-use clinical

equipment into the delivery subsystem. Placing the odour sourcing apparatus after the airflow preparation and control stages was also crucial in keeping the non-disposable hardware sterile, thus avoiding the risks of across subject contamination.

Airflow preparation and control subsystem

The airflow preparation and control apparatus is placed within the control room. The medical grade compressed breathing air supplied by the hospital wall socket is channelled through a one-way manual flow regulator (ARM5 series, SMC Corp., Tokyo, JP) before being input into the control box, architecture of which is shown in Figure 5.11A. Within the control box, air first passes through a flow meter (PFM series, SMC Corp., Tokyo, JP), before being directed to three on-off solenoid valves (VX series, SMC Corp., Tokyo, JP) via a 3-way airflow distributor (QS series, Festo Corp., Essling am Neckar, DE). In keeping with my standard methodology, digital control of the valves and monitoring of the analogue flow meter readings is achieved via a DAQ (USB-6008, National Instruments Corp., Austin TX, USA) connected to standard PC running LabView software (National Instruments Corp., Austin TX, USA). Data logging and control is performed at 100 Hz, and allows synchronization of the valve operation with the TTL pulses produced by the scanner with each acquisition. The software is based on that developed for my previous devices, where five virtual channels are defined for the DAQ to establish digital communication with the three valves and the scanner and analogue communication with the flow meter.

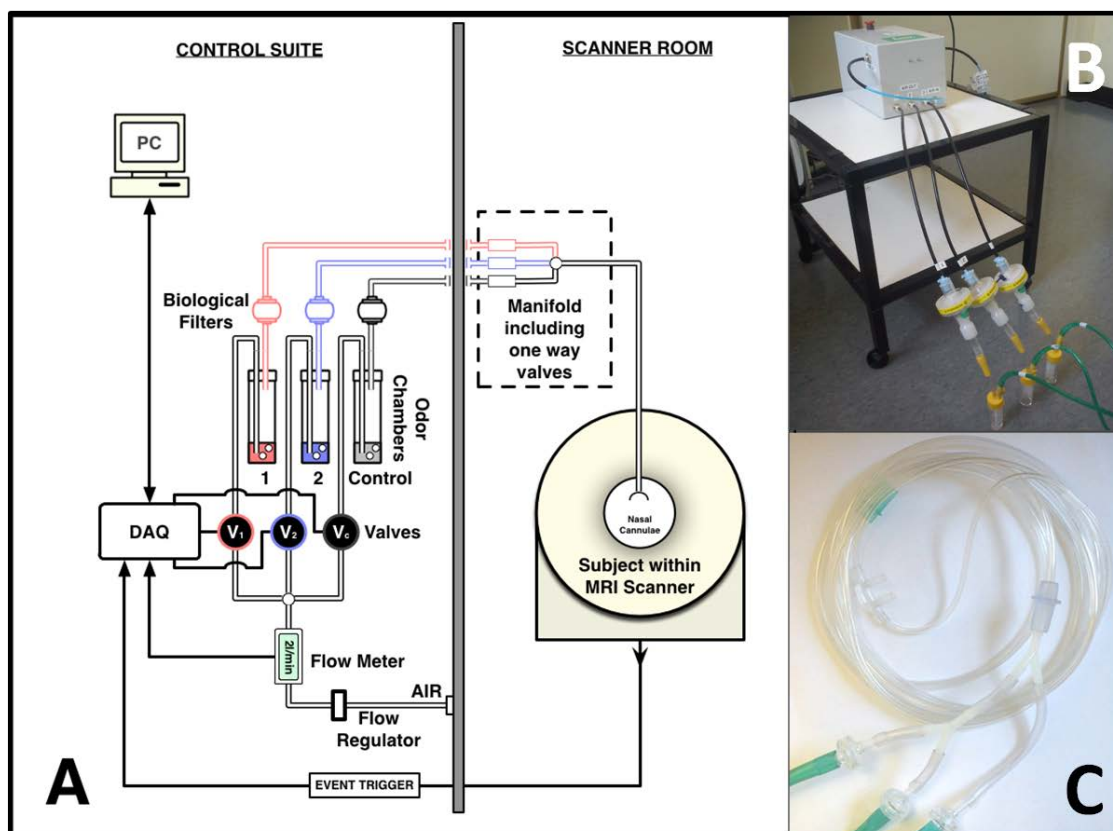


Figure 5.11: (A) The control box consisting of the flow regulator, meter and valves is in the MR scanner console room. Pressurized air is bubbled through the stimulus odorant liquids, thereby presenting the vaporised odor to the subject inside the scanner examination room. **(B)** To further minimize infective risk, all components distal to the control box are disposable, and antimicrobial respiratory filters are fitted (orange arrow). **(C)** The stimulus presentation manifold contains one way valves (blue arrow) to prevent mixing of smells. These are then connected to nasal cannulae fitted to the subject prior to data acquisition.

Odour sourcing subsystem

To allow experimenters to visually ascertain correct delivery of the olfactory stimulation, the odour-sourcing subsystem is also placed within the control room and consists of three single-use mucous specimen traps (Pennine Healthcare, Derby, UK), which were used as disposable odour canisters, each containing 3 mL of liquid odorant. Each canister is connected to an individual solenoid valve outlet via 50 cm of pneumatic tubing. A short length of silicone tubing was added within the canister to ensure that incoming airflow would “touch” the liquid’s surface, which was found to maximize odour transmission in adult pilot studies. Additionally, an anaesthetic grade ventilator circuit breathing filter (Clear-Guard II; Intersurgical Ltd., Wokingham, UK) was fitted to

the inlet of each odour canister, to prevent air from flowing back into the control unit, potentially causing cross-subject contamination (Figure 5.11B). Finally, in contrast to many previously reported designs that used compressors as the main source of pressurized air, my system was directly supplied by medical grade air from the hospital wall supply which is already filtered for particulate and chemical contamination and thus did not require additional pre-filtering stages.

Odour delivery subsystem

The delivery apparatus (Figure 5.11C) is the only subsystem component that extends into the scanner room, is entirely made of disposable clinical equipment and is fully metal free and MR safe. It consists of three 6 m long PVC bubble pipes (Flexicare Medical Ltd., Mountain Ash, UK), each stemming from an individual odour canister. The pipes are then all attached to a manifold composed of two Y junctions and three one-way valves, one for each tube termination (Bio-orb, Reef-One, Norwich, UK). This manifold configuration prevents odours from back-flowing into the tubes, and effectively serves to keep all odour conduits separate until the last 25 cm, where they merge into a neonatal soft-tip curved nasal cannulae (Flexicare Medical Ltd., Mountain Ash, UK).

Operation

The flow rate into the system was set manually to approximately 1.7 l/min via the one-way flow regulator (ARM5 series, SMC Corp., Tokyo, JP) to account for a slow rise that was observed to occur through testing, thus ensuring it would remain below 2 l/min. Previous studies have found a flow rate of 2 l/min ideal as it prevents the subject from experiencing dryness and discomfort, and this notion was confirmed throughout our pilot experiments with adult subjects who also confirmed they could rapidly and easily detect the smells delivered. Airflow into each of the three odour canisters is controlled via the opening and closing of separate on/off pneumatic valves and although the system allows for experimental flexibility, the canisters were chosen to contain a control odour (sterile water), and two other odour agents. In a standard experimental paradigm, the control odour valve is kept open during epochs of rest, while during epochs of stimulation the control valve is closed and the selected odorant is presented by simultaneous opening of the appropriate valve. In

neonates, this system was used to only study the response to a single odour so one valve was kept closed at all times.

Safety

Several safety measures were implemented on the system to avoid the subject from experiencing discomfort throughout the scanning session. These mainly entail means of limiting airflow, which is done (i) manually by adjusting the flow regulator or by means of both software and hardware emergency stop switches which act to close all valves; (ii) automatically by the control software, which interrupts the paradigm if the flow rate exceeds a preset threshold (2.5 l/min). Additionally, the flow rate can also be constantly quantitatively monitored via the flow sensor, whose readings are displayed in real time both on the LabView user interface, and via its LCD display, which is mounted on the top surface of the control box (Figure 5.12).

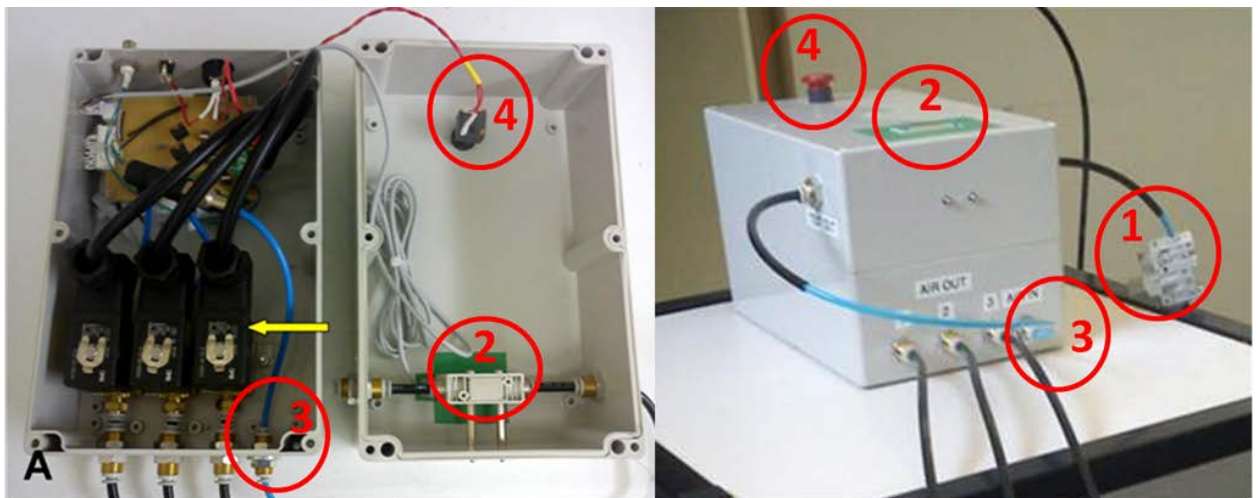


Figure 5.12: The olfactometer has a number of safety measures designed to ensure that the flow into the system (and to the subject) is carefully controlled and cannot exceed a certain level. Flow into the system is set by a lockable flow regulator (1), and then measured by a digital flow meter (2). The airflow then enters the olfactometer via a single channel (3) before it is diverted through one of the three solenoid valves (yellow arrow), which can only be opened one at a time. Should the experimenter have any concerns during data acquisition, an emergency stop button (4) can be pressed which immediately closes all of the valves and shuts off the system.

5.2.2 Experimental Validation

The work was approved by the NHS Research Ethics Committee, and written parental consent was obtained prior to all sessions of data acquisition.

Study population and experimental design

A validation study was conducted on a population consisting of 1 term and 6 term equivalent neonatal subjects (3 male, 4 female) of median PMA at scan 40 weeks (range from 38 + 4 to 42 + 6 weeks), median GA at birth 27 + 3 weeks (range 24 + 3 to 37 + 6 weeks) and median weight at scan 2765 g (range from 2160 to 3420), all recruited from the neonatal intensive care unit and postnatal wards at the Queen Charlotte and Chelsea Hospital, London, UK. Patient information including clinical characteristics and details of their feeding are reported in Table 5.1. None of the infants required forms of respiratory support at the time of the scan, nor had any form of focal brain injury identified on cranial ultrasound scanning. Oral sedation was administered to 6 of the 7 infants studied via chloral hydrate (30–50 mg/kg dose), approximately 20 minutes before the MRI session. All infants were clinically assessed prior to MRI scan by a paediatrician, who was also in attendance throughout the image acquisition period.

To avoid potential effects of stimulus habituation, an event related experimental paradigm was implemented in which infants were presented with the odour for 6 seconds (4 TRs), followed by 24 seconds (16 TRs) of inter-stimulus rest period and 10 repetitions. Since, at the time of scan 4 of the infants recruited were exclusively breastfed, and 3 infants were fed with both formula milk and breastmilk, formula milk was used as a single odorant for stimulation (Aptamil; Nutricia Ltd., Wiltshire, UK), which allowed comparing the responses of infants under different feeding regimes, and different degrees of exposure to the formula milk odour. All of the subjects had been fed one hour or less prior to the imaging session, and none exhibited signs of hunger throughout the study.

fMRI image acquisition and data analysis

Infant preparation, physiological monitoring and image acquisitions were performed as described in Chapter 6 (Section 6.2.2). Lower level (individual subject) and higher level (group) fMRI data analysis were carried out as reported in Chapter 6 (Section 6.2.4).

Results

BOLD fMRI data was successfully collected in all infants despite the presence of sporadic head motions in all datasets, which were, however, not correlated with the timing of stimulus presentation. Evidence from physiological parameters, as well as continuous observation, also indicated that in no instance did infants exhibit adverse reactions to olfactory stimuli throughout the scanning sessions. Lower-level (single-subject) fMRI analysis, summarized in Table 5.2, revealed well localized clusters of functional activity (positive BOLD signal) at the level of the piriform cortices in 5 subjects (in 4 bilaterally), while diffuse patterns of activation were also observed at the level of the cerebellum, in all 7 subjects; of the insular cortex, in 5 subjects, and partially of the orbito-frontal regions, in 3 subjects. Functional deactivation (negative BOLD) was not consistently observed in any subject.

Table 5.2: Spatial localization of identified responses in the study population (“cross” denotes a positive response identified in the subject).

Subject Number	Feeding Regimen	Pattern Activation				
		Piriform Cortex	Thalamus	Cerebellum	Orbito-frontal Areas	Insula
1	Breastfeeding	X	X	X	X	X
2	Breast and Formula feeding	X	X	X	X	-
3	Breast and Formula feeding	X	X	X	-	X
4	Breast and Formula feeding	-	-	X	-	-
5	Breastfeeding	X	-	X	-	X
6	Breastfeeding	X	-	X	-	X
7	Breastfeeding	-	-	X	X	X

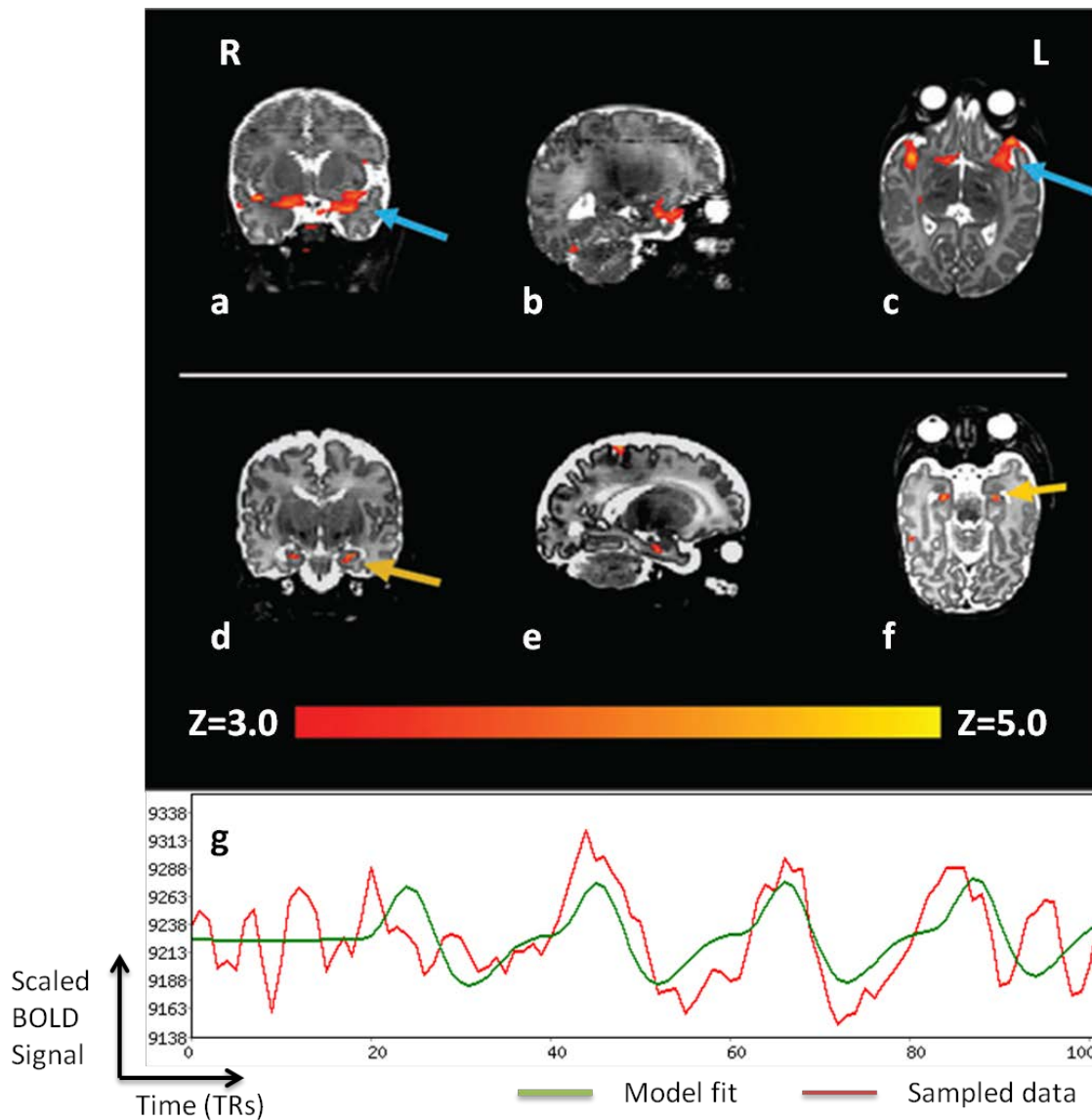


Figure 5.13: (a) Example of neonatal olfactory responses to formula milk odour in two ex-preterm infants at term equivalent age. T2-weighted images with an overlaid thresholded statistical map (corrected cluster significance of $p < 0.05$) showing well-localized functional responses in the piriform cortices bilaterally in an infant at 42 + 6 weeks PMA (blue arrows). (d, e, f): Well localized responses were also identified in the amygdalae as shown in an infant at 39 + 6 weeks PMA (yellow arrows). (g): In the example time series (averaged over the clusters of activation), the sampled Blood oxygen level dependent (BOLD) signal (red) can be seen to closely fit the predicted response as modelled by the pattern of stimulation and an age-appropriate Hemodynamic response function (HRF) model (green).

Higher level (group) analysis highlighted that a distributed, discrete pattern of functional activation resembling that of adult subjects studied with fMRI (Poellinger et al., 2001) was already present in these newborns. Well-localized clusters of functional activation were found in regions of the right piriform cortex, the right thalamus, the left insular area and cerebellum (Figure 5.14),

whereas activity in the orbito-frontal regions identified within 3 subjects in lower-level analysis failed to reach significance at the group level stage.

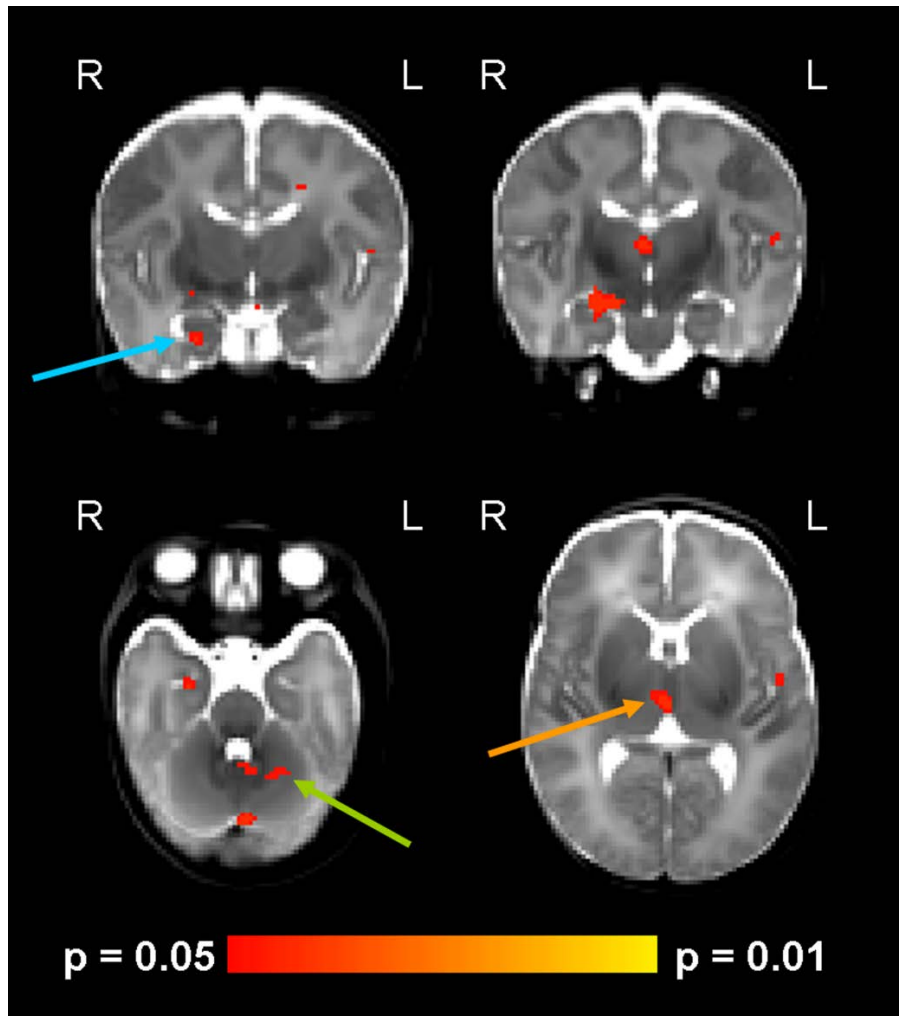


Figure 5.14: Results of a one-sample non-parametric T-test, showing the average activation patterns in 7 infants at term PMA (median age 40+4 weeks, range 38+4 to 42+5). Activation is seen in the right piriform cortex (blue arrow), the right thalamus (orange arrow), left insular area (white arrow), and cerebellum (green arrow).

Flow meter readings indicated that an upward drift in flow occurred in all the studies, and amounted to a total of approximately 0.2 l/min throughout the entire duration of the experiment (Figure 5.15).

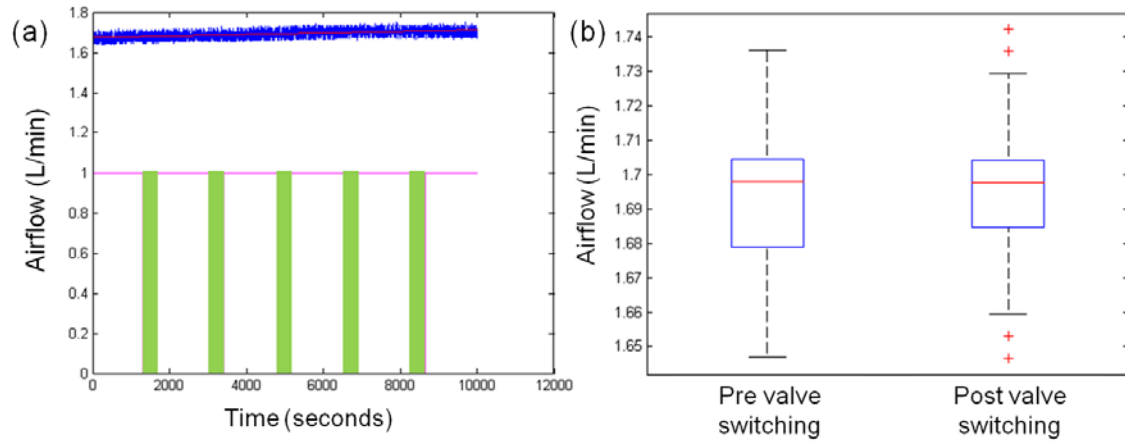


Figure 5.15: (a) Recorded airflow during an experiment performed with a neonatal subject, showing the airflow (blue). The airflow can be seen to remain constant during periods of stimulation (green boxes) and rest (pink line). (b) There was no significant difference in airflow 1.5 seconds before and after the switching of the valves (when the stimulus is initiated). Boxplots show 25th-75th centiles, redline data median, error bars show data ranges, red crosses are data outliers.

Discussion

Based on the simplified design that is becoming increasingly standardized in adult studies, I developed and tested a fully automated and programmable olfactometer suitable for fMRI studies in the neonatal population. The device was built to meet the stringent operational and clinical safety requirements of the neonatal population. The risk of cross-subject contamination was limited by ensuring that the air breathed by each patient would be fully retained within the odour delivery apparatus, which was entirely composed of low-cost, single-use disposable medical equipment. However, the PVC tubing utilized has the inherent disadvantage of introducing a baseline odour into the system (Evans et al., 1993). In keeping with my previous devices, several safety measures were also implemented. These included, in particular, the ability of constantly monitoring the subject's status and airflow supply, also limited by software, and manually overriding the system with both mechanical and software adaptations. The device developed is fully automated and programmable, and, despite some limitations, is capable of providing a reliable and sufficiently reproducible pattern of stimulation. The main limiting factor is represented by the manual regulator, which even when locked into place, does not guarantee a steady flow across studies. While exactly time-locking the instant in which the stimulus is received by the subject is inherently challenging as it requires taking into account patterns of breathing and the rate at which odourant molecules diffuse into the air, an

adult pilot study has proven the ability of my largely simplified system to induce well-localized patterns of BOLD activation which spatially and temporally replicate known adult olfactory responses (Seubert et al., 2013) (Figure 5.16).

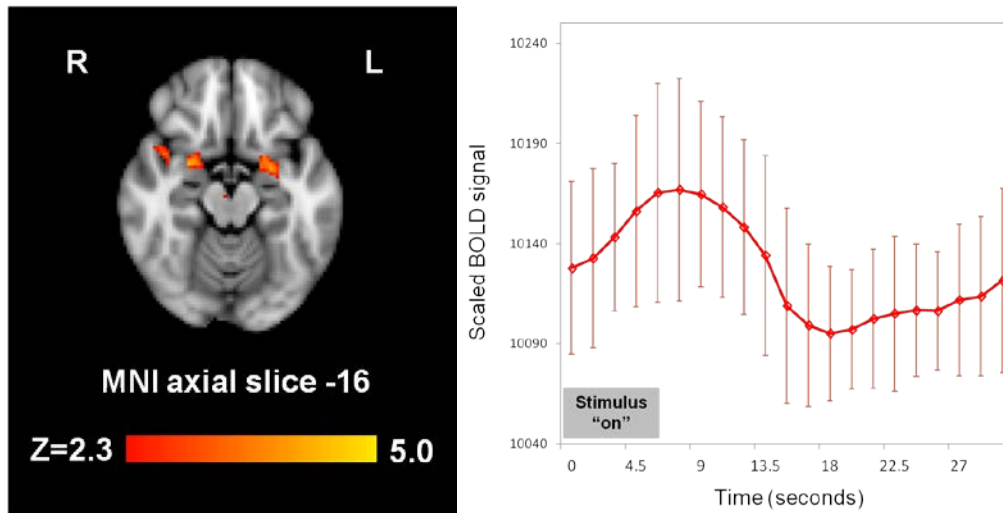


Figure 5.16: Olfactory response to formula milk identified in a 34 year old healthy male volunteer using the described olfactometer system. The identified activity has been overlaid onto a standard anatomical template (the MNI-152 brain), and shows a cluster of activity identical to that described in a recent meta-analysis of published studies in adults (Seubert et al., 2013). Peristimulus plot showing the scaled BOLD signal change following olfactory stimulation with formula milk of 6 seconds duration (shaded box) in a 34 year old adult volunteer. The BOLD signal within the identified cluster is seen to rise with a peak response at 7.5 seconds following initiation of the stimulus, as would be predicted from the time of stimulus onset using a model derived from by convolution with a canonical hemodynamic response function. Data points represent mean signal, error bars are one standard deviation from the mean.

A validation study has been conducted on 7 infants at term equivalent PMA and revealed well-localized patterns of positive BOLD activity in cerebral areas in response to formula milk that are consistent with those associated with the primary olfactory system of the mature adult brain (Poellinger et al., 2001). In particular, activation was found in regions of the piriform cortex, which in adults are associated with lower level olfactory functions such as odour detection and discrimination. The significance of the results is, however, clearly limited by the number of infants

included in the study as well as the heterogeneous background of feeding regimens. These factors suggest that caution is needed in the interpretation of the patterns of response observed within the insula, thalamus and cerebellum. In the adult brain, these are thought to be responsible for the rich perception of odours, by performing higher level processing and association of olfactory information with emotion and memory (Wilson and Sullivan, 2011).

A low dose of sedative medication was administered to 6/7 infants prior to MRI scanning as term newborns are inherently susceptible to physical and auditory perturbations and tend to move exceedingly and become distressed during the scanning process. However positive and well-localized BOLD responses have been identified in sleeping infants, and previous studies (as well as the findings reported in Chapter 6) have not found chloral hydrate to affect the spatio-temporal characteristics of the evoked fMRI response (Arichi et al., 2010, Arichi et al., 2012).

Conclusion

In this chapter I presented the design, construction and validation of an olfactometer based on the common architecture developed within the work of this thesis, and meeting all the requirements to be utilized to conduct fMRI studies of neonatal subjects. In fact, the system is sterile, and able to induce well-localized patterns of functional activation. The validation study conducted on 7 subjects at term equivalent age here presented revealed that the functional representation of smell is already well established by the time of birth, with activity localized to the primary olfactory areas.

Chapter 6: Evolution of functional activity associated with somatosensory stimulation and spontaneous limb movements in the preterm human brain

Overview - The third trimester of human gestation represents a crucial period for the developing brain's motor and somatosensory systems. Using functional MRI and the wrist robotic stimulator described in Chapter 4, activity in preterm infants induced by proprioceptive stimulation and spontaneous peripheral limb movements has been systematically characterized. A maturational trend towards faster and higher amplitude somatosensory responses and more spatially dispersed activity have been found. These results suggest that somatosensory network connectivity increases with maturation, and is influenced by activity-dependent mechanisms. Spontaneous movements have been found to be somatotopically represented at the level of the primary sensorimotor cortices.

Acknowledgment of contributions

The consenting, preparation and scanning of infant subjects was performed in collaboration with the research, nursing and neuroradiologist team of the Centre for the Developing Brain, King's College, St Thomas' Hospital London; formerly part of the Weston group, Imperial College, Queen Charlotte and Chelsea Hospital London.

fMRI data analysis was performed in collaboration with Dr. Tomoki Arichi

This material is currently unpublished

6.1 Introduction

6.1.1 Study hypothesis and aims

I hypothesized that throughout the third trimester of gestation up to term equivalent age (i) the somatosensory system undergoes a pattern of functional maturation which reflects its ability to process externally induced proprioceptive stimulation; (ii) extra-uterine exposure can influence this normal developmental trajectory; (iii) spontaneous movements may be processed at a cortical level and contribute to the early establishment of the primary somatosensory and motor cortices.

To assess these hypotheses I aimed to utilize the previously developed wrist robotic stimulator system to: (i) conduct the first developmental task-fMRI somatosensory study across the preterm and term population in a systematic and objective manner; (ii) characterize the spatio-temporal and magnitude trajectory of the induced BOLD responses; (iii) investigate the effects of post-natal age on BOLD response; (iv) reveal potential neural correlates of spontaneous movements in the preterm and term population.

6.1.2 The early development of somatosensory function

The maturation of the human somatosensory system is a complex process that begins early in foetal life, and entails the progressive formation and consolidation of an intricate but highly organized framework of structural and functional connectivity. The development of ascending somatosensory (and generally all sensory) neural pathways proceeds from the periphery towards higher cortical levels, with the 3rd trimester of gestation characterized by the exuberant growth of higher order neuronal connections through the forming cerebral substrates (de Graaf-Peters and Hadders-Algra, 2006, Kostovic and Jovanov-Milosevic, 2006, Kostovic and Judas, 2007, Kostovic and Judas, 2010, Volpe, 2008). Disruptive events occurring at this juncture such as those associated with preterm birth, often result in neuromotor dysfunctions (Ancel et al., 2006), highlighting the importance of the late in-utero period to the long-term organization and establishment of the entire sensorimotor system. In fact, animal studies suggest that early somatosensory input may be crucial

in the differentiation and establishment not only of the somatosensory cortex (Vanderhaeghen et al., 2000, Khazipov et al., 2004, Schouenborg, 2008, Seelke et al., 2012), but also of the primary motor cortex (Chakrabarty and Martin, 2000, Chakrabarty and Martin, 2005). The latter is thought to receive somatosensory input predominantly through indirect cortico-cortical pathways, which contribute to the establishment of well-defined receptive fields by birth age; these are then fundamental in guiding the somatotopic representation of descending tracts later in life (Chakrabarty and Martin, 2005). Characterizing the normal developmental trajectory of the somatosensory system and understanding its underlying driving mechanisms, could therefore be crucial in identifying early signatures of pathophysiology, and in attaining the causal knowledge necessary to devise early evidence-based therapies (Vanhatalo and Kaila, 2010).

While animal studies have begun to elucidate some of the endogenous and exogenous mechanisms that guide axonal maturation throughout gestation and early postnatal life (Khazipov et al., 2004, Schouenborg, 2008), and structural imaging techniques (as well as post-mortem histological studies) have identified the broad anatomical and temporal milestones of the developing somatosensory neural pathways (Kostovic and Jovanov-Milosevic, 2006, Kostovic and Judas, 2007, Kostovic and Judas, 2010), it is only with the recent advent of functional neuroimaging techniques that researchers and clinicians have been able to directly probe the ability of the emerging neural substrates to transmit and process peripheral somatosensory information. Preliminary clinical studies have revealed that, even in the absence of overt structural insults, abnormal early electrophysiological somatosensory evoked responses can predict the occurrence (although not the degree) of later neurodevelopmental delays with a good specificity, but highly variable sensitivity (13-100%) (reviewed in (Vanhatalo and Lauronen, 2006)). These findings strengthen the notion that a true understanding of the developing somatosensory system is only likely to be achieved by integrating structural and functional information derived from multimodal techniques. This is a major undertaking of current clinical research.

A variety of neuroimaging techniques have been utilized to gather functional information on the developing somatosensory system, with a fundamental trade-off existing between spatial and temporal resolution. Electrophysiological measures offer the ability to probe signals at the

millisecond scale, and to therefore establish a directional, although not necessarily causative, hierarchy of cortical responses to somatosensory stimulation (Nevalainen et al., 2008). However, owing to the intrinsically limited spatial resolution inherent to multichannel techniques, these lack the all-important ability to accurately localize responses to cortical substrates. Despite a relatively poor temporal resolution, fMRI can instead be used to map brain function to the underlying structure across the whole brain with enhanced spatial specificity, offering the potential to largely complement and integrate our understanding of early somatosensory functional development. To date only resting-state fMRI studies have described the longitudinal development of functional connectivity throughout the preterm period (Doria et al., 2010, Smyser et al., 2010) based on the assumption that, contrarily to what is seen in adults, functional sensory “networks” are synchronously activated by endogenous activity originating from spontaneous activation of distal sensory receptors (Colonnese and Khazipov, 2012). However, while this hypothesis is supported by animal studies and inferred from preliminary EEG findings (reviewed in (Colonnese and Khazipov, 2012)), in practice the neural origin of resting-state fluctuations is currently unknown, and this technique may not be indicative of the effective ability of the somatosensory system to transmit and elaborate externally induced (exogenous) stimulation. Largely due to technical and methodological limitations, no task-fMRI study has so far attempted to directly probe the evolution of the cerebral processing of somatosensory information in response to a very specific pattern of stimulation. Such a characterization is intuitively more likely to be indicative of functional integrity than resting state connectivity (or may further support the hypothesis underlying resting state activity), and provide results that are directly comparable with those obtained via electrophysiological techniques. This is particularly appealing in view of the complementary spatio-temporal properties of fMRI and electrophysiology.

Additionally, in keeping with the notion of activity-driven maturation of neural pathways (Kostovic and Jovanov-Milosevic, 2006), afferent input is thought to play a crucial role in guiding the establishment of the somatosensory system throughout the 3rd trimester of gestation. Contrary to popular beliefs, the intra-uterine environment provides a wealth of exogenous somatosensory (and sensory) stimulation, which the human foetus is perfectly optimized to perceive and elaborate

(Vanhatalo and Kaila, 2010). Further, animal models suggest that spontaneous intra-uterine foetal movements may be initiated by spinal reflexes, and serve to ensure that there is a constant influx of cortical somatosensory feedback arising from the peripheral receptors within the limbs and sub-cortical structures (Blumberg and Lucas, 1994, O'Donovan, 1999, Petersson et al., 2003, Khazipov et al., 2004, Colonnese and Khazipov, 2012). In view of these endogenous mechanisms of intra-uterine maturation, multiple theories advocate that preterm birth can lead to an unnatural over-exposure to external stimulation, which in turn may alter feedback from spontaneous movements ultimately affecting somatosensory development through activity-dependent mechanisms (such as aberrant plasticity). Although evidence from electrophysiological human studies is sparse and contradictory, animal studies have indeed demonstrated that exposure to distorted patterns of tactile stimulation can significantly alter the establishment of somatosensory connectivity (Schouenborg, 2008). These findings, if confirmed in humans, may have important implications for therapeutic interventions aimed at improving clinical outcome by providing targeted patterns of stimulation.

In this chapter, I first briefly review the current knowledge on the functional maturation of the somatosensory system during the 3rd trimester of gestation, with a particular focus on the relationship that exists between the observed patterns of functional activity and the development of the underlying structural substrates. I then present the first developmental task-based fMRI study of the preterm somatosensory system, which I conducted utilizing the system and tools presented in Chapter 4, and report the associated findings concerning the spatio-temporal maturation trajectory of BOLD response, and the potential influence of extra-uterine exposure. Finally, I will also report, for the first time, evidence of a spatially localized region of cortical elaboration of spontaneous movements in preterm and term-equivalent infants as revealed by fMRI.

6.1.3 The signature of functional somatosensory maturation

Human and animal studies suggest that peripheral sensory transmission to the thalamus is already well established early in foetal life, with the subsequent formation of thalamocortical connectivity largely occurring throughout the 3rd trimester of gestation (described in chapter 1) (de Graaf-Peters and Hadders-Algra, 2006, Kostovic and Jovanov-Milosevic, 2006, Kostovic and Judas, 2007,

Kostovic and Judas, 2010, Volpe, 2008) therefore thought to play a crucial role in the developmental dynamics of the functional somatosensory responses observed throughout the preterm period.

At around gestation week (GW) 25, ascending thalamocortical neural pathways first reach the subplate, where axons rapidly proliferate as they gradually extend into the forming cortical layers. This marks the beginning of a transient period during which the subplate progressively recedes and dissolves at approximately GW37, and is gradually superseded by the cortex as the main synaptogenetic substrate (Chapter 1) (de Graaf-Peters and Hadders-Algra, 2006, Kostovic and Jovanov-Milosevic, 2006, Kostovic and Judas, 2007, Kostovic and Judas, 2010, Volpe, 2008). This process is also paralleled by the progressive myelination of the ascending neural pathways, which in the pre- and post-central gyri is detectable from ca. GW35 (Iai et al., 1997, Nevalainen et al., 2014).

This hybrid network of connectivity exhibits an overall electrophysiological signature that reflects the maturation of each of its elements (the neural pathways, the cortex and the subplate) (Vanhatalo and Kaila, 2010). Cortical somatosensory evoked potentials (SEPs) observed via EEG during this period are subdivided into 2 main components, each seemingly associated with transient structural configurations specific to this stage of development (i) large, spatially dispersed, slow responses, or delta-brushes, (lasting 100ms to 1s) thought to arise from electrical impulses of immature afferent axonal projections terminating in the subplate, and eventually diffusing into the cortical plate (Hrbek et al., 1973, Vanhatalo and Kaila, 2010) (ii) more conventional SEP responses consisting of low latency (although initially relatively long with respect to adult SEPs), small amplitude, more spacially circumscribed long waveforms commonly referred to as N1 deflections (reviewed in (Vanhatalo and Lauronen, 2006)). From the earliest stages of preterm viability (GW25) until GW26, thalamocortical afferents only extend as far as the subplate layer (de Graaf-Peters and Hadders-Algra, 2006, Kostovic and Jovanov-Milosevic, 2006, Kostovic and Judas, 2007, Kostovic and Judas, 2010, Volpe, 2008), and median-nerve SEPs ($SEPs_{MN}$) are largely dominated by high amplitude delta-brushes (Hrbek et al., 1973, Vanhatalo and Kaila, 2010, Vanhatalo and Lauronen, 2006). From GW26 to GW32, as thalamocortical axons gradually penetrate the overlying cortical layers, and the subplate begins to recede, the amplitude of slow wave responses gradually decreases and N1 $SEPs_{MN}$ become detectable with an initially relatively long latency (90ms) (Hrbek et al.,

1973, Taylor et al., 1996, Nevalainen et al., 2014). As the brain matures, largely due to the arborisation, ingrowth and myelination of thalamocortical afferents, and to the dissolution of the subplate, slow response transients gradually subside and disappear by GW37, while the latency of N1 SEPs_{MN} gradually decreases to ca. 30 ms by term age (Hrbek et al., 1973, Nevalainen et al., 2014). Somatosensory evoked fields to median nerve stimulation (SEFs_{MN}) as studied via MEG in term-equivalent infants, peak at approximately 30ms at the level of the primary somatosensory cortex, which is consistent with EEG findings (reviewed in (Nevalainen et al., 2014)). However, to the best of the author's knowledge, no MEG studies have so far assessed SEFs during preterm age.

Resting-state fMRI studies indicate that the somatosensory and motor networks mature in parallel throughout the preterm period, first emerging unilaterally during early prematurity (29 to 32 wks PMA), to then assume an increasingly bilateral, complex and localized configuration by term equivalent age (Smyser et al., 2010, Doria et al., 2010). Bilateral patterns of activity are predominantly circumscribed to homotopic substrates, which become increasingly correlated throughout preterm cerebral development. This may be indicative of a progressive strengthening of interhemispheric connectivity, which is in keeping with the largely unmyelinated callosal fibres (i.e. long cortico-cortical connectivity) first apparent on DTI at ca. GW28 (Huppi et al., 1998, Hüppi et al., 1998), and found to grow into the cortical plate by GW35 (Kostovic and Jovanov-Milosevic, 2006). This hypothesis is in contrast with that proposed by early task-fMRI studies, which had attributed ipsilateral responses to direct thalamocortical input due to immature lateralization of somatosensory afferents (Erberich et al., 2006). In contrast, although reported only by a minority of newborn MEG studies, SEFs recorded at the level of the ipsilateral primary somatosensory cortex have millisecond latencies with respect to the contralateral response (Nevalainen et al., 2008), further supporting the notion that bilateral activity may indeed be driven by interhemispheric communication, rather than being the result of direct thalamocortical connectivity.

As previously mentioned, resting-state somatosensory networks in the preterm brain are thought to reflect slow fluctuations induced by intermittent spontaneous peripheral sensory activity. In this respect, their aetiology may be substantially different from that of adult resting-state networks, which are thought to instead represent intra-cerebral modulation of ongoing activity (Colonnese and

Khazipov, 2012). Interestingly, in 2007 Milh and colleagues assessed this hypothesis amongst preterm newborns (29-31 wks gestation), by correlating EEG readings with sporadic wrist and ankle movements, as well as with elicited wrist and ankle somatosensory stimulations (Milh et al., 2007). In both cases spatially dispersed delta-brushes were respectively broadly observed in the lateral and medial regions of the contralateral central cortex (which is consistent with the mature homunculus), however the majority of the recorded delta-brushes were not associated with either spontaneous movements or stimulation. The authors concluded that, in keeping with animal studies, spontaneous foetal movements initiated by muscle twitches generated in the spinal cord and subcortical structures (Blumberg and Lucas, 1994, O'Donovan, 1999, Petersson et al., 2003) may in fact serve to elicit sensory feedback at the level of the corresponding somatosensory areas (Khazipov et al., 2004). This mechanism may be in place to ensure the constant influx of neuronal input needed for the early activity-dependent development of the somatosensory system, and guide synaptogenetic plasticity (Feldman et al., 1999, Fox, 2002, Petersson et al., 2003, Milh et al., 2007).

In summary, electrophysiological studies suggest that somatosensory evoked responses progress from the broadly localized, high amplitude, long depolarizations associated with subplate mediated activation (delta-brushes), to lower amplitude, more spatially circumscribed conventional SEP responses observed at around term age, when thalamocortical projections make direct contact with their cortical targets (reviewed in (Vanhatalo and Kaila, 2010)). Resting-state studies suggest that somatosensory functional connectivity is first established unilaterally during early preterm age, and assumes an increasingly bilateral configuration towards term age, potentially due to the strengthening of interhemispheric connectivity (Smyser et al., 2010, Doria et al., 2010). Due to methodological and technical limitations, the maturation of somatosensory evoked responses has not previously been studied with task-fMRI; however such characterization may complement electrophysiological findings with enhanced spatial information. Finally, it is accepted that neuronal input is necessary to guide the early establishment of the somatosensory system (Kostovic and Jovanov-Milosevic, 2006), and animal studies suggest that sustained distorted patterns of external stimulation during the preterm period can markedly alter the formation the somatosensory system through aberrant plasticity (Schouenborg, 2008). It is proposed that spinally triggered spontaneous

movements are an endogenous mechanism in place to provide the early brain with accurately gauged neural feedback from peripheral receptors, and ensure an appropriate activity-driven development of the somatosensory system starting from the early intra-uterine life (Khazipov et al., 2004, Milh et al., 2007). To date a single EEG study has probed this hypothesis directly on preterm infants, and found delta-brush cortical responses to be associated with spontaneous limb movements, although these could not be precisely localized (Milh et al., 2007).

With all this in mind, in this study I aimed to use the tools developed in Chapter 4 and task-fMRI to (i) complement current knowledge, largely based on resting-state networks and EEG studies, by providing the first direct fMRI characterization of the maturation of the functional somatosensory network in response to externally induced stimulation during the preterm period (ii) investigate the potential effects of extra-uterine exposure on the normal course of development (iii) identify the spatial localization of neural correlates in response to spontaneous movements.

6.2 Materials and Methods

The work was approved by the NHS ethics committee (refs: 07/H0707/101 and 12/LO/1247), and written parental consent was obtained for all subjects prior to data collection.

6.2.1 Study population

Subjects were recruited from the Neonatal Intensive Care Units at the Queen Charlotte and Chelsea Hospital London and St Thomas' Hospital London over a 3 year period between 2011 and 2013. The study population consisted of a total of 62 infants (range 30+2 to 43+2 weeks post-menstrual age (PMA)), of which the majority (59/62) had been delivered prematurely. Prior to enrolment all infants were reviewed by an experienced clinician to assess their clinical status, identify any clear contra-indications for MRI scanning. Infants were excluded from the study group if they would require respiratory support during scanning, and if they were known to have a history of brain pathology such as extensive intra-ventricular haemorrhage (IVH) (grade 3 with ventricular dilatation; grade 4 with parenchymal extension), focal intracerebral lesions (such as infarction), severe hydrocephalus, or congenital brain malformations.

6.2.2 Image acquisition

Images were acquired using a 3-Tesla Philips Achieva MRI systems (Best, Netherlands) and an 8 channel phased array head coil, located on the Neonatal Intensive Care Units at the Queen Charlotte and Chelsea and St Thomas' Hospitals. Prior to imaging, a neonatal neurological examination was performed by an experienced practitioner (Mercuri et al., 2005), and clinical (antenatal, birth and post-natal care) and demographic details were recorded from the clinical notes. Oral sedation (chloral hydrate 30–50 mg/kg dose) was administered approximately 20 min before scanning to only 2 of the 42 infants less than 37 weeks PMA, but to the majority (15/20) of infants at term equivalent age. Infants who did not receive sedation were fed immediately prior to the scan and then swaddled to encourage natural sleep. Hearing protection was applied in the form of moulded dental putty in the external auditory meatus and adhesive earmuffs (MiniMuffs, Natus Medical Inc, San Carlos CA, USA), and the infants were immobilized using a vacuum evacuated bag (Med-Vac, CFI Medical Solutions, Fenton MI, USA). All image acquisition sessions were attended by a clinician trained in neonatal resuscitation, and the oxygen saturations, heart rate, and axillary temperature of all infants

were recorded during the imaging session. There were no adverse incidents during the data acquisition period.

High resolution structural 3D MPRAGE T1, turbo spin-echo T2, and Diffusion weighted images were acquired for all infants for review by an expert Neonatal Neuroradiologist (sequence parameters can be found in (Merchant et al., 2009)). fMRI data were collected from all infant subjects with an Gradient echo (GRE) Echo-Planar-Imaging (EPI) sequence lasting 6 minutes and 30 seconds with parameters: (TR) 1500msec; (TE) 45msec; (flip angle) 90 degrees; (resolution (x*y*z)) 2.5*2.5*4mm; minimum 200 volumes.

6.2.3 fMRI experimental design

A safe and reproducible pattern of proprioceptive stimulation was delivered to the infant subjects via the fully automated fMRI compatible wrist robot described in Chapter 4. Wrist flexion/extension was chosen as a specific joint movement which could be readily induced without causing discomfort to an infant, and would minimize other tactile effects such as skin stretching, which could confound the elicited somatosensory cortical responses. Prior to each experiment a 2-point sensor calibration was performed. The stimulation paradigm and parameters were selected based on those used previously to successfully induce statistically significant clusters of BOLD signal response in the preterm and term population (Arichi et al., 2012, Arichi et al., 2010, Allievi et al., 2013). A minimum of 6 blocks of stimulation were presented to all subjects. In 40 infants, spontaneous limb movements were recorded during a subsequent acquisition session using the same device and fibre optic position sensor, after disengagement of the piston.

6.2.4 fMRI data analysis

First-level Individual subject analysis

Data analysis was performed using FSL (FMRIB, Oxford, UK, www.fmrib.ox.ac.uk/fsl) (Smith et al., 2004b). Functional datasets were visually scrutinized for evidence of sustained head motion, severe image distortions and field-of-view misalignments, and corrupted data were discarded accordingly. The remaining datasets were truncated such that epochs affected by isolated bursts of head motion exceeding a total absolute displacement of 1.25mm (equivalent to half-a-voxel in the

acquisition plane) as calculated by MCFLIRT (FSL's intra-modal motion correction tool) were deleted (this is done by convention in fMRI studies (Power et al., 2012)). For passive paradigms of stimulation, following appropriate deletion, the minimum number of volumes used for analysis was 128 (or 4 full blocks of stimulation and rest); for spontaneous movements paradigm datasets were kept only if a minimum of 3 movements were present throughout the scanning session and if, following appropriate deletion, they contained a minimum of 110 volumes. This systematic removal of motion-corrupted data minimized the detrimental impact that false correlations caused by motion artifacts may have on the statistical fMRI analysis despite standard registration and motion estimate regression techniques (Power et al., 2012).

Each of the remaining patient datasets was then pre-processed using the standard pipeline as implemented in FEAT (fMRI Expert Analysis Tool, v5.98), which entails motion correction (using MCFLIRT), slice-timing correction, non-brain tissue removal, spatial smoothing (FWHM 5 mm), global intensity normalization and high-pass temporal filtering (cut-off 50 seconds) (for full details see (Woolrich et al., 2001, Jenkinson et al., 2002)). Signal artefact related to residual motion and physiological noise (such as cardiovascular pulsation and respiratory motion) were also removed by performing additional data de-noising with MELODIC (Model-free FMRI analysis using Probabilistic Independent Component Analysis (PICA, v3.0) (Beckmann and Smith, 2004). Independent components were assessed for their spatial representation and frequency power spectrum, and those that were judged to represent physiological noise or motion artefact were then filtered from the data prior to further statistical analysis (Arichi, 2012).

Time-series statistical analysis in FEAT was carried out using FMRIB's improved linear model (FILM) with local autocorrelation correction (Woolrich et al., 2001). Voxelwise time-series statistical analysis was carried out using the General Linear Model (GLM) as implemented in FEAT by fitting the observed data to a linear combination of the timing of somatosensory stimulation or the normalized velocity of spontaneous movements, convolved with a set of basis functions representing the age-appropriate HRF for both preterm and term equivalent infants (as derived in (Arichi et al., 2012, Arichi, 2012)) and their temporal and dispersion partial derivatives in order to capture the expected range of latency and duration of somatosensory evoked functional responses (Friston et al.,

1998a). The calculated t-statistical image was then converted to a z-statistical score image (and combined in a F-test to identify the full model fit derived from each of the basis functions), and a z-score threshold of 2.3 with a corrected cluster significance level of $p < 0.05$ was then used to generate spatial maps of activated voxels on an individual subject level. To allow better anatomical localisation of the identified functional activity, all of the filtered raw data and their corresponding activation maps were then registered to the individual subject's high resolution structural T2-weighted image using FMRIB's linear image registration tool (FLIRT) version 5.5 (Jenkinson and Smith, 2003).

For the passive stimulation experiment, the lag time and percentage BOLD signal change of the identified functional activity were studied within a region of interest (ROI) defined as the z-score upper quartile in the identified contralateral primary somatosensory cortex cluster. The mean of the parameter estimate values derived from the GLM analysis of the experimental design convolved with each of the basis functions were then calculated for the ROI, multiplied by the original basis function, summed to identify (or reconstruct) a subject specific HRF, and the lag time taken to reach the response peak amplitude identified. The correlation between the left and right peri-rolandic regions (encompassing both the primary motor and somatosensory cortices) during the period of functional stimulation were then calculated for each subject by registration of an anatomical mask defined in standard template space (as defined in (Doria et al., 2010)) to the individual subject's native space. The Pearson's partial correlation coefficient between the mean BOLD signal timeseries within the left and right peri-rolandic regions was then calculated controlling for the whole brain mean signal and the 6 detected head motion parameters using tools implemented in the statistics toolbox of Matlab (The Mathworks, Natick MA, USA). The calculated partial correlation coefficients were then normalized by conversion to a z-statistic using the Fisher's transformation, using tools implemented in the statistics toolbox of Matlab (The Mathworks, Natick MA, USA).

Higher-level group analysis

Passive paradigm datasets were subdivided into 5 age-specific groups (31-32wks, 33-34wks, 35-36wks, term equivalent (37-43wks)), where subjects were categorized in accordance with their PMA at scan as reported in Table 6.1.

Individual subject F-test maps were co-aligned to an age specific spatio-temporal neonatal atlas using FSL's non-linear image registration tool (FNIRT) (Serag et al., 2012). High resolution T2 images were used to guide the sequence of spatial transforms needed to attain an accurate registration between the lower resolution subject-specific fMRI image, and the age specific neonatal templates. The registration pipeline adopted entailed (i) registering the subject-specific T2 image to the subject-specific functional images (mean volume) using a 6DOF linear registration (ii) using this transform to transpose the z-statistic image into the T2 image space (iii) registering the T2 image to standard space using a 12DOF linear registration (iv) using FNIRT to register the T2 image to a standard age specific template using non-linear registration (v) finally applying the resulting non-linear warp to the z-statistic image in the T2 image space to get full alignment into the standard template space suitable for higher level analysis.

For each age group, the aligned f-test z-statistic images were combined to form a single 4D data set, and higher level analysis of each data set was performed using non-parametric t-testing and permutation testing to identify suprathreshold clusters with 5mm of variance smoothing (suggested to improve the estimation of the variance for data sets including up to 20 subjects) as implemented in FSL Randomise (Altman and Bernal, 2001). As described in Chapter 2, this approach was deemed suitable as no assumption could be made concerning the distributions of the f-statistic data sets and noise. Significant clusters were identified at a threshold of $p < 0.05$ with threshold free cluster enhancement (TFCE) and family-wise error correction (FWE) 05 (Nichols and Hayasaka, 2003). Using this approach, the possible confounding effects of sedative medication on the identified responses was also explored in a subset of 10 patients (5 of whom had received sedation for data collection and 5 of whom had not) using a paired t-test (paired by PMA at scan). An identical approach was also utilized to analyze the active-paradigm datasets, where datasets were grossly subdivided into preterm and term and registered to the template corresponding to the median age of each group.

Finally, permutation testing as implemented in FSL Randomise (Altman and Bernal, 2001) was also used to estimate the effects of PNA on the z-statistic maps of functional responses in the 22 infants of up to 35 wks PMA at scan. A GLM was used within randomise with PNA as the

explanatory variable, and PMA as a further confound of no interest. Crucially in the studied groups, PNA and PMA were not correlated (Pearson's r correlation coefficient = 0.18).

Table 6.1: Demographic characteristics of the study population

Subject group	Number of subjects	PMA at scan (weeks + days) (median, ranges)	GA at birth (weeks + days) (median, ranges)	PNA at scan (weeks + days) (median, range)	Birth weight (g) (median, ranges)	Birth occipito- parietal head circumference (cm) (median, ranges)
31-32 weeks	9	32+1 (31+0 - 32+4)	29+5 (27+3 - 30+5)	2+5 (1+2 - 6+2)	1210 (695 - 1650)	27 (24.6 - 29.9)
33-34 weeks	13	34+2 (33+3 - 34+5)	31+3 (27+1 - 33+2)	2+4 (0+4 - 7+4)	1450 (1025 - 2100)	28.1 (26 - 30.5)
35-36 weeks	10	35+6 (35+0 - 36+1)	34 (29+5 - 35+6)	1+6 (0+2 - 6+2)	1640 (850 - 2160)	29.0 (26 - 31.5)
Term equivalent (37-44 weeks)	15	41+1 (37+6 - 43+2)	29+6 (25+3 - 39+5)	11+1 (0+1 - 17+2)	1350 (545 - 3520)	28.25 (22.7 - 33.5)

6.3 Results

6.3.1 Passive movements

Functional MRI data for the passive paradigm were successfully collected from 47 of the 62 infants studied. Data were discarded in 11 infants due to excessive head motion throughout the period of image acquisition which could not be resolved with the measures described above; in 3 infants due to the identification of unexpected pathology (one infant with subependymal heterotopia, one with severe punctate white matter lesions, and one with severe cerebellar hemorrhage); and in 1 infant due to severe image artifacts associated with the EPI acquisition sequence. The final study group therefore consisted of 9 infants studied between 31 and 32+6 weeks PMA at the time of MRI scan (median age: 32+1 weeks PMA); 13 infants between 33 and 34+6 weeks PMA (median age: 34+1 weeks PMA); 10 infants between 35 and 36+6 weeks PMA (median age: 35+6 weeks PMA); and 15 infants at term equivalent age (between 37 and 44 weeks PMA) at the time of data acquisition (median age: 41+1 weeks PMA) (full study population details are shown in Table 6.1).

Preterm maturation

Following induced flexion and extension movement of the right wrist, I identified a positive BOLD contrast response in the contralateral (left) primary somatosensory cortices of all 47 infants studied. Responses were predominately contralateral in the youngest infants studied between 31 and 32 weeks post-menstrual age (PMA), and localized to the peri-rolandic region (encompassing both the primary somatosensory and motor cortices). A trend towards increasing involvement of the accessory motor areas was observed with increasing PMA; firstly involving the supplementary motor area (SMA) at 33 to 34 weeks PMA, and then the ipsilateral primary somatosensory cortex and contralateral thalamus at 35 to 36 weeks PMA. We also saw a maturational trend towards faster and higher amplitude responses which were more spatially dispersed across the peri-rolandic region (Figure 6.1b,c). As in resting-state network ontogenesis, a mature adult-like activity pattern was seen at the conclusion of the third trimester (37-44 weeks PMA or term equivalent age), with well localized functional activity visible in both the left and right peri-rolandic regions, SMA, bilateral basal ganglia and contralateral insular cortex (Fransson et al., 2009a, Mento and Bisiacchi, 2012).

During the late preterm period (35-36wks PMA) I saw a prominent trend towards increasing correlation between the mean time-series of the induced BOLD response in the left and right perirolandic regions (Figure 6.2a). This is consistent with a strengthening of the functional interhemispheric connectivity suggested by resting-state studies (i.e. (Doria et al., 2010)), accompanied by a concurrent increase in structural interhemispheric connectivity which has been revealed at around this period by MR markers of white matter microstructure within the corpus callosum (Fransson et al., 2007a).

I further found that this process is influenced by activity dependent processes up to 35 weeks PMA, with the length of ex-utero exposure (controlling for PMA) linearly related to both the occurrence of functional responses in the ipsilateral cortex and the correlation between BOLD responses in the left and right sensorimotor cortices (Figure 6.2b,c). These findings are in agreement with those of animal studies which show that the later stages of prenatal corpus callosum development are critically dependent on activity-dependent mechanisms driven by both spontaneous and sensory-dependent neuronal activity (Wang et al., 2007). At the end of the third trimester, I found that the development of interhemispheric connectivity appears to be pre-determined by PMA, as a widely dispersed and bilateral response pattern was seen regardless of post-natal age.

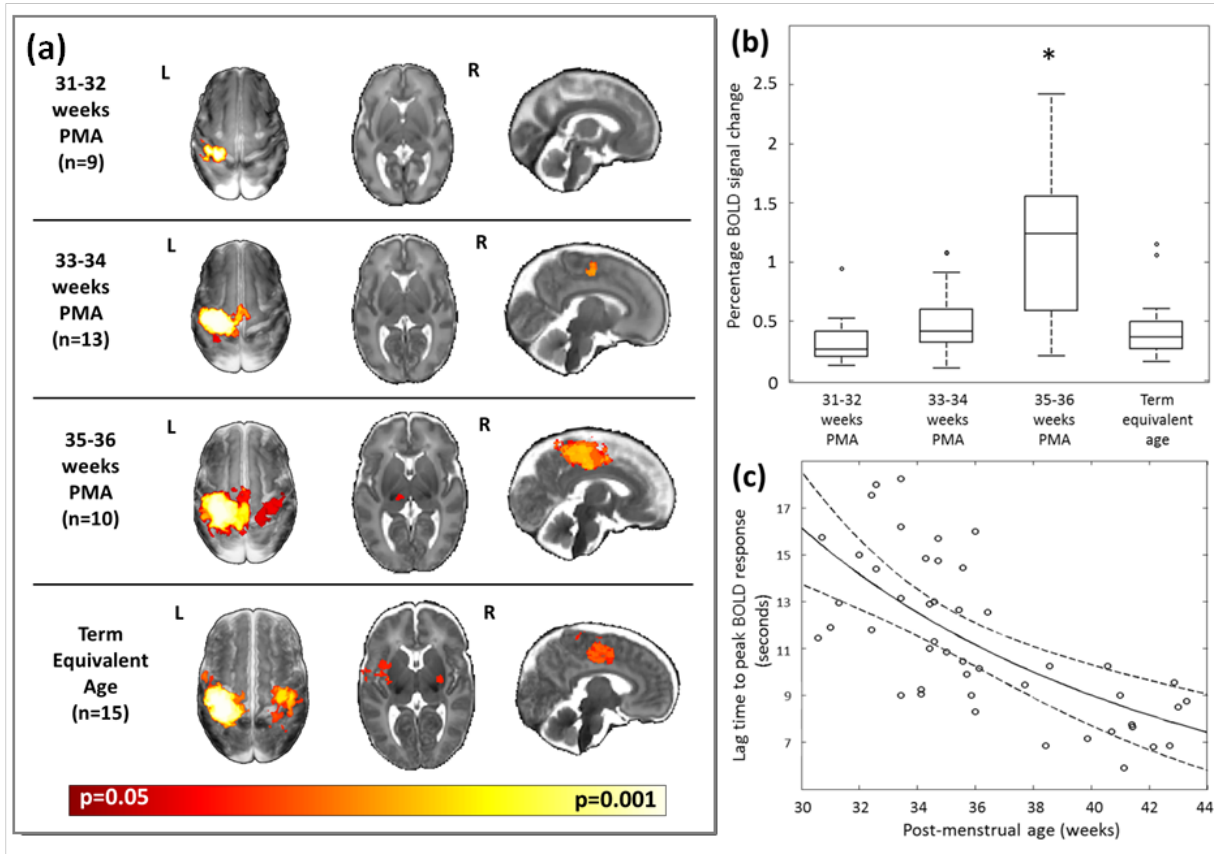


Figure 6.1: Evolution of Somatosensory functional responses to induced movement of the right wrist during the preterm period. **(a).** Following 0.3Hz flexion and extension movements of the right wrist, localized clusters of functional activity were identified in all infants in the contralateral (left) primary somatosensory cortex. Functional responses progresses initially from a contralateral only response pattern in the youngest infants (31-32 weeks post-menstrual age (PMA)); to then include the midline supplementary motor area (SMA) at 33-34 weeks; and the ipsilateral hemisphere at 35-36 weeks. At term equivalent age (37-44 weeks), a mature adult-like activation pattern is seen including the bilateral peri-rolandic regions, basal ganglia, SMA, and contralateral insula (results of one-sample t-test performed using permutation testing and corrected for the Family-Wise Error (FWE) have been overlaid on an age specific 4-D brain T2-weighted brain atlas. **(b).** The amplitude of the BOLD signal change within the identified clusters of activity are significantly higher at the conclusion of the third trimester in comparison to younger infants and at term equivalent age (line represents data median; box represents data lower and upper quartile; * denotes $p < 0.05$ Mann-Whitney test with Holm-Bonferroni correction). **(c).** The lag time to the peak of the identified response decreases systematically with increasing PMA (data shown with robust regression line with prediction error bands).

Term transition

At term equivalent age, we observed a counter-intuitive decrease in both fMRI response amplitude and signal correlation across the bilateral peri-rolandic cortical regions (Figure 6.1c, Figure 6.2a). This change mirrors that seen in response to somatosensory stimulation with electrophysiological techniques at this juncture; where a clear transition from non-specific high

amplitude neuronal bursts of delta-brush activity to more specific somatosensory evoked potentials is observed (Vanhatalo and Lauronen, 2006, Nevalainen et al., 2014, Vanhatalo and Kaila, 2010, Fabrizi et al., 2011). This transition temporally corresponds to the dissolution of the transient subplate zone, which is crucial throughout the third trimester for the establishment of thalamocortical, callosal, and association structural connections (Kostovic and Jovanov-Milosevic, 2006, Vanhatalo and Kaila, 2010, Vanhatalo and Lauronen, 2006).

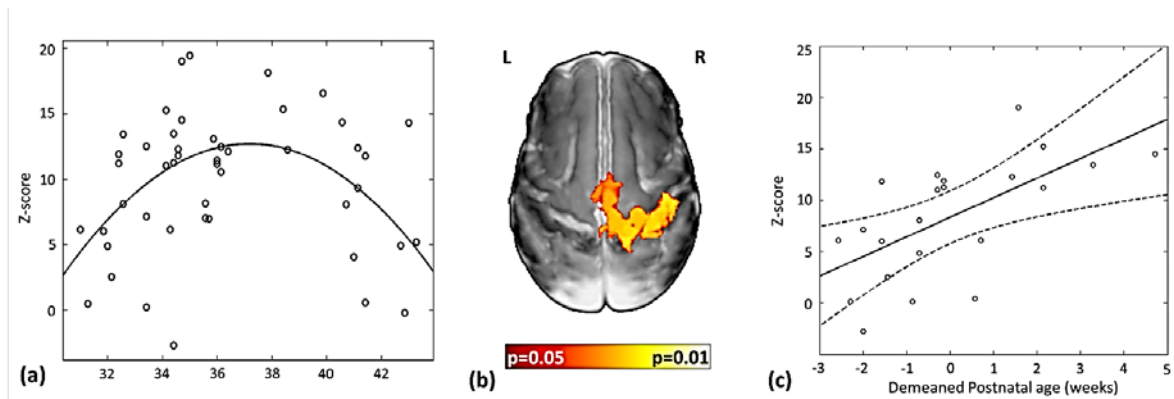


Figure 6.2: Interhemispheric functional connectivity increases during the preterm period and is influenced by ex-utero exposure. **(a).** Correlation between the BOLD contrast timeseries to somatosensory stimulation in the left and right peri-rolandic regions increases rapidly during the preterm period, and reaches a maximum value at 36 weeks PMA. It then falls at term equivalent age, suggesting that connectivity becomes more focal at this time (data represents z-transformed partial correlation coefficient). **(b).** Functional activity in the ipsilateral primary somatosensory cortex and supplementary motor area has a linear relationship in the preterm period (up to 35 weeks PMA, $n=22$) (results of linear model fitting based on post-natal age at scan (and controlling for PMA) are overlaid on an age specific template). **(c).** In the same infants, correlation between the induced BOLD timeseries in the left and right peri-rolandic regions rises with the demeaned post-natal age.

6.3.2 Spontaneous movements

Functional MRI data for the active paradigm were successfully collected from 18 of the 40 infants studied. Datasets from 22 infants were discarded due to either: (i) sustained head motion, (ii) patterns of head motion correlated with spontaneous wrist movements, (iii) less than 3 spontaneous right wrist movements being recorded throughout the period of image acquisition. The final study group consisted of 10 preterm infants (median age: 33+3 weeks PMA, age range: 32+2 to 36+3 weeks PMA) and 8 term infants (median age: 41 weeks PMA, age range: 37+5 to 43 weeks PMA).

Spontaneous right wrist movements in preterm infants were significantly associated with widely dispersed brain activity across both the primary somatosensory and motor cortices, nearly identical to that seen following induced wrist movement (Figure 6.3c). At term equivalent age, spontaneous movements were associated with a more distinct and anterior pattern of functional activity, further supporting a tendency towards increasing functional localisation of activity at this stage (Figure 6.3d).

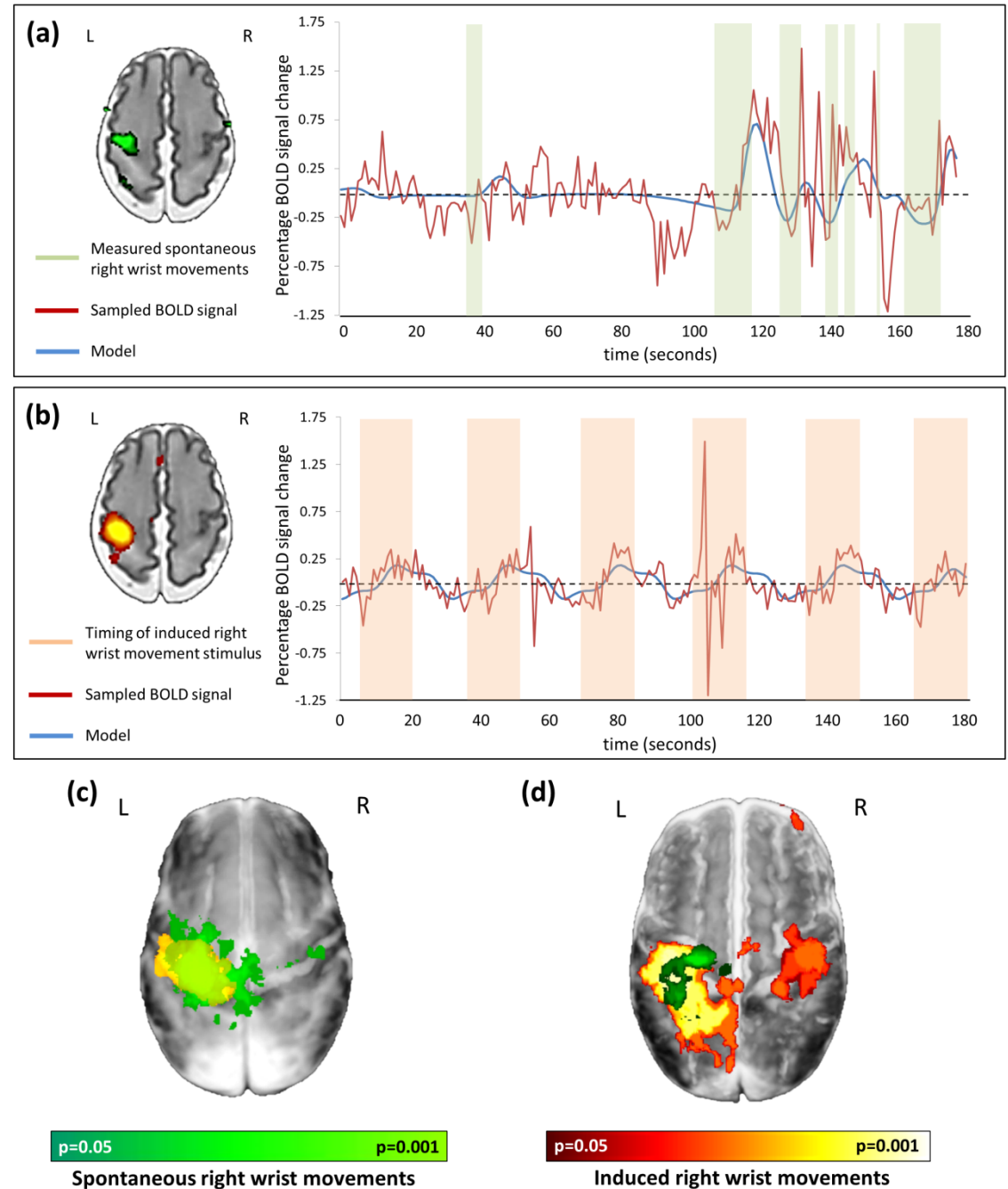


Figure 6.3: Spontaneous limb movements in the preterm and neonatal brain are associated with functional brain activity in the peri-rolandic cortices. **(a).** Using a precise fibre-optic position sensor, spontaneous right wrist movements during image acquisition were recorded (green blocks) and convolved with an age-specific Hemodynamic Response Function (HRF) to model (blue line) the acquired BOLD signal (red line) (Arichi, 2012, Arichi et al., 2012). In this example preterm infant (32+3 weeks PMA), spontaneous movements of the right wrist were significantly correlated with the BOLD signal in the left peri-rolandic cortex (green cluster). **(b).** This same approach also shows the reproducible pattern of induced right wrist movement (orange blocks), with the measured BOLD signal closely fitting the design model. In the same infant, a localised cluster (red-yellow) can be seen in the left primary somatosensory cortex. **(c).** Spontaneous movements in preterm infants (median age 33+3 weeks PMA, n=10) are associated with a large cluster of activity (green) in the bilateral peri-rolandic regions which is nearly identical to that seen following somatosensory stimulation (red-yellow). **(d).** At term equivalent age (median age 41 weeks PMA, n=8), functional activity associated with spontaneous movements (green) is more focal and anterior to that seen following somatosensory stimulation (red-yellow).

6.4 Discussion

6.4.1 Maturation of BOLD responses to somatosensory stimulation

This study utilized task-fMRI and a set of population-tailored experimental tools including a preterm specific hemodynamic response function (HRF) (Arichi, 2012, Arichi et al., 2012) and a custom MR-safe proprioceptive stimulus (Allievi et al., 2013) to precisely characterize for the first time, the maturation of functional responses to external somatosensory stimulation within the developing human brain. I found that, throughout the preterm period, infants exhibit an increasingly complex and bilateral pattern of functional activation at the level of the perirolandic cortical regions, paralleled by a growing involvement of the accessory motor areas. As preterm infants reach term equivalent age, clusters of functional activity become increasingly specific to the cortical regions commonly associated with somatosensory processing in the adult brain. Both the peak amplitude and the cross-hemispheric correlation of BOLD responses within the primary sensorimotor regions are seen to steadily increase throughout the preterm period, to subsequently decrease at term equivalent age.

As hypothesized by previous resting-state studies, and supported by evidence from structural imaging, I suggest that the progressive emergence of bilateral patterns of functional responses may reflect the growth and strengthening of the underlying interhemispheric connectivity. In fact, MR measures of white matter microstructure (such as fractional anisotropy (FA)) within the corpus callosum have been described to steadily rise during the third trimester of gestation, indicating that the maturational increase in functional connectivity is accompanied (and subserved) by an associated increase in structural connectivity (Dudink et al., 2007, Rose et al., 2014). I propose that the enhanced localization of activation observed at term age, and the concurrent decrease in cross-hemispheric BOLD signal correlation may be caused by: (i) a systematic pruning of redundant neuronal projections which accompanies the specialization of cortical regions (Volpe, 2008) (ii) a transition from spatially diffused, high-amplitude subplate mediated responses, to lower amplitude focal responses following cortical ingrowth and arborisation of thalamocortical projections and disappearance of the subplate layer (as observed in EEG studies) (Vanhatalo and Kaila, 2010, Vanhatalo and Lauronen, 2006) (iii) a combination of the above.

6.4.2 Spatio-temporal maturation of BOLD responses

Prior to this study, only Arichi and colleagues had investigated somatosensory neural correlates via task-fMRI on cohorts of preterm infants of up to 35 weeks PMA, with the vast majority of subjects exhibiting positive BOLD responses circumscribed only to the contralateral somatosensory cortex (9/11 subjects 29 to 34 wks PMA, 6/6 range 32-34 wks PMA, and 10/10 32-35 wks PMA). This is partially consistent with my results, as 35 wks PMA corresponds to the age at which bilateral responses only begin to emerge. In keeping with my findings, bilateral activation has instead been extensively reported amongst term age infants by all previous task-fMRI studies (Heep et al., 2009, Erberich et al., 2003b, Erberich et al., 2006, Arichi et al., 2010). My results are also largely consistent with the developmental trajectory of the HRF delineated by Arichi and colleagues via somatosensory task-fMRI, as I observe a clear maturational trend towards a decreased time-to-peak in the evoked BOLD response (despite a counter-intuitive decrease in peak amplitude at term age, discussed below (Section 6.4.3)).

Animal models suggest that during the preterm period, resting-state network activity may be predominantly driven by peripheral sensory input, rather than by cortico-cortical circuitry as in adults (Colonnese and Khazipov, 2012). It is therefore advocated that the somatosensory resting-state network may be indicative of the underlying afferent functional connectivity. I directly probed the maturation of the somatosensory system in response to proprioceptive stimulation, and my findings are in substantial agreement with the reported spatio-temporal maturation of the somatosensory resting-state network (Doria et al., 2010), thus partially validating this hypothesis. The results of Doria and colleagues obtained via both ICA and seed-based correlation analysis show that the somatosensory resting-state network emerges unilaterally in early preterm infants (defined as 29 to 32 wks PMA) to then gradually and progressively assume a bilateral configuration from preterm age (defined as 33 to 36 wks PMA) through term equivalent age, with an increasing involvement of the supplementary motor area and thalamic nuclei (the latter apparent on seed-based correlation analysis only).

My results highlight a similar maturational trend towards increasing bilateral activation, and also precisely identify a growing involvement of well circumscribed lower accessory motor areas (such

as the thalamus, basal ganglia and the insular cortex). In comparison, resting-state function identified via seed-based partial correlation analysis seems to grossly encompass entire anatomical patterns of thalamocortical connections. While it is possible that this different spatial specificity arises from statistical conventions, the higher signal-to-noise ratio (SNR) of task-fMRI, or registration effects, I propose that it could also be due to the ability of task-fMRI to more directly and finely probe cerebral responses exclusively associated with the transmission and elaboration of a very specific ascending peripheral input. In contrast, seed-based resting-state somatosensory networks have the potential to overestimate such functional connectivity (Colonnese and Khazipov, 2012), as the underlying signal fluctuations may not only arise from spontaneous firing of peripheral receptors, but that also originate from spontaneous intra-cerebral activity directly induced by higher brain structures (such as the thalamus, the brainstem and the subplate) (Vanhatalo and Kaila, 2010). This hypothesis is supported by electrophysiological studies, which suggest that early slow-evoked responses resembling those generated by (endogenous or exogenous) peripheral sensory activation (delta brushes), although not necessarily spatially circumscribed to the sensorimotor areas, can also be triggered endogenously via intrinsic intracortical mechanisms (Vanhatalo and Kaila, 2010). In the absence of a precise model detailing the time course of intracortical and induced activity, these patterns may be difficult to discern based on spatial and frequency correlation of BOLD signal alone. In this respect I propose that although resting-state fMRI may be indicative of the large-scale functional connectivity within the somatosensory network, it does not provide a true estimate of its ability to transmit and process peripheral information; and that such insight is more likely to be directly gained via task-fMRI.

In keeping with the findings described in Chapter 4, the somatosensory system exhibits an early somatotopic differentiation both at the level of the cortex and of the underlying structural connectivity. This might also serve to explain the enhanced spatial specificity of activation patterns described via my task-fMRI study compared to that revealed by ICA based resting-state analysis (and seed-based resting-state study of Doria and colleagues (2010) where the seed region was extended to the entire somatosensory cortex (Doria et al., 2010)), where the somatosensory network was considered as a unit. By making prior assumptions on the anatomical location of seed regions

associated with the functional representation of specific body parts, Smyser and colleagues (2010) were able to split the resting-state motor network into somatotopically organized sub-networks representing the “face”, “foot” and “hand” (Smyser et al., 2010). In this respect the “hand” motor resting-state network could be comparable to my findings, however it was only studied at 4 wks intervals. From the reported results, it can only be assumed that the hand network’s bilateral representation and involvement of the supplementary motor area occur sometime between the 34 wks PMA and term equivalent age, which is consistent with my findings. In contrast, the “leg” and “face” networks seem to already achieve such mature organization by 34 wks PMA (Smyser et al., 2010).

Taken together previous resting-state studies therefore suggest that, although possible, interpreting resting-state function of individual body parts is inherently challenging. Choosing an arbitrarily large number of ICA components has the potential to over-segregate the somatosensory network and reveal fictitious “artificial sub-networks”, seed-based correlation techniques can be performed selecting an almost infinite amount of neighbouring seed regions, which, if appropriately thresholded, may all reveal different correlation maps (Smith et al., 2009). I instead propose that task-fMRI has the potential to assess the functional connectivity directly associated with the processing of somatosensory information arising from individual body parts. In view of the important role of early somatosensory processing in the establishment of the later primary motor map, I suggest that this kind of specificity may hold important clinical value in accurately predicting the nature of later neuro-developmental outcome.

6.4.3 Preterm to term transition

As previously discussed, at the transition between late preterm and term equivalent age, I observed an enhanced localization of functional responses, accompanied by a concurrent decrease in BOLD signal correlation across bilateral peri-rolandic cortical regions (defined by an anatomical mask) and a reduction in the amplitude of BOLD response. It has been proposed that this may be due to an early period of synaptic pruning associated with the specialization of cortical regions (Volpe, 2008), and/or to the shift in the neural origin of responses reported by EEG studies, with a transition

at circa GW37 from subplate mediated spatially dispersed high-amplitude activation to a more localized low-amplitude SEP response directly associated with thalamocortical ingrowths (Nevalainen et al., 2014, Vanhatalo and Kaila, 2010, Vanhatalo and Lauronen, 2006).

However this effect was not observed or reported by previous resting-state fMRI studies; this may be due to several reasons. Firstly, despite the rapid maturational changes that occur during the preterm period, in previous fMRI studies preterm infants have been subdivided into larger age groups; Doria and colleagues (2010) combined subjects from 33 to 36 wks PMA, possibly leading to an underestimation of the spatial complexity of the cortical responses already present at 35-36 wks PMA (which in turn may have masked the transition from late preterm to term age) (Doria et al., 2010). Secondly, although possibly representative of large scale functional connectivity, the resting-state somatosensory network may be relatively insensitive to functional responses associated with the processing of ascending information from specific body parts, and thus may not capture the enhanced spatial localization of somatotopic responses that accompanies the progressive differentiation of neuronal afferents. Thirdly, although Doria and colleagues utilized my same anatomical masks to estimate the sensorimotor cross hemispheric correlation of the BOLD signal, they found a linearly increasing trend through term age; however in my study cross hemispheric signal correlation was guided by higher amplitude, regular BOLD signal fluctuations induced by passive wrist movements, rendering the correlation analysis more specific to patterns of functional activation in response to a precise stimulus. Moreover, the creation of high-amplitude signal waveforms intuitively renders the analysis less sensitive to artefactual signals, and increases SNR possibly providing a better estimate of functional correlation.

6.4.4 Effects of extra-uterine experience

Infants delivered prematurely are constantly exposed to environmental stimulation, and there is now increasing evidence that this additional sensory experience may influence structural cerebral growth, and shape the early maturation of functional connectivity in several sensory networks (Groppo et al., 2012). In infants up to 35 weeks PMA I found that increased post-natal experience was associated with a significant increase in the activation of the ipsilateral peri-rolandic regions

accompanied by a growing involvement of the supplementary motor area, as well as a significant increase in cross hemispheric signal correlation. This can be interpreted as an activity-driven maturation of inter-hemispheric connectivity, which is in keeping with animal studies suggesting a critical role of sensory experience in the perinatal establishment of the corpus callosum (Wang et al., 2007).

Interestingly, EEG studies of spontaneous cerebral activity have predominantly found that extra-uterine experience may delay the normal bioelectrical trajectory of cerebral maturation (e.g. (Nunes et al., 2014)), while resting-state fMRI studies have found no significant difference in any functional connectivity network between term-born and preterm infants at term equivalent age. Structural studies of white matter tracts have reported somewhat conflicting results. An early DTI study by Hüppi and colleagues (Hüppi et al., 1998) revealed that preterm birth is associated with lower fractional anisotropy (FA) of the internal capsule (IC) suggesting extra-uterine experience induces a developmental delay. On the contrary, a more recent study by Gimenez and colleagues found that preterm infants exhibit higher FA at the level of the sagittal stratum, suggesting that, possibly due to an enhanced extra-uterine exposure to somatosensory stimulation, the preterm brain undergoes a process of accelerated white matter tract maturation (Gimenez et al., 2008).

A prior electrophysiological study aimed to directly assess the influence of extra-uterine exposure on the somatosensory system, and found no significant effect of PNA on somatosensory-evoked potentials in infants born preterm without neurological deficits (Tombini et al., 2009). Guzzetta and colleagues have consistently demonstrated that massage therapy can accelerate the maturation of spontaneous electrical brain activity (Guzzetta et al., 2009). My results indicate the existence of a critical period where activity dependent mechanisms accelerate the maturation of somatosensory functional connectivity, but that by the end of the third trimester of gestation preterm infants achieve similar bilateral patterns of functional responses independently of their extra-uterine exposure. Although by no means conclusive, these findings may help explain some of the inconsistencies revealed by previous studies of the somatosensory network; and suggest that the effects of extra-uterine experience on the maturation of the somatosensory system should be closely studied during the early preterm period (up to 35 weeks PMA).

Clinical relevance

While taken together these results and the work of Guzzetta and colleagues (2009) suggest that there may be scope for therapeutic intervention to guide early preterm brain plasticity (Guzzetta et al., 2009), animal studies have shown that prolonged, sustained sensory stimulation during the neonatal period may also discourage cerebral angiogenesis, leading to possible adverse long-term effects on clinical outcome (Whiteus et al., 2014). The possible implications of such detrimental interactions, and the difficulty in devising and validating appropriate interventional strategies, have led physicians to generally use a minimum-touch policy, although Newborn Individualized Developmental Care and Assessment Programs (NIDCAP) have been shown to provide better clinical outcomes (Als et al., 2011). However I propose that clinicians have the opportunity to develop and implement strategies of interaction that can shape and guide the development of connectivity as best as possible in the clinical context. Devising therapeutic strategies is however a challenging task, since the infant's sensory experience in the newborn intensive care unit (NICU) environment, including exposure to bright lights, high sound levels, and frequent noxious interventions, may also have deleterious effects on the immature brain and alter its development (Sehgal and Stack, 2006). In this context, techniques such as task dependent fMRI may offer a characterization of functional physiological milestones that integrate the structural prognostic and diagnostic features currently under investigation (Panigrahy et al., 2012). Physicians may thus be offered a holistic approach to monitor and evaluate the implementation of clinical interventions for each infant.

6.4.5 Correlates of spontaneous movements

Animal studies suggest that spontaneous movements during preterm age may be triggered by the spine, and represent an endogenous mechanism to ensure appropriate activity-dependent development of the somatosensory system through a constant influx of peripheral feedback (Khazipov et al., 2004). This hypothesis is supported by the findings of Milh and colleagues (2007), who reported a correlation between spontaneous hand and foot movements and dispersed cortical

delta-brushes in preterm infants, which resemble typical somatosensory evoked responses (Milh et al., 2007). It should however also be noted that preterm infants make infrequent but complex spontaneous movements, which are qualitatively abnormal following brain injury (Gao et al., 2011, Spittle et al., 2008); this suggests that higher level neural circuitry may also be involved in their modulation and/or initiation.

By precisely measuring the timing and velocity of position changes of the right wrist during BOLD fMRI acquisition it has been possible, for the first time, to precisely identify and localize intra-cerebral correlates of spontaneous limb movement (Figure 6.3a). In both preterm and term infants these were circumscribed to the peri-rolandic sensorimotor cortex, and appeared to lie adjacent, and follow a pattern of specialization similar to the responses identified via proprioceptive stimulation previously reported. Although the temporal limitations of fMRI prevent us from definitively ascertaining their role, my results together with the findings of Milh and colleagues (2007), suggest that as in rodents at this juncture (Khazipov et al., 2004), spontaneous peripheral movements play a fundamental role in triggering bursts of cortical activity during early brain development in a somatotopic manner (Milh et al., 2007). This may guide early establishment and differentiation of the somatosensory and primary motor cortices.

6.5 Limitations and Considerations

Sedation effects

A low dose of sedative medication was administered to the majority of infants and term equivalent age, whereas preterm infants were predominately scanned during natural sleep. Term subjects are inherently more susceptible to physical and auditory perturbations and tend to move exceedingly and become distressed during the scanning process. In this specific subject group sedation has the additional significant benefit of reducing severe head motion which can cause the systematic identification of false patterns of functional activity (Power et al., 2012, Satterthwaite et al., 2012, Van Dijk et al., 2012b) and is the leading cause of fMRI data corruption. Although we cannot definitively exclude a possible effect of chloral hydrate induced sedation on the cerebral

hemodynamics which underlie the described functional responses, we did not identify a significant difference in the spatial distribution of functional responses using a paired t-test (paired by age at scan) analysis of a small subset of age matched subjects (1 preterm subject, 4 term equivalent subjects) who were and were not sedated for data acquisition. Furthermore, previous work has found that chloral hydrate sedation does not affect either the topology of identified resting-state networks or baseline cerebral blood flow (CBF) in neonatal subjects (Doria et al., 2010, Arichi et al., 2012, Arichi, 2012).

Population effects

While all subjects included in the study population were diagnosed as being without overt clinical problems at the time of scan, preterm infants are inherently susceptible to cerebral palsy and other neuro-developmental deficits (Doria et al., 2010) which may only become apparent after the first year of age. Although unlikely, negative clinical outcome cannot be completely discarded without a long-term follow-up study.

Robotic stimulator

Proprioceptive stimulation was delivered systematically to the infants inside the MRI scanner via the custom robotic device presented in Chapter 4. Although the proprioceptive stimulus induced by the joint movement was accompanied by a slight tactile stimulation due to handle-to-hand contact, this contact was permanent so likely affected the rest periods in a similar way as during motion. Furthermore, the wrist flexion/extension device design starkly reduced the tactile effects of skin stretching caused by the balloon stimulator employed in previous studies (Arichi et al., 2010). The robotic stimulator was also accurately controlled to provide a pattern of wrist motion that was both precise in terms of frequency and amplitude of motion and repeatable across subjects. The device-mounted position sensor allowed experimenters to log and verify the robustness of the movement induced to all subjects and to monitor in real time whether the device actuation was being obstructed by items in the scanner and/or opposed by strong spontaneous movements/reflexes during scans. However, subtle spontaneous movements of the passively stimulated wrist as well as spontaneous movements of other limbs could not be monitored during scans.

In view of the sensor's response linearity (as shown in Chapter 4) a quick 2 point calibration with the device in place was carried out prior to each scanning session, which was both time efficient and sufficient to guarantee robustness of readings. Following this initial calibration the within-session signal differential was constant, thus ensuring that the full range of motion was produced by the stimulator throughout experiments. The calibration was also used for the subsequent active session.

6.6 Conclusion

I have characterized the key stages of somatosensory and motor system development in the human brain during the period equivalent to the third trimester of gestation using task-fMRI and an MR-safe robotic interface which can provide both proprioceptive stimulation (via induced wrist movement) and record peripheral spontaneous motor behavior.

My findings show a systematic maturational trend in the spatial and temporal characteristics of somatosensory functional responses, which, at least in the early to mid-preterm period, appears to be influenced by activity-dependent mechanisms. These findings highlight the critical importance of this period for motor and somatosensory development, and hint towards the potential for exploiting activity dependent plasticity following injury to the preterm brain.

I further showed for the first time using fMRI, that spontaneous movements in early life are associated with functional activity at the level of the primary sensorimotor cortices. This is in keeping with what was observed in rodent studies (Khazipov et al., 2004) and indicates that spontaneous movements may serve to promote the early establishment of the sensorimotor system through activity dependent mechanisms.

Chapter 7: Conclusions and Future work

Some of the concepts within this chapter have been previously published in a review article:

Allievi AG, Arichi T, Gordon AL, Burdet E. Technology-aided assessment of sensori-motor function in early infancy. *Frontiers in Neurology (Neuropediatrics)* 2014; 5: 197-207.

7.1 Motivation and Aims

Studying the preterm brain via functional magnetic resonance imaging (fMRI) techniques offers a unique opportunity to probe the emergence of brain function during early human development at a fine spatial resolution. In addition to elucidating some fundamental neuroscientific questions, this can provide clinicians with much needed early diagnostic and prognostic information about a vulnerable demographic that can be affected by long term neuro-developmental impairment. However, owing to methodological challenges; including the inability to induce TR-locked, robust and consistent patterns of stimulation, fMRI studies of the preterm population have so far reported a discouraging sequence of inconsistent and contradictory findings. This is especially true for studies aimed at assessing the early function of the sensorimotor system; which is of particular relevance given the susceptibility of preterm infants to develop neuromotor dysfunctions such as cerebral palsy (Larroque et al., 2008a). While in adult fMRI studies sensory stimuli are commonly presented via computer-controlled robotic systems (Gassert et al., 2006b, Tsekos et al., 2007), transposing this approach to the preterm population is a challenging task, which requires a careful consideration of multiple safety and practical aspects.

7.1.1 Technical aims

The first goal of this thesis was to address these challenges and to develop an approach that would provide researchers and clinicians with low-cost, safe and reliable solutions to conduct task-dependent fMRI studies of the preterm population. This required developing a computer-controlled

system which is clean, efficient, simple and modular; such that the core architecture could be used in different configurations, or with interchangeable interfaces, to perform multisensory stimulation.

Furthermore the system had:

- i. to be able to induce stimulation patterns of constant amplitude and frequency, which are sufficient to elicit robust functional responses.
- ii. to be capable of presenting a stimulation type and pattern which is appropriate for the neuro-developmental stage of the study population.
- iii. to allow remote monitoring of the operation of the stimulus to ensure stimulation is presented in a consistent and safe manner.
- iv. to not induce additional head movements which can cause image artefacts.

Moreover the system's interface should:

- i. not contain ferrous materials and be fully MRI/fMRI compatible.
- ii. be mechanically safe to avoid distress or possible harm to the infant.
- iii. be able to provide stimulation synchronised with fMRI acquisition.
- iv. be light, small and flexible enough to avoid the infant suffering movement restriction or discomfort.
- v. be easily cleanable to prevent infection spreading from one infant to another.

7.1.2 Neuroscientific aims

The second goal of this thesis was to utilize the systems developed to conduct task-dependent fMRI studies of the preterm population. As previously mentioned, characterizing the function of the sensorimotor system is of particular importance in preterm infants, and that was therefore the primary focus of this thesis. This required probing some fundamental traits of early brain functionality, which had never previously been studied in humans at a fine spatial resolution:

- i. The emergence of the somatotopic organization of the sensorimotor cortices
- ii. The intracerebral correlates of spontaneous motor activity

- iii. The maturational trajectory of the somatosensory system throughout the preterm period and how this can be influenced by extra-uterine exposure

A final goal of this thesis was to exploit the modularity of the developed stimulation system to further study the emerging functionality of the olfactory system, which despite its clear developmental importance had never been previously been studied in newborns.

7.2 Summary of Contributions

7.2.1 Technical contributions

Throughout the work of this thesis I developed a standard approach and a set of tools meeting all of the technical requirements described above, which is easy to operate, to maintain and, eventually, to replicate. The developed system can be compartmentalized into a modular control unit, and a range of interfaces. For the purpose of rendering the developed system accessible to clinicians and experimenters, the control unit was assembled from readily available off-the-shelf components such as electro-valves, a data acquisition card and a signal conditioner. The developed system architectures allows: (i) delivering TR-locked pneumatic actuation of constant amplitude and frequency (ii) online monitoring and logging of stimulation patterns (iii) interchanging interfaces and/or pneumatic valves to induce multisensory stimulation. Some interfaces had to be custom-made to meet the technical and safety requirements described above. The systems and interfaces are all the first of their kind to be suitable for fMRI studies of preterm infants. The technical innovations presented within this thesis are:

- a. A set of systems based on a common approach, and meeting the requirements described in section 1.1, these include:
 - An ankle and wrist stimulator able to induce proprioceptive stimulation through a specific and repeatable pattern of joint flexion/extension, and to provide real-time feedback on joint position;

- An olfactometer system able to present 3 separate odours, and to constantly monitor the input airflow;
 - A pacifier system able to induce specific patterns of orocutaneous stimulation, and to provide real-time feedback on the pacifier's intraluminal pressure.
- b. A set of interfaces specifically designed to meet the requirements described in section 1.1. including:
- The ankle and wrist robotic stimulators, which are actuated by an inexpensive, commercial LEGO piston, and featuring a custom fibre optic position sensor.
 - The olfactometer odour delivery system, which is made from disposable, medical grade material, thus ensuring full sterility of the equipment.
 - The pacifier interface which can be coupled to standard NICU pacifiers in an airtight manner, such that new sterile pacifiers can be used for each patient. Additionally the interface can both induce actuation via a custom balloon, and monitor intraluminal pressure both during both sucking and actuation via a custom mounted pressure sensor. Another non-MR safe actuated pacifier system has been described in the literature, which can however not monitor pressure during actuation.
- c. The sensors had to either be implemented in a novel manner, or to be engineered based on simple operating principles. These include:
- A commercial fibre-optic pressure sensor, which however had to be glued within a nylon screw to ensure that it could be coupled in an airtight manner to the pacifier chamber.
 - A custom fibre-optic goniometer, manufactured from a segmented mono-core fibre-optic lead, and based on the modulation of light intensity caused by the bending of the segmented fibre. While this concept had previously been explored for strain measurements (Kuang et al., 2002), this was the first implementation of such sensing principle for position measurements.

Finally the approach presented can be expanded to other fMRI-guided robotic applications by simply designing new interfaces and/or control algorithms. The position sensor developed for the wrist interface is a novel, low-cost solution which can be easily adapted and implemented in other robotic devices.

7.2.2 Neuroscientific contributions

The tools developed throughout this thesis were utilized to conduct task-dependent fMRI studies to probe, for the first time, several aspects of the emerging function of the human newborn brain at a fine spatial resolution. The major neuroscientific contributions of each study, carried out in collaboration with the Centre for the Developing Brain, are summarized below.

Contributions to the neuroscientific body of knowledge are here reported in terms of (i) findings description, (ii) integration of current knowledge, (iii) expansion of current knowledge:

- a. The first study to reveal the emergence of a spatially well-localized somatosensory “homunculus” from the early preterm period.:
 - **Findings:** Functional responses associated with passively induced ankle and wrist movements are spatially distinct and well-localized to the medial and lateral perirolandic regions respectively, contralateral to the side of stimulation.
 - **Knowledge integration:** This finding is in agreement with the broad spatial localization of somatosensory evoked responses previously identified via electrophysiological studies (Milh et al., 2007). It is in agreement with rodent studies, which have revealed that the fine somatotopic arrangement of the primary somatosensory cortex is established around post-natal day 10-15 (equivalent to the mid-late preterm period of human infants); with the vibrissae’s receptive fields maturing before others (Seelke et al., 2012).
 - **Knowledge expansion:** This is the first clear indication that a mature, spatially defined, somatotopic arrangement of the primary somatosensory cortex emerges early during the preterm period in human infants. This study has therefore provided new insights on the structural and functional congruence of the developing human.

- b. The first study to reveal the spatial localization of functional activity associated with spontaneous (wrist) movements.
 - **Findings:** Functional activity associated with spontaneous wrist movements is well localized to the perirolandic cortical region, and adjacent to the areas of activation associated with passive wrist stimulation.
 - **Knowledge integration:** This finding is in agreement with previous rodent studies, which have identified “spindle” bursts of functional activity in response to spontaneous movements (Khazipov et al., 2004). It is also consistent with a previous electrophysiological study in preterm human infants, which has found that similar, although broadly localized, patterns of functional activity are induced by both passive stimulation and spontaneous movements of the hand and foot (Milh et al., 2007).
 - **Knowledge expansion:** This study has provided first-time evidence that spontaneous movements are represented in a somatotopic manner. This strengthens the notion that spontaneous movements may sub-serve the early activity-driven development of the sensorimotor system (Milh et al., 2007). However the potential cortical involvement in the modulation and initiation of spontaneous movements cannot be excluded.
- c. The first task-fMRI study to characterize the maturation of functional somatosensory processing during the preterm period.
 - **Findings:** Throughout the preterm period somatosensory-evoked BOLD fMRI responses become faster, of higher amplitude, more spatially dispersed, and increasingly bilateral. Results further indicate that somatosensory network connectivity increases with maturation, and, during the early stages of preterm development, is influenced by extra-uterine experience. At term age, responses become more spatially circumscribed and of lower amplitude.

- **Knowledge integration:** The spatial trajectory of BOLD responses up to the late preterm period is in keeping with that reported by previous resting-state developmental studies. The decrease in response signal observed at term equivalent age may be explained by the concurrent disappearance of the subplate and the shift from high amplitude to low amplitude somatosensory-evoked responses reported by previous electrophysiological studies (reviewed in (Vanhatalo and Lauronen, 2006)). Previous literature has not comprehensively explored or demonstrated the effects of extra-uterine exposure on the functional somatosensory development of the preterm brain.
 - **Knowledge expansion:** In addition to providing a first-time characterization of the spatio-temporal maturation of functional somatosensory processing, this study highlights the importance of the preterm period for motor and somatosensory development, and indicates that, especially during the early stages of preterm neurodevelopment, it may be possible to exploit activity dependent plasticity following injury to the preterm brain.
- d. The first study to reveal the functional correlates of the olfactory system in preterm newborns studied at term equivalent age.
- **Findings:** Functional responses to formula milk odorant cues elicit functional activity at the level of the primary olfactory areas in newborns studied at term equivalent age.
 - **Knowledge integration:** Functional correlates of odour processing in the preterm brain have not been previously described in the literature, although behavioural and structural evidence indicates that the olfactory system is already highly functional at birth (e.g. (Browne, 2008)).
 - **Knowledge expansion:** These findings suggest that functionality of a sensory system, which plays a crucial role in early development, is already well-established by the time of birth.

7.3 Neuroscientific and clinical horizons

Taken together, the inconsistencies afflicting previous literature and the results here presented strongly suggest that it is crucial to deliver a repeatable pattern of sensory stimulation through fMRI-compatible systems if reliable cross-sectional and longitudinal task-fMRI studies are to be conducted. Throughout the work of this thesis it has been possible to probe some neuroscientific theories at a fine spatial resolution by means of task-dependent fMRI. The findings presented in Chapter 4 suggest that a seemingly well-defined somatotopic map of the somatosensory system emerges early during human foetal development, and it would therefore be intriguing to perform an fMRI mapping study in younger preterm infants, which might reveal the onset of functional differentiation in the sensorimotor system. So far, findings have been restricted to the wrist and ankle joints, but other interfaces such as the pacifier, corresponding to infants' most important activity, could be utilized to gain further insights on the early formation of the whole-body homunculus. Animal studies suggest that effector organs, which are important for early development, achieve a specific functional representation before others, and it is possible that the human brain follows a similar developmental trajectory. These effects cannot be inferred from structure alone, as cortical ingrowth of ascending neural pathways occurs quasi simultaneously in all sensory regions, although findings in rodents further suggest that structural and functional congruence of emerging cortical substrates is achieved gradually during an ex-utero period equivalent to the mid-late preterm period (post-natal day 10-15) (Seelke et al., 2012).

The custom wrist stimulator presented in this thesis was utilized to achieve the first task-fMRI characterization of the sensorimotor system development throughout the late preterm and term period, providing a first spatially-specific insight into the cerebral substrates involved in the processing of peripheral sensorimotor activity, and complementing the fine temporal information previously acquired through neurophysiological techniques. Through combining spatial information about the localization of functional brain activity derived from fMRI experiments with that derived from other analysis techniques such as diffusion MRI tractography, there is the opportunity to investigate the early establishment and organization of the framework of both structural and functional connectivity. This might also provide invaluable clinical information concerning the

sequelae of early cerebral insults, with some of reported heterogeneity in neurodevelopmental outcome following damage to specific anatomical substrates potentially explained by the underlying stage of functional maturation at the time of injury.

In this respect, it might be fruitful to characterize the longitudinal functional maturation of the entire somatosensory system via multimodal functional imaging techniques. Achieving a “template of normal functional brain maturation” throughout the preterm period could then help identify abnormalities in infants via standardized functional imaging protocols including task-fMRI paradigms. For instance, a combination of diffusion imaging and task-fMRI could be used to dynamically evaluate the functional representation of each limb in infants at risk of CP. If deviations from the normal developmental pattern are identified, then therapeutic strategies could be implemented, and their effectiveness constantly monitored. This could be a powerful method to improve clinical outcome, as neural plasticity appears to be activity-guided especially during the early preterm period (as indicated by our results presented in Chapter 6). This notion becomes even more compelling, when it is considered that there have been a number of case reports of children in which dramatic sustained alterations in functional neuroanatomy have been seen following focal brain injury earlier in life. The added benefit of characterizing and monitoring the normal maturation of the entire somatosensory system is that therapy-induced patterns of aberrant plasticity could be identified and averted, as increasing the functional representation of any individual limb could disrupt that of other body parts. While we have begun to characterize the maturation of the functional representation of the wrist (as was described in Chapter 6), future studies on larger cohorts of preterm infants with multimodal stimulations and clinical follow-up will be required before normal and abnormal templates of somatosensory function can be defined. A potential avenue to building a tool for the early detection of functional abnormalities would be to individually characterize fMRI output measures such as the location, intensity and temporal course of functional responses throughout different stages of normal or pathological development. These could be used to build and train a classifier (i.e. a Support Vector Machine or a Neural Network) where features extracted from fMRI (and potentially other imaging techniques) output measure are related to observed explanatory variables such as PNA/GA and clinical outcome. This classifier would contain

a description of normal and abnormal functional development, and could ultimately be used as a predictor of pathology.

This method could be used in the future to study the contribution of functional somatosensory deficits in the sensory mis-perception in subjects affected by a broad range of neurological conditions other than CP. This would require carefully selecting the study population, by including infants whose siblings may be affected by a neurodevelopmental disorder such as autism, or those with other congenital risk factors. For instance children with autistic spectrum disorders are known to exhibit hyper- and hypo-responsivity to touch (and to sensory stimuli in general); however controversy exists as to whether this is due to a perceptual dysfunction or is merely representative of an emotional response. Our approach could be used to broaden the base knowledge in this field, and determine whether hypo/hyper sensitivity to touch correlates with similarly altered functional somatosensory responses. Although the primary focus of the work of this thesis lies on the functional development of the sensorimotor system, it was demonstrated that the approach I presented is inherently flexible and can be used to investigate responses to several other types of sensory stimulations. I suggest that, similar to what was done here for the sensorimotor system; a developmental functional study can be performed encompassing other sensory systems which are known become established early in foetal life and are envisaged to be important to early post-natal human development. The approach is currently being utilized to study the olfactory, auditory and visual systems. As the robotic stimulation system is easily scalable, it can also be used to carry out more extensive longitudinal developmental studies going from early post-natal age to early adolescence, which would serve to describe the entire trajectory of pathophysiology and recovery. In addition to characterizing each sensory system separately, the stimulators could also be combined to investigate the neurological contribution to tasks requiring multi-sensory integration.

While it may not be feasible to perform the aforementioned fMRI studies in all hospitals, it is likely that the results acquired in specific specialist centres may still provide dramatic new insights about the pathophysiological processes underlying the development of later cerebral palsy which will therefore be of relevance to all working in the field.

So far we have predominantly considered using fMRI in conjunction with diffusion imaging; however a future prospect is to integrate these findings with the highly complementary information derived from multimodal functional imaging techniques with greater temporal resolution such as MEG and EEG. A simultaneous EEG-fMRI recording could be exploited to provide a more accurate interpretation of our findings presented in Chapter 6. I envisage that future research will continue to unravel the mechanisms that underlie early functional brain development through an increasingly integrated approach that uses complementary neuro-imaging techniques. An accurate spatio-temporal depiction of cerebral development could then be used to interpret and expand on current physiological and structural knowledge, allowing scientists and medical experts to achieve a more thorough understanding of the genesis of both fundamental neuroscientific notions and early pathophysiology. In this context I propose that task-fMRI can be used to provide a general mapping of the location and intensity of functional responses, while electrophysiological techniques (such as EEG and EMG) can reveal causal relations between identified regions of activity. For instance, we have proposed that the maturing bilateral representation of somatosensory responses is indicative of the strengthening of interhemispheric connectivity, while this effect has been previously ascribed to an immature lateralization of thalamocortical afferents (Erberich et al., 2006). EEG could be used to precisely characterize the latency between homologous cross-hemispheric responses and help resolve this uncertainty. This concept could be further probed via a concurrent EEG-fMRI investigation carried out in subjects with impaired cross-hemispheric structural connectivity such as those with agenesis of the corpus callosum. Establishing the role of cortico-cortical or thalamocortical afferents may be especially important in view of the potential for promoting bilateral responses during the early preterm period through activity-dependent mechanisms. Furthermore, simultaneous EEG and task-fMRI analysis could help establish a direct relationship between the characteristics of somatosensory evoked potentials and those of the corresponding BOLD response. A possible interpretation of the decreased intensity, decreased lag-time and increased spatial specificity of BOLD responses observed at term age is that it results from the ingrowth of thalamocortical afferents and dissolution of the subplate. This transition temporally corresponds to the disappearance from the EEG of specific developmental patterns of activity known as delta brushes (Vanhatalo and Lauronen, 2006, Vanhatalo and Kaila, 2010, Nevalainen et al., 2014). It is

possible that features of the BOLD response are tightly coupled with particular EEG signatures, and directly probing this concept could be crucial in defining an increasingly integrated framework through which brain function can be characterized. This would also allow a definitive exploration on long accepted clinical notions, such as the systematic decrease in SEP latency throughout maturation, which has so far been primarily ascribed to the myelination of afferent pathways (Vanhatalo and Lauronen, 2006, Vanhatalo and Kaila, 2010, Nevalainen et al., 2014). Infants exhibiting sustained latent SEP responses have been found to have an increased incidence of neurodevelopmental delay, however this biomarker suffers from poor sensitivity amongst preterm infants (reviewed in (Vanhatalo and Lauronen, 2006)). It is possible that identifying the spatio-temporal features of BOLD responses (and associated functional pathways identified via diffusion imaging) that accompany the decrease in SEP latency may reveal the existence of mechanisms other than axonal myelination (e.g. specialization of thalamic nuclei, enhanced interhemispheric connectivity) which may increase the current prognostic value of somatosensory-evoked functional responses.

In summary, fMRI and advanced imaging techniques such as tractography and EEG may therefore present an accurate means with which to characterize and monitor neural (re)organization and neuroplasticity following brain injury, and furthermore to longitudinally monitor how they may be influenced by activity and therapeutic intervention. While electrophysiological and structural studies of the preterm brain are relatively widespread, the work of this thesis has now begun to pave the way for a fine spatial mapping of early brain function. Due to temporal and practical constraints, the population study presented focused on the sensorimotor characterization of a single joint; however, the approach developed is flexible enough to be utilized in the future to build a complete sensory map of the maturing human brain. I therefore suggest refining and adapting the current fMRI-compatible system to perform multi-sensorial experiments in an objective and standardized manner, which would provide results that are not only reliable, but also comparable across research centres. In fact, my device has started to be used by other centres such as the Department of Neonatology at the medical center of the University of Utrecht, Netherlands. The currently developed systems are also suitable to perform fMRI conditioning studies in preterm infants. For instance the pacifier and

olfactometer interfaces could be combined to reveal the effects that milk odour has on non-nutritive-sucking patterns, and verify the involvement of higher level neural processing.

7.4 Stimulus systems: considerations and future improvements

A major and ongoing goal of the described work is to provide clinicians and investigators with accessible and reliable solutions to probe the development of brain function in preterm infants using task-dependent fMRI protocols. In this thesis I therefore adopted a modular and flexible system composed of a control unit and a range of pneumatically actuated custom interfaces, which can provide computer-controlled stimulation to the preterm infant in a safe and robust manner. While a large proportion of previous task-dependent fMRI studies of the preterm population have focussed on the visual and auditory systems, my primary aim was to develop tools that would allow assessment of the early sensori-motor system, especially in view of the increased susceptibility of preterm infants to neuromotor dysfunctions such as cerebral palsy (Larroque et al., 2008b). This was done by designing and producing robotic interfaces capable of inducing proprioceptive stimulation through precise ankle and wrist joint movement, while also providing real-time feedback on joint position. I utilized a commercial inexpensive plastic piston produced by LEGO as the only actuator, while a position sensor was custom made from a simple mono-core fibre optic cable. Designing the body of the devices required careful geometrical consideration not only to ensure appropriate actuation and sensing, but also to guarantee the infant's safety and comfort. The final interfaces are therefore relatively unconventionally sized and shaped, and require using rapid prototyping solutions to be manufactured. With 3D printing technology becoming increasingly available, my interfaces can be replicated from the source drawings without requiring specific engineering skills, and could be adapted to consider additional constraints.

By implementing a simple open-loop control, and modelling actuation delays into the fMRI analysis, both devices were demonstrated to be capable of eliciting statistically significant and well-localized patterns of positive BOLD responses to passive joint stimulation in a robust and repeatable manner across all individual subjects included in the work of this thesis (51 wrist, 5 ankle). Moreover, in no instance did infants exhibit physiological signs of distress during sessions of

passive joint stimulation, which indicates that the interface design and the elicited range of movements are well suited to the preterm demographic and biomechanics. The inconsistent findings regarding both the nature and localization of responses reported by previous studies indicate that the developed system solves a concrete problem, and represents the only currently reported solution to reliably conducting somatosensory task-fMRI studies of the preterm population.

While the designed devices have been demonstrated to be suitable for the current purpose of systematically identifying and characterizing functional activity in response to a specific pattern of stimulation, however for more complex experiments (such as those aimed at investigating specific effects of stimulus modulation), the current sensing, actuation and control could be modified to consider these additional constraints. At present only the frequency, but not the velocity or amplitude of movement, is dynamically controlled. However, should experimenters require all three parameters be altered during the course of the experiment, a different actuator should be considered. In fact, the piston's stick-slip effect, the inherently low bandwidth of pneumatic drive, the slow time response of the solenoid valves, and the low proportion of air volume contained within the driving piston, are all limiting factors when trying to achieve a controllable velocity profile. A potential solution would be to develop custom pneumatic stepper motors; however one must also consider the weight, size and flexibility limitations imposed by our specific application. Alternatively, hydraulic actuation or transmission could be considered, although as described in Chapter 4, although such systems are more difficult to maintain, may be prompt to leakage and are not inherently back-driveable.

If accurate position control is required, this may require completing or improving the sensing unit. The only similar commercial solution identified suffers from poor spatial resolution (and is excessively expensive). MR safe digital optical encoders are generally unsuitable for our application due to the size restrictions imposed by their sensing principle. As part of an ongoing project conducted in collaboration with the department of Mechanical Engineering at Imperial College, we are developing an optical micro-encoder based on fiberscope technology. This camera-based encoder allows inferring displacement in two directions by tracking a projected grid pattern, and is an ideal solution for our application as it is extremely compact, flexible and does not produce

resistive forces. However, its acquisition rate is limited by the frame rate that can be achieved with current (complementary metal-oxide semiconductor) CMOS image sensors. An alternative to position sensing, is to equip the devices with torque and/or force sensors, which would allow capturing the forces exerted by the infant without requiring disengaging the piston. However the design of the current interface would need to be carefully modified (perhaps introducing an air chamber at the level of the handle) before this solution could be implemented.

With the aim of exploring the intracerebral elaboration of further sensory information that is crucial to the early human development, I designed and manufactured an fMRI compatible instrumented pacifier and olfactometer. The pacifier system is capable of both producing orocutaneous stimulation and monitoring spontaneous sucking activity, and validation tests on adult subjects demonstrate that this equipment can be reliably used with fMRI to identify patterns of functional activation associated with both conditions. The interface is fully MR safe, inherently sterile, and is capable of accurately measuring intra-luminal pacifier pressure both during epochs of stimulation and rest. Preliminary testing with two infant subjects have confirmed this, but have also highlighted the difficulty of ensuring that the pacifier is kept within the infant's mouth throughout experimental sessions. Subject to the parents' approval, this could be resolved by implementing a weak elastic strap which could be placed around the infants' head to hold it in place.

The olfactometer system was designed to be safe and fully sterile, with its odour delivery subsystem assembled from disposable, low cost medical grade equipment that is readily available in the clinical setting. Based on a simple design, the olfactometer developed is simple to operate and replicate, and was shown to produce reproducible patterns of olfactory stimulation, with well-localized functional activity identified in neonates studied at term equivalent age. However, unlike proprioceptive and orocutaneous stimulation, the presentation of odour stimuli is inherently difficult to quantify and characterize. A real time measurement of the diffusion of odorant molecules within the air being inhaled by the subject would be ideal, but cannot be achieved within the MRI scanner, as the necessary equipment is not MR safe. However modelling of these parameters via a mass spectrometer, and utilizing the subject's breathing pattern as further analytical confound could potentially account for these effects and improve analysis accuracy. The manual pressure gauge

currently used could be replaced with a pressure regulator to prevent the slow flow drift observed during experiments.

Last but not least, in view of the large proportion of fMRI data sets which are routinely discarded due to motion corruption, there is a clear need for a better head immobilization system than the currently than the foam-packed vacuum-evacuated MRI infant immobilizer (MedVac, Fenton, MI, USA) currently being used. Several options are currently being investigated such as a custom made soft helmet with a chin strap and head strap, however safety requirements, ethical approval and parent consent all clearly need to be fulfilled before alternative methods of head immobilization can be implemented.

References

- ADDE, L., HELBOSTAD, J., JENSENIUS, A. R., LANGAAS, M. & STØEN, R. 2013. Identification of fidgety movements and prediction of CP by the use of computer-based video analysis is more accurate when based on two video recordings. *Physiotherapy Theory and Practice*, 29, 469-475.
- ADDE, L., HELBOSTAD, J. L., JENSENIUS, A. R., TARALDSEN, G., GRUNEWALDT, K. H. & STØEN, R. 2010. Early prediction of cerebral palsy by computer-based video analysis of general movements: a feasibility study. *Developmental Medicine & Child Neurology*, 52, 773-778.
- ADDE, L., HELBOSTAD, J. L., JENSENIUS, A. R., TARALDSEN, G. & STØEN, R. 2009. Using computer-based video analysis in the study of fidgety movements. *Early Hum Dev*, 85, 541-547.
- ADIBELLI, Z. H., SONGU, M. & ADIBELLI, H. 2011. Paranasal sinus development in children: A magnetic resonance imaging analysis. *Am J Rhinol Allergy*, 25, 30-35.
- AGUIRRE, G. K., ZARAHN, E. & D'ESPOSITO, M. 1998a. The inferential impact of global signal covariates in functional neuroimaging analyses. *NeuroImage*, 8, 302-6.
- AGUIRRE, G. K., ZARAHN, E. & D'ESPOSITO, M. 1998b. The variability of human, BOLD hemodynamic responses. *Neuroimage*, 8, 360-9.
- ALJABAR, P., BHATIA, K. K., MURGASOVA, M., HAJNAL, J. V., BOARDMAN, J. P., SRINIVASAN, L., RUTHERFORD, M. A., DYET, L. E., EDWARDS, A. D. & RUECKERT, D. 2008. Assessment of brain growth in early childhood using deformation-based morphometry. *Neuroimage*, 39, 348-58.
- ALJABAR, P., WOLZ, R., SRINIVASAN, L., COUNSELL, S. J., RUTHERFORD, M. A., EDWARDS, A. D., HAJNAL, J. V. & RUECKERT, D. 2011. A combined manifold learning analysis of shape and appearance to characterize neonatal brain development. *IEEE transactions on medical imaging*, 30, 2072-86.
- ALLEN, M. C. 2002. Preterm outcomes research: a critical component of neonatal intensive care. *Ment Retard Dev Disabil Res Rev*, 8, 221-233.
- ALLEN, M. C. 2008. Neurodevelopmental outcomes of preterm infants. *Curr Opin Neurol*, 21, 123-128.
- ALLIEVI, A. G., MELENDEZ-CALDERON, A., ARICHI, T., EDWARDS, A. D. & BURDET, E. 2013. An fMRI compatible wrist robotic interface to study brain development in neonates. *Ann Biomed Eng*, 41, 1181-92.
- ALS, H., DUFFY, F. H., MCANULTY, G. B., FISCHER, C. B., KOSTA, S., BUTLER, S. C., PARAD, R. B., BLICKMAN, J. G., ZURAKOWSKI, D. & RINGER, S. A. 2011. Is the Newborn Individualized Developmental Care and Assessment Program (NIDCAP) effective for preterm infants with intrauterine growth restriction? *J Perinatol*, 31, 130-6.
- ALTMAN, N. R. & BERNAL, B. 2001. Brain activation in sedated children: auditory and visual functional MR imaging. *Radiology*, 221, 56-63.
- AMARO, E., JR. & BARKER, G. J. 2006. Study design in fMRI: basic principles. *Brain Cogn*, 60, 220-32.

- ANCEL, P. Y., LIVINEC, F., LARROQUE, B., MARRET, S., ARNAUD, C., PIERRAT, V., DEHAN, M., N'GUYEN, S., ESCANDE, B., BURGUET, A., THIRIEZ, G., PICAUD, J. C., ANDRE, M., BREART, G. & KAMINSKI, M. 2006. Cerebral palsy among very preterm children in relation to gestational age and neonatal ultrasound abnormalities: the EPIPAGE cohort study. *Pediatrics*, 117, 828-35.
- ANDERSON, A. W., MAROIS, R., COLSON, E. R., PETERSON, B. S., DUNCAN, C. C., EHRENKRANZ, R. A., SCHNEIDER, K. C., GORE, J. C. & MENT, L. R. 2001. Neonatal auditory activation detected by functional magnetic resonance imaging. *Magn Reson Imaging*, 19, 1-5.
- AOYAMA, S., TOSHIMA, T., SAITO, Y., KONISHI, N., MOTOSHIGE, K., ISHIKAWA, N., NAKAMURA, K. & KOBAYASHI, M. 2010. Maternal breast milk odour induces frontal lobe activation in neonates: a NIRS study. *Early human development*, 86, 541-5.
- ARICHI, T. 2012. *Characterization of the haemodynamic response function (HRF) in the neonatal brain using functional MRI*. Ph.D., Imperial College London.
- ARICHI, T., COUNSELL, S. J., ALLIEVI, A. G., CHEW, A. T., MARTINEZ-BIARGE, M., MONDI, V., TUSOR, N., MERCHANT, N., BURDET, E., COWAN, F. M. & EDWARDS, A. D. 2014. The effects of hemorrhagic parenchymal infarction on the establishment of sensori-motor structural and functional connectivity in early infancy. *Neuroradiology*, 1-10.
- ARICHI, T., FAGIOLO, G., VARELA, M., MELENDEZ-CALDERON, A., ALLIEVI, A., MERCHANT, N., TUSOR, N., COUNSELL, S. J., BURDET, E., BECKMANN, C. F. & EDWARDS, A. D. 2012. Development of BOLD signal hemodynamic responses in the human brain. *Neuroimage*.
- ARICHI, T., MORAUX, A., MELENDEZ, A., DORIA, V., GROPPA, M., MERCHANT, N., COMBS, S., BURDET, E., LARKMAN, D. J., COUNSELL, S. J., BECKMANN, C. F. & EDWARDS, A. D. 2010. Somatosensory cortical activation identified by functional MRI in preterm and term infants. *Neuroimage*, 49, 2063-71.
- ASHBURNER, J. 2012. SPM: a history. *Neuroimage*, 62, 791-800.
- ATTWELL, D. & IADECOLA, C. 2002. The neural basis of functional brain imaging signals. *Trends in neurosciences*, 25, 621-5.
- AZOULAY, R., FALLET-BIANCO, C., GAREL, C., GRABAR, S., KALIFA, G. & ADAMSBAUM, C. 2006. MRI of the olfactory bulbs and sulci in human fetuses. *Pediatr Radiol*, 36, 97-107.
- BALL, G., COUNSELL, S. J., ANJARI, M., MERCHANT, N., ARICHI, T., DORIA, V., RUTHERFORD, M. A., EDWARDS, A. D., RUECKERT, D. & BOARDMAN, J. P. 2010. An optimised tract-based spatial statistics protocol for neonates: applications to prematurity and chronic lung disease. *Neuroimage*, 53, 94-102.
- BANDETTINI, P. A. 2001. Selection of the optimal pulse sequence for functional MRI. *Functional Magnetic Resonance Imaging*. Oxford: Oxford University Press.
- BANDETTINI, P. A. & COX, R. W. 2000. Event-related fMRI contrast when using constant interstimulus interval: theory and experiment. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, 43, 540-8.
- BANDETTINI, P. A., JESMANOWICZ, A., WONG, E. C. & HYDE, J. S. 1993. Processing strategies for time-course data sets in functional MRI of the human brain. *Magn Reson Med*, 30, 161-73.

- BANDETTINI, P. A., WONG, E. C., HINKS, R. S., TIKOFSKY, R. S. & HYDE, J. S. 1992. Time course EPI of human brain function during task activation. *Magn Reson Med*, 25, 390-7.
- BARBOSA, V. M., CAMPBELL, S. K. & BERBAUM, M. 2007. Discriminating infants from different developmental outcome groups using the Test of Infant Motor Performance (TIMP) item responses. *Pediatr Phys Ther*, 19, 28-39.
- BARBOSA, V. M., CAMPBELL, S. K., SMITH, E. & BERBAUM, M. 2005. Comparison of Test of Infant Motor Performance (TIMP) item responses among children with cerebral palsy, developmental delay, and typical development. *American Journal of Occupational Therapy*, 59, 446-456.
- BARLOW, S. M. 2009a. Central pattern generation involved in oral and respiratory control for feeding in the term infant. *Curr Opin Otolaryngol Head Neck Surg*, 17, 187-93.
- BARLOW, S. M. 2009b. Oral and respiratory control for preterm feeding. *Curr Opin Otolaryngol Head Neck Surg*, 17, 179-86.
- BARLOW, S. M., BURCH, M., VENKATESAN, L., HAROLD, M. & ZIMMERMAN, E. 2012. Frequency Modulation and Spatiotemporal Stability of the sCPG in Preterm Infants with RDS. *International journal of pediatrics*, 2012, 581538.
- BARLOW, S. M., FINAN, D. S., LEE, J. & CHU, S. 2008. Synthetic orocutaneous stimulation entrains preterm infants with feeding difficulties to suck. *J Perinatol*, 28, 541-8.
- BARLOW, S. M., LEE, J., WANG, J., ODER, A., HALL, S., KNOX, K., WEATHERSTONE, K. & THOMPSON, D. 2014. Frequency-modulated orocutaneous stimulation promotes non-nutritive suck development in preterm infants with respiratory distress syndrome or chronic lung disease. *Journal of perinatology : official journal of the California Perinatal Association*, 34, 136-42.
- BARLOW, S. M., LUND, J. P., ESTEP, M. & KOLTA, A. 2010. Central pattern generators for orofacial movements and speech. In: BRUDZYNSKI, S. M. (ed.) *Handbook of Mammalian Vocalization: An Integrative Neuroscience Approach*. Academic Press.
- BARTOCCI, M., WINBERG, J., PAPENDIECK, G., MUSTICA, T., SERRA, G. & LAGERCRANTZ, H. 2001. Cerebral hemodynamic response to unpleasant odors in the preterm newborn measured by near-infrared spectroscopy. *Pediatr Res*, 50, 324-30.
- BARTOCCI, M., WINBERG, J., RUGGIERO, C., BERGQVIST, L. L., SERRA, G. & LAGERCRANTZ, H. 2000. Activation of olfactory cortex in newborn infants after odor stimulation: a functional near-infrared spectroscopy study. *Pediatr Res*, 48, 18-23.
- BATTIN, M. & RUTHERFORD, M. A. 2001. Magnetic resonance imaging of the brain in preterm infants: 24 weeks' gestation to term. In: RUTHERFORD, M. A. (ed.) *MRI of the Neonatal Brain*. Saunders Ltd.
- BATTIN, M. R., MAALOUF, E. F., COUNSELL, S. J., HERLIHY, A. H., RUTHERFORD, M. A., AZZOPARDI, D. & EDWARDS, A. D. 1998. Magnetic resonance imaging of the brain in very preterm infants: visualization of the germinal matrix, early myelination, and cortical folding. *Pediatrics*, 101, 957-62.
- BAX, M., GOLDSTEIN, M., ROSENBAUM, P., LEVITON, A., PANETH, N., DAN, B., JACOBSSON, B. & DAMIANO, D. 2005. Proposed definition and classification of cerebral palsy, April 2005. *Dev Med Child Neurol*, 47, 571-6.

- BAX, M. C. 1964. Terminology and Classification of Cerebral Palsy. *Dev Med Child Neurol*, 6, 295-297.
- BEAINO, G., KHOSHNOOD, B., KAMINSKI, M., PIERRAT, V., MARRET, S., MATIS, J., LEDESERT, B., THIRIEZ, G., FRESSON, J., ROZE, J. C., ZUPAN-SIMUNEK, V., ARNAUD, C., BURGUET, A., LARROQUE, B., BREART, G., ANCEL, P. Y. & GROUP, E. S. 2010. Predictors of cerebral palsy in very preterm infants: the EPIPAGE prospective population-based cohort study. *Dev Med Child Neurol*, 52, e119-25.
- BECKMANN, C. F. 2012. Modelling with independent components. *Neuroimage*, 62, 891-901.
- BECKMANN, C. F., DELUCA, M., DEVLIN, J. T. & SMITH, S. M. 2005. Investigations into resting-state connectivity using independent component analysis. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 360, 1001-1013.
- BECKMANN, C. F., JENKINSON, M. & SMITH, S. M. 2003. General multilevel linear modeling for group analysis in FMRI. *Neuroimage*, 20, 1052-63.
- BECKMANN, C. F. & SMITH, S. M. 2004. Probabilistic independent component analysis for functional magnetic resonance imaging. *IEEE Trans Med Imaging*, 23, 137-52.
- BENIGNUS, V. & PRAH, J. 1980. A computer-controlled vapor-dilution olfactometer. *Behavior Research Methods & Instrumentation*, 12, 535-540.
- BENNET, L., VAN DEN HEUIJ, L., J. M. D., DRURY, P., WASSINK, G. & JAN GUNN, A. 2013. Neural plasticity and the Kennard principle: does it work for the preterm brain? *Clin Exp Pharmacol Physiol*, 40, 774-84.
- BERNS, G. S., SONG, A. W. & MAO, H. 1999. Continuous functional magnetic resonance imaging reveals dynamic nonlinearities of "dose-response" curves for finger opposition. *J Neurosci*, 19, RC17.
- BEST, A. R. & WILSON, D. A. 2003. A postnatal sensitive period for plasticity of cortical afferents but not cortical association fibers in rat piriform cortex. *Brain Res*, 961, 81-7.
- BINGHAM, P. M., ABASSI, S. & SIVIERI, E. 2003. A pilot study of milk odor effect on nonnutritive sucking by premature newborns. *Arch Pediatr Adolesc Med*, 157, 72-5.
- BISWAL, B., YETKIN, F. Z., HAUGHTON, V. M. & HYDE, J. S. 1995. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, 34, 537-41.
- BISWAL, B. B., ELDTRETH, D. A., MOTES, M. A. & RYPMA, B. 2010. Task-dependent individual differences in prefrontal connectivity. *Cereb Cortex*, 20, 2188-97.
- BLANCHARD, C., ROLL, R., ROLL, J. P. & KAVOUNOUDIAS, A. 2011. Combined contribution of tactile and proprioceptive feedback to hand movement perception. *Brain Res*, 1382, 219-229.
- BLASI, A., MERCURE, E., LLOYD-FOX, S., THOMSON, A., BRAMMER, M., SAUTER, D., DEELEY, Q., BARKER, G. J., RENVALL, V., DEONI, S., GASSTON, D., WILLIAMS, S. C., JOHNSON, M. H., SIMMONS, A. & MURPHY, D. G. 2011a. Early specialization for voice and emotion processing in the infant brain. *Curr Biol*, 21, 1220-4.
- BLASI, A., MERCURE, E., LLOYD-FOX, S., THOMSON, A., BRAMMER, M., SAUTER, D., DEELEY, Q., BARKER, G. J., RENVALL, V., DEONI, S., GASSTON, D., WILLIAMS, S. C. R., JOHNSON, M.

- H., SIMMONS, A. & MURPHY, D. G. M. 2011b. Early specialization for voice and emotion processing in the infant brain. *Current biology : CB*, 21, 1220-4.
- BLUMBERG, M. S. & LUCAS, D. E. 1994. Dual mechanisms of twitching during sleep in neonatal rats. *Behav Neurosci*, 108, 1196-202.
- BOARDMAN, J. P., GANESAN, V., RUTHERFORD, M. A., SAUNDERS, D. E., MERCURI, E. & COWAN, F. 2005. Magnetic Resonance Image Correlates of Hemiparesis After Neonatal and Childhood Middle Cerebral Artery Stroke. *Pediatrics*, 115, 321-326.
- BOIRON, M., DA NOBREGA, L., ROUX, S., HENROT, A. & SALIBA, E. 2007. Effects of oral stimulation and oral support on non-nutritive sucking and feeding performance in preterm infants. *Developmental medicine and child neurology*, 49, 439-44.
- BORN, A. P., MIRANDA, M. J., ROSTRUP, E., TOFT, P. B., PEITERSEN, B., LARSSON, H. B. & LOU, H. C. 2000. Functional magnetic resonance imaging of the normal and abnormal visual system in early life. *Neuropediatrics*, 31, 24-32.
- BORN, A. P., ROSTRUP, E., MIRANDA, M. J., LARSSON, H. B. & LOU, H. C. 2002. Visual cortex reactivity in sedated children examined with perfusion MRI (FAIR). *Magn Reson Imaging*, 20, 199-205.
- BORN, P., LETH, H., MIRANDA, M. J., ROSTRUP, E., STENSGAARD, A., PEITERSEN, B., LARSSON, H. B. & LOU, H. C. 1998. Visual activation in infants and young children studied by functional magnetic resonance imaging. *Pediatr Res*, 44, 578-83.
- BORN, P., ROSTRUP, E., LETH, H., PEITERSEN, B. & LOU, H. C. 1996. Change of visually induced cortical activation patterns during development. *Lancet*, 347, 543.
- BORRAMEO, S., HERNANDEZ-TAMAMES, J. A., LUNA, G., MACHADO, F., MALPICA, N. & TOLEDANO, A. 2010. Objective Assessment of Olfactory Function Using Functional Magnetic Resonance Imaging (fMRI). *Instrumentation and Measurement, IEEE Transactions on*, 59, 2602-2608.
- BOSANQUET, M., COPELAND, L., WARE, R. & BOYD, R. 2013. A systematic review of tests to predict cerebral palsy in young children. *Dev Med Child Neurol*, 55, 418-26.
- BOUGHTER, J. D. & BACHMANOV, A. A. 2007. Behavioral genetics and taste. *BMC neuroscience*, 8 Suppl 3, S3.
- BOYNTON, G. M., ENGEL, S. A., GLOVER, G. H. & HEEGER, D. J. 1996. Linear systems analysis of functional magnetic resonance imaging in human V1. *J Neurosci*, 16, 4207-21.
- BRAMMER, M. J. 2001. Head motion and its correction. In: JEZZARD MATTHEWS, P. M., SMITH, S. M., P (ed.) *Functional MRI: An introduction to methods*. New York, USA: Oxford University Press.
- BRETT, M., JOHNSRUDE, I. S. & OWEN, A. M. 2002. The problem of functional localization in the human brain. *Nature reviews. Neuroscience*, 3, 243-9.
- BRIGGS, R. W., DY-LIACCO, I., MALCOLM, M. P., LEE, H., PECK, K. K., GOPINATH, K. S., HIMES, N. C., SOLTYSIK, D. A., BROWNE, P. & TRAN-SON-TAY, R. 2004. A pneumatic vibrotactile stimulation device for fMRI. *Magn Reson Med*, 51, 640-3.
- BRODMANN, K. & GAREY, L. 1999. *Brodmann's Localisation in the cerebral cortex*, London, Imperial College Press : Distributed by World Scientific Pub.

- BROWN, T. G. 1914. On the nature of the fundamental activity of the nervous centres; together with an analysis of the conditioning of rhythmic activity in progression, and a theory of the evolution of function in the nervous system. *The Journal of physiology*, 48, 18-46.
- BROWNE, J. V. 2008. Chemosensory Development in the Fetus and Newborn. *Newborn and Infant Nursing Reviews*, 8, 180-186.
- BUCKNER, R. L., BANDETTINI, P. A., OCRAVEN, K. M., SAVOY, R. L., PETERSEN, S. E., RAICHLE, M. E. & ROSEN, B. R. 1996. Detection of cortical activation during averaged single trials of a cognitive task using functional magnetic resonance imaging. *Proc Natl Acad Sci U S A*, 93, 14878-14883.
- BUCKNER, R. L., KRIENEN, F. M. & YEO, B. T. 2013. Opportunities and limitations of intrinsic functional connectivity MRI. *Nat Neurosci*, 16, 832-7.
- BUCKNER, R. L. & VINCENT, J. L. 2007. Unrest at rest: default activity and spontaneous network correlations. *Neuroimage*, 37, 1091-6; discussion 1097-9.
- BULLMORE, E., BRAMMER, M., WILLIAMS, S. C., RABE-HESKETH, S., JANOT, N., DAVID, A., MELLERS, J., HOWARD, R. & SHAM, P. 1996. Statistical methods of estimation and inference for functional MR image analysis. *Magn Reson Med*, 35, 261-77.
- BULLMORE, E. & SPORNS, O. 2012. The economy of brain network organization. *Nat Rev Neurosci*, 13, 336-349.
- BUTZ, M., WORGOTTER, F. & VAN OOOYEN, A. 2009. Activity-dependent structural plasticity. *Brain Research Reviews*, 60, 287-305.
- BUXTON, R. B. 2009. Introduction to Functional Magnetic Resonance: Principles and Techniques.
- BUXTON, R. B. 2012. Dynamic models of BOLD contrast. *Neuroimage*, 62, 953-961.
- BUXTON, R. B., ULUDAG, K., DUBOWITZ, D. J. & LIU, T. T. 2004. Modeling the hemodynamic response to brain activation. *Neuroimage*, 23 Suppl 1, S220-33.
- BUXTON, R. B., WONG, E. C. & FRANK, L. R. 1998. Dynamics of blood flow and oxygenation changes during brain activation: the balloon model. *Magn Reson Med*, 39, 855-64.
- BYRANT, J., ROY, S. & BOUGHTER, J. 2007. A proposed new function of the mouse cerebellum: temporal modulation of brain stem pattern generator activity. *Society for Neuroscience*, 78.
- BYSTRON, I., BLAKEMORE, C. & RAKIC, P. 2008. Development of the human cerebral cortex: Boulder Committee revisited. *Nat Rev Neurosci*, 9, 110-122.
- CAMPBELL, S. K. 2005. The Test of Infant Motor Performance. Test User's Manual Version 2.0. *Chicago: Infant Motor Performance Scales*.
- CAMPBELL, S. K., WRIGHT, B. D. & LINACRE, J. M. 2002. Development of a functional movement scale for infants. *J Appl Meas*, 3, 190-204.
- CAULI, B. & HAMEL, E. 2010. Revisiting the role of neurons in neurovascular coupling. *Frontiers in neuroenergetics*, 2, 9.
- CERNOCH, J. M. & PORTER, R. H. 1985. Recognition of maternal axillary odors by infants. *Child Dev*, 56, 1593-8.

- CHAKRABARTY, S. & MARTIN, J. H. 2000. Postnatal development of the motor representation in primary motor cortex. *J Neurophysiol*, 84, 2582-2594.
- CHAKRABARTY, S. & MARTIN, J. H. 2005. Motor But Not Sensory Representation in Motor Cortex Depends on Postsynaptic Activity During Development and in Maturity. 94, 3192-3198.
- CHEN, J. J. & PIKE, G. B. 2009a. BOLD-specific cerebral blood volume and blood flow changes during neuronal activation in humans. *NMR Biomed*, 22, 1054-62.
- CHEN, J. J. & PIKE, G. B. 2009b. Origins of the BOLD post-stimulus undershoot. *Neuroimage*, 46, 559-68.
- CHINZEI, K. & MILLER, K. MRI guided surgical robot. Proceedings of the 2001 Australian Conference on Robotics and Automation, Sydney, Australia, November, 2001. 14-15.
- COLE, D. M., SMITH, S. M. & BECKMANN, C. F. 2010. Advances and pitfalls in the analysis and interpretation of resting-state fMRI data. *Front Syst Neurosci*, 4, 8.
- COLONNESE, M. & KHAZIPOV, R. 2012. Spontaneous activity in developing sensory circuits: Implications for resting state fMRI. *Neuroimage*, 62, 2212-21.
- COWAN, F., RUTHERFORD, M., GROENENDAAL, F., EKEN, P., MERCURI, E., BYDDER, G. M., MEINERS, L. C., DUBOWITZ, L. M. S. & DE VRIES, L. S. 2003. Origin and timing of brain lesions in term infants with neonatal encephalopathy. *The Lancet*, 361, 736-742.
- COX, R. W. 2012. AFNI: what a long strange trip it's been. *NeuroImage*, 62, 743-7.
- DALE, A. M. 1999. Optimal experimental design for event-related fMRI. *Human brain mapping*, 8, 109-14.
- DALE, A. M. & BUCKNER, R. L. 1997. Selective averaging of rapidly presented individual trials using fMRI. *Human brain mapping*, 5, 329-40.
- DE GRAAF-PETERS, V. B. & HADDERS-ALGRA, M. 2006. Ontogeny of the human central nervous system: what is happening when? *Early Hum Dev*, 82, 257-66.
- DE VRIES, L. S., VAN HAASTERT, I. C., BENDERS, M. J. N. L. & GROENENDAAL, F. 2011. Myth: Cerebral palsy cannot be predicted by neonatal brain imaging. *Semin Fetal Neonatal Med*, 16, 279-287.
- DECHENT, P., SCHUTZE, G., HELMS, G., MERBOLDT, K. D. & FRAHM, J. 2011. Basal cerebral blood volume during the poststimulation undershoot in BOLD MRI of the human brain. *J Cereb Blood Flow Metab*, 31, 82-9.
- DEHAENE-LAMBERTZ, G., DEHAENE, S. & HERTZ-PANNIER, L. 2002. Functional neuroimaging of speech perception in infants. *Science*, 298, 2013-5.
- DEMPSEY, M. F., CONDON, B. & HADLEY, D. M. 2001. Investigation of the factors responsible for burns during MRI. *J Magn Reson Imaging*, 13, 627-31.
- DENNIS, M. 2010. Margaret Kennard (1899-1975): Not a 'Principle' of brain plasticity but a founding mother of developmental neuropsychology. *Cortex*, 46, 1043-1059.
- DEONI, S. C. L. 2010. Quantitative relaxometry of the brain. *Topics in magnetic resonance imaging : TMRI*, 21, 101-13.
- DEONI, S. C. L., MERCURE, E., BLASI, A., GASSTON, D., THOMSON, A., JOHNSON, M., WILLIAMS, S. C. R. & MURPHY, D. G. M. 2011. Mapping infant brain myelination with magnetic

- resonance imaging. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 31, 784-91.
- DONALDSON, D. I. & BUCKNER, R. 2001. Effective Paradigm Design. In: MATTHEWS, P. M., JEZZARD, P. & SMITH, S. M. (eds.) *Functional Magnetic Resonance Imaging: An Introduction to Methods*. Oxford: Oxford University Press.
- DORIA, V., BECKMANN, C. F., ARICHI, T., MERCHANT, N., GROPPA, M., TURKHEIMER, F. E., COUNSELL, S. J., MURGASOVA, M., ALJABAR, P., NUNES, R. G., LARKMAN, D. J., REES, G. & EDWARDS, A. D. 2010. Emergence of resting state networks in the preterm human brain. *Proc Natl Acad Sci U S A*, 107, 20015-20.
- DOYLE, L. W. & ANDERSON, P. J. 2010. Adult outcome of extremely preterm infants. *Pediatrics*, 126, 342-51.
- DUDINK, J., LEQUIN, M., VAN PUL, C., BUIJS, J., CONNEMAN, N., VAN GOUDOEVEER, J. & GOVAERT, P. 2007. Fractional anisotropy in white matter tracts of very-low-birth-weight infants. *Pediatr Radiol*, 37, 1216-23.
- EDWARDS, A. D. & ARTHURS, O. J. 2011. Paediatric MRI under sedation: is it necessary? What is the evidence for the alternatives? *Pediatric radiology*, 41, 1353-64.
- ELSTER, A. D. & BURDETTE, J. H. 2001. Questions & Answers in Magnetic Resonance Imaging. 333.
- ERBERICH, S. G., FRIEDLICH, P., SERI, I., NELSON, M. D. & BLÜML, S. 2003a. Functional MRI in neonates using neonatal head coil and MR compatible incubator. *NeuroImage*, 20, 683-92.
- ERBERICH, S. G., FRIEDLICH, P., SERI, I., NELSON, M. D., JR. & BLÜML, S. 2003b. Functional MRI in neonates using neonatal head coil and MR compatible incubator. *Neuroimage*, 20, 683-92.
- ERBERICH, S. G., PANIGRAHY, A., FRIEDLICH, P., SERI, I., NELSON, M. D. & GILLES, F. 2006. Somatosensory lateralization in the newborn brain. *Neuroimage*, 29, 155-61.
- ERNST, T. & HENNIG, J. 1994. Observation of a fast response in functional MR. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, 32, 146-9.
- ESTEP, M., BARLOW, S. M., VANTIPALLI, R., FINAN, D. & LEE, J. 2008. Non-Nutritive Suck Parameter in Preterm Infants with RDS. *J Neonatal Nurs*, 14, 28-34.
- EVANS, W. J., KOBAL, G., LORIG, T. S. & PRAH, J. D. 1993. Suggestions for Collection and Reporting of Chemosensory (Olfactory) Event-Related Potentials. *Chem Senses*, 18, 751-756.
- EYRE, J. A., MILLER, S., CLOWRY, G. J., CONWAY, E. A. & WATTS, C. 2000. Functional corticospinal projections are established prenatally in the human foetus permitting involvement in the development of spinal motor centres. *Brain*, 123 (Pt 1), 51-64.
- FABRIZI, L., SLATER, R., WORLEY, A., MEEK, J., BOYD, S., OLHEDE, S. & FITZGERALD, M. 2011. A shift in sensory processing that enables the developing human brain to discriminate touch from pain. *Curr Biol*, 21, 1552-8.
- FAIR, D. A., SCHLAGGAR, B. L., COHEN, A. L., MIEZIN, F. M., DOSENBACH, N. U., WENGER, K. K., FOX, M. D., SNYDER, A. Z., RAICHLE, M. E. & PETERSEN, S. E. 2007. A method for using

- blocked and event-related fMRI data to study "resting state" functional connectivity. *Neuroimage*, 35, 396-405.
- FELDMAN, D. E., NICOLL, R. A. & MALENKA, R. C. 1999. Synaptic plasticity at thalamocortical synapses in developing rat somatosensory cortex: LTP, LTD, and silent synapses. *J Neurobiol*, 41, 92-101.
- FIELD, T., HERNANDEZ-REIF, M., FEIJO, L. & FREEDMAN, J. 2006. Prenatal, perinatal and neonatal stimulation: a survey of neonatal nurseries. *Infant Behavior & Development*, 29, 24-31.
- FINAN, D. S. & BARLOW, S. M. 1996. The actifier: a device for neurophysiological studies of orofacial control in human infants. *J Speech Hear Res*, 39, 833-8.
- FINAN, D. S. & BARLOW, S. M. 1998. Intrinsic dynamics and mechanosensory modulation of non-nutritive sucking in human infants. *Early Hum Dev*, 52, 181-97.
- FINGER, S. & WOLF, C. 1988. The Kennard Effect before Kennard - the Early History of Age and Brain-Lesions. *Arch Neurol*, 45, 1136-1142.
- FOX, K. 2002. Anatomical pathways and molecular mechanisms for plasticity in the barrel cortex. *Neuroscience*, 111, 799-814.
- FOX, M. D. & RAICHLE, M. E. 2007. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nature reviews. Neuroscience*, 8, 700-11.
- FOX, M. D., SNYDER, A. Z., VINCENT, J. L., CORBETTA, M., VAN ESSEN, D. C. & RAICHLE, M. E. 2005. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc Natl Acad Sci U S A*, 102, 9673-8.
- FOX, P. T. & RAICHLE, M. E. 1986. Focal physiological uncoupling of cerebral blood flow and oxidative metabolism during somatosensory stimulation in human subjects. *Proc Natl Acad Sci U S A*, 83, 1140-4.
- FRAHM, J., BAUDEWIG, J., KALLENBERG, K., KASTRUP, A., MERBOLDT, K. D. & DECHENT, P. 2008. The post-stimulation undershoot in BOLD fMRI of human brain is not caused by elevated cerebral blood volume. *NeuroImage*, 40, 473-81.
- FRAHM, J., KRÜGER, G., MERBOLDT, K. D. & KLEINSCHMIDT, A. 1996. Dynamic uncoupling and recoupling of perfusion and oxidative metabolism during focal brain activation in man. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, 35, 143-8.
- FRANSSON, P., ADEN, U., BLENNOW, M. & LAGERCRANTZ, H. 2011. The functional architecture of the infant brain as revealed by resting-state FMRI. *Cereb Cortex*, 21, 145-54.
- FRANSSON, P., SKIÖLD, B., ENGSTRÖM, M., HALLBERG, B., MOSSKIN, M., ADEN, U., LAGERCRANTZ, H. & BLENNOW, M. 2009a. Spontaneous brain activity in the newborn brain during natural sleep--an fMRI study in infants born at full term. *Pediatr Res*, 66, 301-5.
- FRANSSON, P., SKIÖLD, B., ENGSTRÖM, M., HALLBERG, B., MOSSKIN, M., ADEN, U., LAGERCRANTZ, H. & BLENNOW, M. 2009b. Spontaneous brain activity in the newborn brain during natural sleep--an fMRI study in infants born at full term. *Pediatric research*, 66, 301-5.

- FRANSSON, P., SKIÖLD, B., HORSCH, S., NORDELL, A., BLENNOW, M., LAGERCRANTZ, H. & ADEN, U. 2007a. Resting-state networks in the infant brain. *Proc Natl Acad Sci U S A*, 104, 15531-6.
- FRANSSON, P., SKIÖLD, B., HORSCH, S., NORDELL, A., BLENNOW, M., LAGERCRANTZ, H. & ADEN, U. 2007b. Resting-state networks in the infant brain. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 15531-6.
- FRISTON, K., JEZZARD, P. & TURNER, R. 1994a. Analysis of functional MRI time-series. *Hum Brain Mapp*, 1, 153-171.
- FRISTON, K. J., ASHBURNER, J., FRITH, C. D., POLINE, J.-B., HEATHER, J. D. & FRACKOWIAK, R. S. J. 1995a. Spatial registration and normalization of images. *Human Brain Mapping*, 3, 165-189.
- FRISTON, K. J., FLETCHER, P., JOSEPHS, O., HOLMES, A., RUGG, M. D. & TURNER, R. 1998a. Event-related fMRI: characterizing differential responses. *Neuroimage*, 7, 30-40.
- FRISTON, K. J., FRITH, C. D., TURNER, R. & FRACKOWIAK, R. S. 1995b. Characterizing evoked hemodynamics with fMRI. *Neuroimage*, 2, 157-65.
- FRISTON, K. J., HOLMES, A. P., WORSLEY, K. J., POLINE, J.-P., FRITH, C. D. & FRACKOWIAK, R. S. J. 1994b. Statistical parametric maps in functional imaging: A general linear approach. *Human Brain Mapping*, 2, 189-210.
- FRISTON, K. J., JEZZARD, P. & TURNER, R. 1994c. Analysis of functional MRI time-series. *Human Brain Mapping*, 1, 153-171.
- FRISTON, K. J., JOSEPHS, O., REES, G. & TURNER, R. 1998b. Nonlinear event-related responses in fMRI. *Magn Reson Med*, 39, 41-52.
- FRISTON, K. J., STEPHAN, K. E., LUND, T. E., MORCOM, A. & KIEBEL, S. 2005. Mixed-effects and fMRI studies. *Neuroimage*, 24, 244-52.
- FRISTON, K. J., WILLIAMS, S., HOWARD, R., FRACKOWIAK, R. S. & TURNER, R. 1996. Movement-related effects in fMRI time-series. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, 35, 346-55.
- FRISTON, K. J., WORSLEY, K. J., FRACKOWIAK, R. S., MAZZIOTTA, J. C. & EVANS, A. C. 1994d. Assessing the significance of focal activations using their spatial extent. *Human brain mapping*, 1, 210-20.
- GAILLARD, W. D., GRANDIN, C. B. & XU, B. 2001. Developmental aspects of pediatric fMRI: considerations for image acquisition, analysis, and interpretation. *Neuroimage*, 13, 239-49.
- GAO, W., GILMORE, J. H., GIOVANELLO, K. S., SMITH, J. K., SHEN, D., ZHU, H. & LIN, W. 2011. Temporal and spatial evolution of brain network topology during the first two years of life. *PLoS One*, 6, e25278.
- GAO, W., GILMORE, J. H., SHEN, D., SMITH, J. K., ZHU, H. & LIN, W. 2013. The synchronization within and interaction between the default and dorsal attention networks in early infancy. *Cereb Cortex*, 23, 594-603.
- GAO, W., ZHU, H., GIOVANELLO, K. S., SMITH, J. K., SHEN, D., GILMORE, J. H. & LIN, W. 2009. Evidence on the emergence of the brain's default network from 2-week-old to 2-year-old healthy pediatric subjects. *Proc Natl Acad Sci U S A*, 106, 6790-5.

- GASSERT, R., BURDET, E. & CHINZEI, K. 2008. Opportunities and challenges in MR-compatible robotics: reviewing the history, mechatronic components, and future directions of this technology. *IEEE Eng Med Biol Mag*, 27, 15-22.
- GASSERT, R., MOSER, R., BURDET, E. & BLEULER, H. 2006a. MRI/fMRI-compatible robotic system with force feedback for interaction with human motion. *Mechatronics, IEEE/ASME Transactions on*, 11, 216-224.
- GASSERT, R., YAMAMOTO, A., CHAPUIS, D., DOVAT, L., BLEULER, H. & BURDET, E. 2006b. Actuation methods for applications in MR environments. *Concepts in Magnetic Resonance Part B: Magnetic Resonance Engineering*, 29B, 191-209.
- GENOVESE, C. R., LAZAR, N. A. & NICHOLS, T. 2002. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. *NeuroImage*, 15, 870-8.
- GERMANN, W. J. & STANFIELD, C. L. 2004. Principles of Human Physiology. 830.
- GIMENEZ, M., MIRANDA, M. J., BORN, A. P., NAGY, Z., ROSTRUP, E. & JERNIGAN, T. L. 2008. Accelerated cerebral white matter development in preterm infants: a voxel-based morphometry study with diffusion tensor MR imaging. *Neuroimage*, 41, 728-34.
- GJEDDE, A. 2001. Brain energy metabolism and the physiological basis of the haemodynamic response. In: JEZZARD, P., MATTHEWS, P. M. & SMITH, S. M. (eds.) *Functional MRI: an introduction to methods*. Oxford: Oxford University Press.
- GLOVER, G. H. 1999. Deconvolution of impulse response in event-related BOLD fMRI. *Neuroimage*, 9, 416-29.
- GOEBEL, R. 2012. BrainVoyager--past, present, future. *NeuroImage*, 62, 748-56.
- GOLASZEWSKI, S. M., ZSCHIEGNER, F., SIEDENTOPF, C. M., UNTERRAINER, J., SWEENEY, R. A., EISNER, W., LECHNER-STEINLEITNER, S., MOTTAGHY, F. M. & FELBER, S. 2002. A new pneumatic vibrator for functional magnetic resonance imaging of the human sensorimotor cortex. *Neurosci Lett*, 324, 125-8.
- GRAHAM, A. M., FISHER, P. A. & PFEIFER, J. H. 2013. What sleeping babies hear: a functional MRI study of interparental conflict and infants' emotion processing. *Psychol Sci*, 24, 782-9.
- GREAVES, S., IMMS, C., DODD, K. & KRUMLINDE-SUNDHOLM, L. 2013. Development of the Mini-Assisting Hand Assessment: evidence for content and internal scale validity. *Dev Med Child Neurol*, 55, 1030-7.
- GREAVES, S., IMMS, C., KRUMLINDE-SUNDHOLM, L., DODD, K. & ELIASSON, A. C. 2012. Bimanual behaviours in children aged 8-18 months: a literature review to select toys that elicit the use of two hands. *Res Dev Disabil*, 33, 240-50.
- GREICIUS, M. D., KRASNOW, B., REISS, A. L. & MENON, V. 2003. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 253-8.
- GROENENDAAL, F., TERMOTE, J. U. M., VAN DER HEIDE-JALVING, M., VAN HAASTERT, I. C. & DE VRIES, L. S. 2010. Complications affecting preterm neonates from 1991 to 2006: what have we gained? *Acta paediatrica (Oslo, Norway : 1992)*, 99, 354-8.
- GROPPO, M., RICCI, D., BASSI, L., MERCHANT, N., DORIA, V., ARICHI, T., ALLSOP, J. M., RAMENGHI, L., FOX, M. J., COWAN, F. M., COUNSELL, S. J. & EDWARDS, A. D. 2012. Development of the optic radiations and visual function after premature birth. *Cortex*.

- GUZZETTA, A., BALDINI, S., BANCALE, A., BARONCELLI, L., CIUCCI, F., GHIRRI, P., PUTIGNANO, E., SALE, A., VIEGI, A., BERARDI, N., BOLDRINI, A., CIONI, G. & MAFFEI, L. 2009. Massage accelerates brain development and the maturation of visual function. *J Neurosci*, 29, 6042-51.
- HACK, M. 2009. Adult outcomes of preterm children. *Journal of developmental and behavioral pediatrics : JDBP*, 30, 460-70.
- HACK, M., ESTABROOK, M. M. & ROBERTSON, S. S. 1985. Development of sucking rhythm in preterm infants. *Early Hum Dev*, 11, 133-40.
- HADDERS-ALGRA, M. 2004. General movements: A window for early identification of children at high risk for developmental disorders. *Journal of Pediatrics*, 145, S12-S18.
- HADDERS-ALGRA, M. 2011. Challenges and limitations in early intervention. *Developmental medicine and child neurology*, 53 Suppl 4, 52-5.
- HAFSTROM, M. & KJELLMER, I. 2001. Non-nutritive sucking in sick preterm infants. *Early Hum Dev*, 63, 37-52.
- HAGENBEEK, R. E. & VAN STRIEN, J. W. 2002. Left-right and upper-lower visual field asymmetries for face matching, letter naming, and lexical decision. *Brain and cognition*, 49, 34-44.
- HAJNAL, J. V., MYERS, R., OATRIDGE, A., SCHWIESO, J. E., YOUNG, I. R. & BYDDER, G. M. 1994. Artifacts due to stimulus correlated motion in functional imaging of the brain. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, 31, 283-91.
- HANDWERKER, D. A., GONZALEZ-CASTILLO, J., D'ESPOSITO, M. & BANDETTINI, P. A. 2012. The continuing challenge of understanding and modeling hemodynamic variation in fMRI. *NeuroImage*, 62, 1017-23.
- HANDWERKER, D. A., OLLINGER, J. M. & D'ESPOSITO, M. 2004. Variation of BOLD hemodynamic responses across subjects and brain regions and their effects on statistical analyses. *NeuroImage*, 21, 1639-51.
- HARMER, J., SANCHEZ-PANCHUELO, R. M., BOWTELL, R. & FRANCIS, S. T. 2012. Spatial location and strength of BOLD activation in high-spatial-resolution fMRI of the motor cortex: a comparison of spin echo and gradient echo fMRI at 7 T. *NMR in biomedicine*, 25, 717-25.
- HARRIS, J. J., REYNELL, C. & ATTWELL, D. 2011. The physiology of developmental changes in BOLD functional imaging signals. *Dev Cogn Neurosci*, 1, 199-216.
- HEEP, A., SCHEEF, L., JANKOWSKI, J., BORN, M., ZIMMERMANN, N., SIVAL, D., BOS, A., GIESEKE, J., BARTMANN, P., SCHILD, H. & BOECKER, H. 2009. Functional magnetic resonance imaging of the sensorimotor system in preterm infants. *Pediatrics*, 123, 294-300.
- HERMUNDSTAD, A. M., BASSETT, D. S., BROWN, K. S., AMINOFF, E. M., CLEWETT, D., FREEMAN, S., FRITHSEN, A., JOHNSON, A., TIPPER, C. M., MILLER, M. B., GRAFTON, S. T. & CARLSON, J. M. 2013. Structural foundations of resting-state and task-based functional connectivity in the human brain. *Proc Natl Acad Sci U S A*, 110, 6169-74.
- HILLMAN, E. M., DEVOR, A., BOUCHARD, M. B., DUNN, A. K., KRAUSS, G. W., SKOCH, J., BACSKAI, B. J., DALE, A. M. & BOAS, D. A. 2007. Depth-resolved optical imaging and microscopy of

- vascular compartment dynamics during somatosensory stimulation. *Neuroimage*, 35, 89-104.
- HINTZ, S. R., KENDRICK, D. E., VOHR, B. R., POOLE, W. K. & HIGGINS, R. D. 2005. Changes in neurodevelopmental outcomes at 18 to 22 months' corrected age among infants of less than 25 weeks' gestational age born in 1993-1999. *Pediatrics*, 115, 1645-51.
- HIRANO, Y., STEFANOVIC, B. & SILVA, A. C. 2011. Spatiotemporal evolution of the functional magnetic resonance imaging response to ultrashort stimuli. *J Neurosci*, 31, 1440-7.
- HOGUE, R. D. & PIKE, G. B. 2001. Quantitative measurement using fMRI. In: JEZZARD, P., MATTHEWS, P. M. & SMITH, S. M. (eds.) *Functional MRI an introduction to methods*. New York, USA: Oxford University Press.
- HOLMES, A. & FRISTON, K. 1998. Generalisability, Random Effects & Population Inference. *Neuroimage*, 7, S754.
- HRBEK, A., KARLBERG, P. & OLSSON, T. 1973. Development of visual and somatosensory evoked responses in pre-term newborn infants. *Electroencephalogr Clin Neurophysiol*, 34, 225-32.
- HU, X., LE, T. H. & UGURBIL, K. 1997. Evaluation of the early response in fMRI in individual subjects using short stimulus duration. *Magn Reson Med*, 37, 877-84.
- HU, X. & YACIOUB, E. 2012. The story of the initial dip in fMRI. *NeuroImage*, 62, 1103-8.
- HUETTEL, S. A. 2004. Non-linearities in the blood-oxygenation-level dependent (BOLD) response measured by functional magnetic resonance imaging (fMRI). *Conf Proc IEEE Eng Med Biol Soc*, 6, 4413-6.
- HUETTEL, S. A., SONG, A. W. & MCCARTHY, G. 2009. *Functional Magnetic Resonance Imaging*, Sinauer Associates.
- HÜPPI, P. S., MAIER, S. E., PELED, S., ZIENTARA, G. P., BARNES, P. D., JOLESZ, F. A. & VOLPE, J. J. 1998. Microstructural development of human newborn cerebral white matter assessed in vivo by diffusion tensor magnetic resonance imaging. *Pediatric research*, 44, 584-90.
- HUPPI, P. S., WARFIELD, S., KIKINIS, R., BARNES, P. D., ZIENTARA, G. P., JOLESZ, F. A., TSUJI, M. K. & VOLPE, J. J. 1998. Quantitative magnetic resonance imaging of brain development in premature and mature newborns. *Ann Neurol*, 43, 224-35.
- IAI, M., YAMAMURA, T. & TAKASHIMA, S. 1997. Early expression of proteolipid protein in human fetal and infantile cerebri. *Pediatr Neurol*, 17, 235-9.
- ILLIG, K. R. 2007. Developmental changes in odor-evoked activity in rat piriform cortex. *Neuroscience*, 145, 370-6.
- ISOBE, K., KUSAKA, T., NAGANO, K., OKUBO, K., YASUDA, S., KONDO, M., ITOH, S. & ONISHI, S. 2001. Functional imaging of the brain in sedated newborn infants using near infrared topography during passive knee movement. *Neuroscience letters*, 299, 221-4.
- JASANOFF, A. 2007. Bloodless FMRI. *Trends Neurosci*, 30, 603-10.
- JENKINSON, M. 2001. Registration, atlases and cortical flattening. In: JEZZARD, P., MATTHEWS, P. M. & SMITH, S. M. (eds.) *Functional MRI an introduction to methods*. New York, USA: Oxford University Press.

- JENKINSON, M., BANNISTER, P., BRADY, M. & SMITH, S. 2002. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage*, 17, 825-41.
- JENKINSON, M., BECKMANN, C. F., BEHRENS, T. E. J., WOOLRICH, M. W. & SMITH, S. M. 2012. FSL. *NeuroImage*, 62, 782-90.
- JENKINSON, M. & SMITH, S. M. 2003. Registration, brain atlases and cortical flattening. In: JEZZARD, P., MATTHEWS, P. M. & SMITH, S. M. (eds.) *Functional Magnetic Resonance Imaging: An Introduction to Methods*. Oxford: Oxford University Press.
- JEZZARD, P. & BALABAN, R. S. 1995. Correction for geometric distortion in echo planar images from B0 field variations. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, 34, 65-73.
- JEZZARD, P. & CLARE, S. 1999. Sources of distortion in functional MRI data. *Human brain mapping*, 8, 80-5.
- JOHNSON, B. N. & SOBEL, N. 2007. Methods for building an olfactometer with known concentration outcomes. *Journal of neuroscience methods*, 160, 231-45.
- JOHNSON, M. H. 1990. Cortical maturation and the development of visual attention in early infancy. *Journal of cognitive neuroscience*, 2, 81-95.
- JOHNSON, S., HENNESSY, E., SMITH, R., TRIKIC, R., WOLKE, D. & MARLOW, N. 2009. Academic attainment and special educational needs in extremely preterm children at 11 years of age: the EPICure study. *Archives of disease in childhood. Fetal and neonatal edition*, 94, F283-9.
- JOHNSON, S., HOLLIS, C., KOCHHAR, P., HENNESSY, E., WOLKE, D. & MARLOW, N. 2010. Psychiatric disorders in extremely preterm children: longitudinal finding at age 11 years in the EPICure study. *Journal of the American Academy of Child and Adolescent Psychiatry*, 49, 453-63.e1.
- JOHNSTON, M. V. 2009. Plasticity in the developing brain: implications for rehabilitation. *Dev Disabil Res Rev*, 15, 94-101.
- JONES, T. & RABINER, E. A. 2012. The development, past achievements, and future directions of brain PET. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*, 32, 1426-54.
- KAISER, W. A., FISCHER, H., VAGNER, J. & SELIG, M. 2000. Robotic system for biopsy and therapy of breast lesions in a high-field whole-body magnetic resonance tomography unit. *Invest Radiol*, 35, 513-9.
- KAPELLOU, O., COUNSELL, S. J., KENNEA, N., DYET, L., SAEED, N., STARK, J., MAALOUF, E., DUGGAN, P., AJAYI-OBE, M., HAJNAL, J., ALLSOP, J. M., BOARDMAN, J., RUTHERFORD, M. A., COWAN, F. & EDWARDS, A. D. 2006. Abnormal cortical development after premature birth shown by altered allometric scaling of brain growth. *PLoS medicine*, 3, e265.
- KARNISKI, W. 1992. The late somatosensory evoked potential in premature and term infants. I. Principal component topography. *Electroencephalogr Clin Neurophysiol*, 84, 32-43.

- KARNISKI, W., WYBLE, L., LEASE, L. & BLAIR, R. C. 1992. The late somatosensory evoked potential in premature and term infants. II. Topography and latency development. *Electroencephalogr Clin Neurophysiol*, 84, 44-54.
- KAVOUNOUDIAS, A., ROLL, J. P., ANTON, J. L., NAZARIAN, B., ROTH, M. & ROLL, R. 2008. Proprio-tactile integration for kinesthetic perception: an fMRI study. *Neuropsychologia*, 46, 567-75.
- KENNARD, M. A. 1936. Age and other factors in motor recovery from precentral lesions in monkeys. *American Journal of Physiology -- Legacy content*, 115, 138-146.
- KENNARD, M. A. 1942. Cortical reorganization of motor function: Studies on series of monkeys of various ages from infancy to maturity. *Archives of Neurology & Psychiatry*, 48, 227-240.
- KHAZIPOV, R., SIROTA, A., LEINEKUGEL, X., HOLMES, G. L., BEN-ARI, Y. & BUZSAKI, G. 2004. Early motor activity drives spindle bursts in the developing somatosensory cortex. *Nature*, 432, 758-61.
- KIM, B., BOES, J. L., BLAND, P. H., CHENEVERT, T. L. & MEYER, C. R. 1999. Motion correction in fMRI via registration of individual slices into an anatomical volume. *Magn Reson Med*, 41, 964-72.
- KIM, D. & KIM, S. K. 2012. Comparing patterns of component loadings: principal component analysis (PCA) versus independent component analysis (ICA) in analyzing multivariate non-normal data. *Behav Res Methods*, 44, 1239-1243.
- KIM, S.-G. & OGAWA, S. 2012. Biophysical and physiological origins of blood oxygenation level-dependent fMRI signals. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*, 32, 1188-206.
- KIM, S. A., LEE, Y. J. & LEE, Y. G. 2011. Predictive Value of Test of Infant Motor Performance for Infants based on Correlation between TIMP and Bayley Scales of Infant Development. *Ann Rehabil Med*, 35, 860-6.
- KIVINIEMI, V., KANTOLA, J.-H., JAUHIAINEN, J., HYVÄRINEN, A. & TERVONEN, O. 2003. Independent component analysis of nondeterministic fMRI signal sources. *NeuroImage*, 19, 253-60.
- KONISHI, Y., TAGA, G., YAMADA, H. & HIRASAWA, K. 2002. Functional brain imaging using fMRI and optical topography in infancy. *Sleep Med*, 3 Suppl 2, S41-3.
- KOSEKI, Y., WASHIO, T., CHINZEI, K. & ISEKI, H. 2002. Endoscope manipulator for trans-nasal neurosurgery, optimized for and compatible to vertical field open MRI. *Medical Image Computing and Computer-Assisted Intervention-Miccai 2002, Pt 1*, 2488, 114-121.
- KOSTOVIC, I. & JOVANOVIĆ-MILOSEVIC, N. 2006. The development of cerebral connections during the first 20-45 weeks' gestation. *Semin Fetal Neonatal Med*, 11, 415-22.
- KOSTOVIC, I. & JUDAS, M. 2007. Transient patterns of cortical lamination during prenatal life: do they have implications for treatment? *Neurosci Biobehav Rev*, 31, 1157-68.
- KOSTOVIC, I. & JUDAS, M. 2010. The development of the subplate and thalamocortical connections in the human foetal brain. *Acta Paediatr*, 99, 1119-27.
- KOSTOVIĆ, I., JUDAS, M., RADOS, M. & HRABAC, P. 2002. Laminar organization of the human fetal cerebrum revealed by histochemical markers and magnetic resonance imaging. *Cerebral cortex (New York, N.Y. : 1991)*, 12, 536-44.

- KOSTOVIC, I. & RAKIC, P. 1990. Developmental history of the transient subplate zone in the visual and somatosensory cortex of the macaque monkey and human brain. *The Journal of comparative neurology*, 297, 441-70.
- KRUBITZER, L. & HUFFMAN, K. J. 2000. Arealization of the neocortex in mammals: Genetic and epigenetic contributions to the phenotype. *Brain Behavior and Evolution*, 55, 322-335.
- KRÜGER, G., KASTRUP, A. & GLOVER, G. H. 2001. Neuroimaging at 1.5 T and 3.0 T: comparison of oxygenation-sensitive magnetic resonance imaging. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, 45, 595-604.
- KRUSE, M., MICHELSEN, S. I., FLACHS, E. M., BRONNUM-HANSEN, H., MADSEN, M. & ULDALL, P. 2009. Lifetime costs of cerebral palsy. *Dev Med Child Neurol*, 51, 622-8.
- KUANG, K. S. C., CANTWELL, W. J. & SCULLY, P. J. 2002. An evaluation of a novel plastic optical fibre sensor for axial strain and bend measurements. *Measurement Science and Technology*, 13, 1523-1534.
- KUEHN, B. M. 2009. Fda warning: Remove drug patches before mri to prevent burns to skin. *JAMA: The Journal of the American Medical Association*, 301, 1328-1328.
- KUKLISOVA-MURGASOVA, M., ALJABAR, P., SRINIVASAN, L., COUNSELL, S. J., DORIA, V., SERAG, A., GOUSIAS, I. S., BOARDMAN, J. P., RUTHERFORD, M. A., EDWARDS, A. D., HAJNAL, J. V. & RUECKERT, D. 2011. A dynamic 4D probabilistic atlas of the developing brain. *Neuroimage*, 54, 2750-63.
- KUSAKA, T., ISOBE, K., MIKI, T., UENO, M., KOYANO, K., NAKAMURA, S., NAKAMURA, M., KONISHI, Y., KUBOI, T., KATO, I., OKUBO, K., YASUDA, S., NISHIDA, T. & ITOH, S. 2011. Functional lateralization of sensorimotor cortex in infants measured using multichannel near-infrared spectroscopy. *Pediatric research*, 69, 430-5.
- KWONG, K. K., BELLIVEAU, J. W., CHESLER, D. A., GOLDBERG, I. E., WEISSKOFF, R. M., PONCELET, B. P., KENNEDY, D. N., HOPPEL, B. E., COHEN, M. S., TURNER, R. & ET AL. 1992. Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. *Proc Natl Acad Sci U S A*, 89, 5675-9.
- LAGERCRANTZ, H. & RINGSTEDT, T. 2001. Organization of the neuronal circuits in the central nervous system during development. *Acta paediatrica (Oslo, Norway : 1992)*, 90, 707-15.
- LARROQUE, B., ANCEL, P.-Y., MARRET, S., MARCHAND, L., ANDRÉ, M., ARNAUD, C., PIERRAT, V., ROZÉ, J.-C., MESSER, J., THIRIEZ, G., BURGUET, A., PICAUD, J.-C., BRÉART, G. & KAMINSKI, M. 2008a. Neurodevelopmental disabilities and special care of 5-year-old children born before 33 weeks of gestation (the EPIPAGE study): a longitudinal cohort study. *Lancet*, 371, 813-20.
- LARROQUE, B., ANCEL, P. Y., MARRET, S., MARCHAND, L., ANDRE, M., ARNAUD, C., PIERRAT, V., ROZE, J. C., MESSER, J., THIRIEZ, G., BURGUET, A., PICAUD, J. C., BREART, G. & KAMINSKI, M. 2008b. Neurodevelopmental disabilities and special care of 5-year-old children born before 33 weeks of gestation (the EPIPAGE study): a longitudinal cohort study. *Lancet*, 371, 813-20.
- LE BIHAN, D., URAYAMA, S., ASO, T., HANAKAWA, T. & FUKUYAMA, H. 2006. Direct and fast detection of neuronal activation in the human brain with diffusion MRI. *Proc Natl Acad Sci U S A*, 103, 8263-8.

- LEE, M. H., SMYSER, C. D. & SHIMONY, J. S. 2013. Resting-state fMRI: a review of methods and clinical applications. *AJNR Am J Neuroradiol*, 34, 1866-1872.
- LEE, W., DONNER, E. J., NOSSIN-MANOR, R., WHYTE, H. E., SLED, J. G. & TAYLOR, M. J. 2012. Visual functional magnetic resonance imaging of preterm infants. *Dev Med Child Neurol*, 54, 724-9.
- LEWIS, C. M., BALDASSARRE, A., COMMITTERI, G., ROMANI, G. L. & CORBETTA, M. 2009. Learning sculpts the spontaneous activity of the resting human brain. *Proc Natl Acad Sci U S A*, 106, 17558-63.
- LI, W., WAIT, S. D., OGG, R. J., SCOGGINS, M. A., ZOU, P., WHELESS, J. & BOOP, F. A. 2013. Functional magnetic resonance imaging of the visual cortex performed in children under sedation to assist in presurgical planning. *J Neurosurg Pediatr*, 11, 543-6.
- LIN, W., ZHU, Q., GAO, W., CHEN, Y., TOH, C. H., STYNER, M., GERIG, G., SMITH, J. K., BISWAL, B. & GILMORE, J. H. 2008. Functional connectivity MR imaging reveals cortical functional connectivity in the developing brain. *AJNR Am J Neuroradiol*, 29, 1883-9.
- LLINÁS, R. & RIBARY, U. 2001. Consciousness and the brain. The thalamocortical dialogue in health and disease. *Annals of the New York Academy of Sciences*, 929, 166-75.
- LOGOTHETIS, N. K. 2008. What we can do and what we cannot do with fMRI. *Nature*, 453, 869-78.
- LOGOTHETIS, N. K., GUGGENBERGER, H., PELED, S. & PAULS, J. 1999. Functional imaging of the monkey brain. *Nature neuroscience*, 2, 555-62.
- LOGOTHETIS, N. K. & PFEUFFER, J. 2004. On the nature of the BOLD fMRI contrast mechanism. *Magn Reson Imaging*, 22, 1517-31.
- LOGOTHETIS, N. K. & WANDELL, B. A. 2004. Interpreting the BOLD signal. *Annual review of physiology*, 66, 735-69.
- LORIG, T. S., ELMES, D. G., ZALD, D. H. & PARDO, J. V. 1999. A computer-controlled olfactometer for fMRI and electrophysiological studies of olfaction. *Behav Res Methods Instrum Comput*, 31, 370-5.
- LOWEN, S. B. & LUKAS, S. E. 2006. A low-cost, MR-compatible olfactometer. *Behav Res Methods*, 38, 307-13.
- LUNDSTROM, J. N., GORDON, A. R., ALDEN, E. C., BOESVELDT, S. & ALBRECHT, J. 2010. Methods for building an inexpensive computer-controlled olfactometer for temporally-precise experiments. *Int J Psychophysiol*, 78, 179-89.
- MACLAREN, J., HERBST, M., SPECK, O. & ZAITSEV, M. 2013. Prospective motion correction in brain imaging: a review. *Magn Reson Med*, 69, 621-636.
- MALONEK, D. & GRINVALD, A. 1996. Interactions between electrical activity and cortical microcirculation revealed by imaging spectroscopy: implications for functional brain mapping. *Science (New York, N.Y.)*, 272, 551-4.
- MANDEVILLE, J. B., MAROTA, J. J., KOSOFISKY, B. E., KELTNER, J. R., WEISSLEDER, R., ROSEN, B. R. & WEISSKOFF, R. M. 1998. Dynamic functional imaging of relative cerebral blood volume during rat forepaw stimulation. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, 39, 615-24.

- MANGHAM, L. J., PETROU, S., DOYLE, L. W., DRAPER, E. S. & MARLOW, N. 2009. The cost of preterm birth throughout childhood in England and Wales. *Pediatrics*, 123, e312-27.
- MANSFIELD, P. 1977. Multi-planar image formation using NMR spin echoes. *Journal of Physics C: Solid State Physics*, 10, L55-L58.
- MARCAR, V. L., STRASSLE, A. E., LOENNEKER, T., SCHWARZ, U. & MARTIN, E. 2004. The influence of cortical maturation on the BOLD response: an fMRI study of visual cortex in children. *Pediatr Res*, 56, 967-74.
- MARTIN, E., JOERI, P., LOENNEKER, T., EKATODRAMIS, D., VITACCO, D., HENNIG, J. & MARCAR, V. L. 1999. Visual processing in infants and children studied using functional MRI. *Pediatr Res*, 46, 135-40.
- MARTIN, E., THIEL, T., JOERI, P., LOENNEKER, T., EKATODRAMIS, D., HUISMAN, T., HENNIG, J. & MARCAR, V. L. 2000. Effect of pentobarbital on visual processing in man. *Human brain mapping*, 10, 132-9.
- MARTINEZ-BIARGE, M., DIEZ-SEBASTIAN, J., KAPELLOU, O., GINDNER, D., ALLSOP, J. M., RUTHERFORD, M. A. & COWAN, F. M. 2011. Predicting motor outcome and death in term hypoxic-ischemic encephalopathy. *Neurology*, 76, 2055-2061.
- MASAMUNE, K., KOBAYASHI, E., MASUTANI, Y., SUZUKI, M., DOHI, T., ISEKI, H. & TAKAKURA, K. 1995. Development of an MRI-compatible needle insertion manipulator for stereotactic neurosurgery. *J Image Guid Surg*, 1, 242-8.
- MATTHEWS, P. M. 2001. An introduction to functional magnetic resonance imaging of the brain. In: JEZZARD, P., MATTHEWS, P. M. & SMITH, S. M. (eds.) *Functional MRI : an introduction to methods*. Oxford: Oxford University Press.
- MCKEOWN, M. J., MAKEIG, S., BROWN, G. G., JUNG, T. P., KINDERMANN, S. S., BELL, A. J. & SEJNOWSKI, T. J. 1998. Analysis of fMRI data by blind separation into independent spatial components. *Human brain mapping*, 6, 160-88.
- MCROBBIE, D. W. 2007. *MRI from picture to proton*, Cambridge, UK ; New York, Cambridge University Press.
- MCROBBIE, D. W., MOORE, E. A., GRAVES, M. J. & PRINCE, M. R. 2006. *MRI from Picture to Proton*.
- MEDOFF-COOPER, B., SHULTS, J. & KAPLAN, J. 2009. Sucking behavior of preterm neonates as a predictor of developmental outcomes. *J Dev Behav Pediatr*, 30, 16-22.
- MENNES, M., KELLY, C., COLCOMBE, S., CASTELLANOS, F. X. & MILHAM, M. P. 2013. The extrinsic and intrinsic functional architectures of the human brain are not equivalent. *Cerebral cortex (New York, N.Y. : 1991)*, 23, 223-9.
- MENT, L. R., HIRTZ, D. & HUPPI, P. S. 2009a. Imaging biomarkers of outcome in the developing preterm brain. *Lancet Neurol*, 8, 1042-1055.
- MENT, L. R., HIRTZ, D. & HÜPPI, P. S. 2009b. Imaging biomarkers of outcome in the developing preterm brain. *The Lancet. Neurology*, 8, 1042-55.
- MENTO, G. & BISIACCHI, P. S. 2012. Neurocognitive development in preterm infants: insights from different approaches. *Neurosci Biobehav Rev*, 36, 536-55.
- MERCHANT, N., GROVES, A., LARKMAN, D. J., COUNSELL, S. J., THOMSON, M. A., DORIA, V., GROPPA, M., ARICHI, T., FOREMAN, S., HERLIHY, D. J., HAJNAL, J. V., SRINIVASAN, L.,

- FORAN, A., RUTHERFORD, M., EDWARDS, A. D. & BOARDMAN, J. P. 2009. A patient care system for early 3.0 Tesla magnetic resonance imaging of very low birth weight infants. *Early Hum Dev*, 85, 779-83.
- MERCURI, E., RICCI, D., PANE, M. & BARANELLO, G. 2005. The neurological examination of the newborn baby. *Early Hum Dev*, 81, 947-56.
- MIEZIN, F. M., MACCOTTA, L., OLLINGER, J. M., PETERSEN, S. E. & BUCKNER, R. L. 2000. Characterizing the hemodynamic response: effects of presentation rate, sampling procedure, and the possibility of ordering brain activity based on relative timing. *Neuroimage*, 11, 735-59.
- MILH, M., KAMINSKA, A., HUON, C., LAPILLONNE, A., BEN-ARI, Y. & KHAZIPOV, R. 2007. Rapid cortical oscillations and early motor activity in premature human neonate. *Cereb Cortex*, 17, 1582-94.
- MILLER, J. L., SONIES, B. C. & MACEDONIA, C. 2003. Emergence of oropharyngeal, laryngeal and swallowing activity in the developing fetal upper aerodigestive tract: an ultrasound evaluation. *Early Hum Dev*, 71, 61-87.
- MIZUNO, K. & UEDA, A. 2005. Neonatal feeding performance as a predictor of neurodevelopmental outcome at 18 months. *Developmental Medicine & Child Neurology*, 47, 299-304.
- MONTI, M. M. 2011. Statistical Analysis of fMRI Time-Series: A Critical Review of the GLM Approach. *Frontiers in human neuroscience*, 5, 28.
- MOORE, C. I., STERN, C. E., CORKIN, S., FISCHL, B., GRAY, A. C., ROSEN, B. R. & DALE, A. M. 2000. Segregation of somatosensory activation in the human rolandic cortex using fMRI. *J Neurophysiol*, 84, 558-569.
- MORI, S. & VAN ZIJL, P. C. M. 2002. Fiber tracking: principles and strategies - a technical review. *NMR Biomed*, 15, 468-480.
- MORITA, T., KOCHIYAMA, T., YAMADA, H., KONISHI, Y., YONEKURA, Y., MATSUMURA, M. & SADATO, N. 2000. Difference in the metabolic response to photic stimulation of the lateral geniculate nucleus and the primary visual cortex of infants: a fMRI study. *Neurosci Res*, 38, 63-70.
- MUMFORD, J. A. & NICHOLS, T. 2006. Modeling and inference of multisubject fMRI data. *IEEE Eng Med Biol Mag*, 25, 42-51.
- MUMFORD, J. A. & POLDRACK, R. A. 2007. Modeling group fMRI data. *Social cognitive and affective neuroscience*, 2, 251-7.
- MUNTENER, M., PATRICIU, A., PETRISOR, D., MAZILU, D., BAGGA, H., KAVOUSSI, L., CLEARY, K. & STOIANOVICI, D. 2006. Magnetic resonance imaging compatible robotic system for fully automated brachytherapy seed placement. *Urology*, 68, 1313-1317.
- MURAMOTO, S., YAMADA, H., SADATO, N., KIMURA, H., KONISHI, Y., KIMURA, K., TANAKA, M., KOCHIYAMA, T., YONEKURA, Y. & ITO, H. 2002. Age-dependent change in metabolic response to photic stimulation of the primary visual cortex in infants: functional magnetic resonance imaging study. *J Comput Assist Tomogr*, 26, 894-901.
- MURESAN, L., RENKEN, R., ROERDINK, J. B. & DUIFHUIS, H. 2005. Automated correction of spin-history related motion artefacts in fMRI: simulated and phantom data. *IEEE Trans Biomed Eng*, 52, 1450-1460.

- NEVALAINEN, P., LAURONEN, L., SAMBETH, A., WIKSTRÖM, H., OKADA, Y. & PIHKO, E. 2008. Somatosensory evoked magnetic fields from the primary and secondary somatosensory cortices in healthy newborns. *NeuroImage*, 40, 738-45.
- NEVALAINEN, P., RAHKONEN, P., PIHKO, E., LANO, A., VANHATALO, S., ANDERSSON, S., AUTTI, T., VALANNE, L., METSARANTA, M. & LAURONEN, L. 2014. Evaluation of somatosensory cortical processing in extremely preterm infants at term with MEG and EEG. *Clin Neurophysiol*.
- NICHOLS, T. & HAYASAKA, S. 2003. Controlling the familywise error rate in functional neuroimaging: a comparative review. *Statistical methods in medical research*, 12, 419-46.
- NICHOLS, T. E. 2012. Multiple testing corrections, nonparametric methods, and random field theory. *NeuroImage*, 62, 811-5.
- NICHOLS, T. E. & HOLMES, A. P. 2002. Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum Brain Mapp*, 15, 1-25.
- NOBLE, Y. & BOYD, R. 2012. Neonatal assessments for the preterm infant up to 4 months corrected age: a systematic review. *Dev Med Child Neurol*, 54, 129-39.
- NUNES, M. L., KHAN, R. L., GOMES FILHO, I., BOOIJ, L. & DA COSTA, J. C. 2014. Maturation changes of neonatal electroencephalogram: a comparison between intra uterine and extra uterine development. *Clin Neurophysiol*, 125, 1121-1128.
- O'DONOVAN, M. J. 1999. The origin of spontaneous activity in developing networks of the vertebrate nervous system. *Curr Opin Neurobiol*, 9, 94-104.
- OVERG, G. K., CAMPBELL, S. K., GIROLAMI, G. L., USTAD, T., JØRGENSEN, L. & KAARESEN, P. I. 2012. Study protocol: an early intervention program to improve motor outcome in preterm infants: a randomized controlled trial and a qualitative study of physiotherapy performance and parental experiences. *BMC pediatrics*, 12, 15.
- OGAWA, S. & LEE, T. M. 1990. Magnetic resonance imaging of blood vessels at high fields: in vivo and in vitro measurements and image simulation. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, 16, 9-18.
- OGAWA, S., LEE, T. M., KAY, A. R. & TANK, D. W. 1990a. Brain Magnetic-Resonance-Imaging with Contrast Dependent on Blood Oxygenation. *Proc Natl Acad Sci U S A*, 87, 9868-9872.
- OGAWA, S., LEE, T. M., KAY, A. R. & TANK, D. W. 1990b. Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *Proc Natl Acad Sci U S A*, 87, 9868-72.
- OGAWA, S., LEE, T. M., NAYAK, A. S. & GLYNN, P. 1990c. Oxygenation-sensitive contrast in magnetic resonance image of rodent brain at high magnetic fields. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, 14, 68-78.
- OGAWA, S., MENON, R. S., TANK, D. W., KIM, S. G., MERKLE, H., ELLERMANN, J. M. & UGURBIL, K. 1993. Functional brain mapping by blood oxygenation level-dependent contrast magnetic resonance imaging. A comparison of signal characteristics with a biophysical model. *Biophys J*, 64, 803-12.

- OGAWA, S., TANK, D. W., MENON, R., ELLERMANN, J. M., KIM, S. G., MERKLE, H. & UGURBIL, K. 1992. Intrinsic signal changes accompanying sensory stimulation: functional brain mapping with magnetic resonance imaging. *Proc Natl Acad Sci U S A*, 89, 5951-5.
- PALMER, F. B. 2004. Strategies for the early diagnosis of cerebral palsy. *J Pediatr*, 145, S8-S11.
- PANIGRAHY, A., WISNOWSKI, J. L., FURTADO, A., LEPORÉ, N., PAQUETTE, L. & BLUML, S. 2012. Neuroimaging biomarkers of preterm brain injury: toward developing the preterm connectome. *Pediatr Radiol*, 42 Suppl 1, S33-61.
- PAULING, L. & CORYELL, C. D. 1936. The Magnetic Properties and Structure of Hemoglobin, Oxyhemoglobin and Carbonmonoxyhemoglobin. *Proceedings of the National Academy of Sciences of the United States of America*, 22, 210-6.
- PENNOCK, J. 2001. Patient preparation, safety and hazards in imaging infants and children. In: RUTHERFORD, M. A. (ed.) *MRI of the Neonatal Brain*. Saunders Ltd.
- PERANI, D., SACCUMAN, M. C., SCIFO, P., ANWANDER, A., AWANDER, A., SPADA, D., BALDOLI, C., POLONIATO, A., LOHMANN, G. & FRIEDERICI, A. D. 2011a. Neural language networks at birth. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 16056-61.
- PERANI, D., SACCUMAN, M. C., SCIFO, P., ANWANDER, A., SPADA, D., BALDOLI, C., POLONIATO, A., LOHMANN, G. & FRIEDERICI, A. D. 2011b. Neural language networks at birth. *Proc Natl Acad Sci U S A*, 108, 16056-61.
- PERANI, D., SACCUMAN, M. C., SCIFO, P., SPADA, D., ANDREOLLI, G., ROVELLI, R., BALDOLI, C. & KOELSCH, S. 2010. Functional specializations for music processing in the human newborn brain. *Proc Natl Acad Sci U S A*, 107, 4758-63.
- PETERS, A. M., BROOKES, M. J., HOOGENRAAD, F. G., GOWLAND, P. A., FRANCIS, S. T., MORRIS, P. G. & BOWTELL, R. 2007. T2* measurements in human brain at 1.5, 3 and 7 T. *Magnetic resonance imaging*, 25, 748-53.
- PETERSSON, P., WALDENSTROM, A., FAHRAEUS, C. & SCHOUENBORG, J. 2003. Spontaneous muscle twitches during sleep guide spinal self-organization. *Nature*, 424, 72-5.
- PIHKO, E. & LAURONEN, L. 2004. Somatosensory processing in healthy newborns. *Experimental neurology*, 190 Suppl S2-7.
- PIHKO, E., NEVALAINEN, P., STEPHEN, J., OKADA, Y. & LAURONEN, L. 2009. Maturation of somatosensory cortical processing from birth to adulthood revealed by magnetoencephalography. *Clin Neurophysiol*, 120, 1552-61.
- PIKE, A. A., MARLOW, N. & REBER, C. 1999. Maturation of the flash visual evoked potential in preterm infants. *Early human development*, 54, 215-22.
- PITCHER, J. B., RILEY, A. M., DOELTGEN, S. H., KURYLOWICZ, L., ROTHWELL, J. C., MCALLISTER, S. M., SMITH, A. E., CLOW, A., KENNAWAY, D. J. & RIDDING, M. C. 2012. Physiological Evidence Consistent with Reduced Neuroplasticity in Human Adolescents Born Preterm. *Journal of Neuroscience*, 32, 16410-16416.
- PLATT, M. J., CANS, C., JOHNSON, A., SURMAN, G., TOPP, M., TORRIOLI, M. G. & KRAGELOHMANN, I. 2007. Trends in cerebral palsy among infants of very low birthweight (<1500 g) or born prematurely (<32 weeks) in 16 European centres: a database study. *Lancet*, 369, 43-50.

- POELLINGER, A., THOMAS, R., LIO, P., LEE, A., MAKRIS, N., ROSEN, B. R. & KWONG, K. K. 2001. Activation and habituation in olfaction--an fMRI study. *NeuroImage*, 13, 547-60.
- POLINE, J.-B. & BRETT, M. 2012. The general linear model and fMRI: does love last forever? *NeuroImage*, 62, 871-80.
- POORE, M., BARLOW, S. M., WANG, J., ESTEP, M. & LEE, J. 2008. Respiratory treatment history predicts suck pattern stability in preterm infants. *J Neonatal Nurs*, 14, 185-192.
- POPP, R., SOMMER, M., MULLER, J. & HAJAK, G. 2004. Olfactometry in fMRI studies: odor presentation using nasal continuous positive airway pressure. *Acta Neurobiol Exp (Wars)*, 64, 171-6.
- POWER, J. D., BARNES, K. A., SNYDER, A. Z., SCHLAGGAR, B. L. & PETERSEN, S. E. 2012. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *Neuroimage*, 59, 2142-54.
- PRECHTL, H. F. 1990. Qualitative changes of spontaneous movements in fetus and preterm infant are a marker of neurological dysfunction. *Early human development*, 23, 151-8.
- PRIETO, C. R., CARDENAS, H., SALVATIERRA, A. M., BOZA, C., MONTES, C. G. & CROXATTO, H. B. 1996. Sucking pressure and its relationship to milk transfer during breastfeeding in humans. *J Reprod Fertil*, 108, 69-74.
- PURVES, D., AUGUSTINE, G. J., FITZPATRICK, D., KATZ, L. C., LAMANTIA, A.-S., MCNAMARA, J. O. & WILLIAMS, S. M. 2001. *Neuroscience*, Sunderland (MA), Sinauer Associates.
- RADOS, M., JUDAS, M. & KOSTOVIC, I. 2006. In vitro MRI of brain development. *Eur J Radiol*, 57, 187-198.
- REES, S., WALKER, D. & JENNINGS, E. 2010. Development of the Somatosensory System In: LAGERCRANTZ, H., HANSON, M. A., MENT, L. R. & PEEBLES, D. M. (eds.) *The Newborn Brain: Neuroscience and Clinical Applications*. Cambridge: Cambridge University Press.
- ROBERTSON, C. M. T., WATT, M.-J. & YASUI, Y. 2007. Changes in the prevalence of cerebral palsy for children born very prematurely within a population-based program over 30 years. *JAMA*, 297, 2733-40.
- ROSAZZA, C., BUNTZ, A., RIEß, T., WÖLL, D., ZUMBUSCH, A. & ROLS, M.-P. 2013. Intracellular tracking of single-plasmid DNA particles after delivery by electroporation. *Molecular therapy : the journal of the American Society of Gene Therapy*, 21, 2217-26.
- ROSE, J., VASSAR, R., CAHILL-ROWLEY, K., GUZMAN, X. S., STEVENSON, D. K. & BARNEA-GORALY, N. 2014. Brain microstructural development at near-term age in very-low-birth-weight preterm infants: an atlas-based diffusion imaging study. *Neuroimage*, 86, 244-56.
- ROZE, E., HARRIS, P. A., BALL, G., ELORZA, L. Z., BRAGA, R. M., ALLSOP, J. M., MERCHANT, N., PORTER, E., ARICHI, T., EDWARDS, A. D., RUTHERFORD, M. A., COWAN, F. M. & COUNSELL, S. J. 2012. Tractography of the corticospinal tracts in infants with focal perinatal injury: comparison with normal controls and to motor development. *Neuroradiology*, 54, 507-16.
- RUBEN, R. J. 1995. The ontogeny of human hearing. *Int J Pediatr Otorhinolaryngol*, 32 Suppl, S199-204.
- RUTHERFORD, M., COUNSELL, S., ALLSOP, J., BOARDMAN, J., KAPELLOU, O., LARKMAN, D., HAJNAL, J., EDWARDS, D. & COWAN, F. 2004. Diffusion-Weighted Magnetic Resonance

- Imaging in Term Perinatal Brain Injury: A Comparison With Site of Lesion and Time From Birth. *Pediatrics*, 114, 1004-1014.
- SAINI, S., DESTEFANO, N., SMITH, S., GUIDI, L., AMATO, M. P., FEDERICO, A. & MATTHEWS, P. M. 2004. Altered cerebellar functional connectivity mediates potential adaptive plasticity in patients with multiple sclerosis. *J Neurol Neurosurg Psychiatry*, 75, 840-6.
- SALEH, S., ADAMOVICH, S. V. & TUNIK, E. 2012. Resting state functional connectivity and task-related effective connectivity changes after upper extremity rehabilitation: a pilot study. *Conf Proc IEEE Eng Med Biol Soc*, 2012, 4559-62.
- SATTERTHWAITE, T. D., WOLF, D. H., LOUGHEAD, J., RUPAREL, K., ELLIOTT, M. A., HAKONARSON, H., GUR, R. C. & GUR, R. E. 2012. Impact of in-scanner head motion on multiple measures of functional connectivity: relevance for studies of neurodevelopment in youth. *NeuroImage*, 60, 623-32.
- SAVIC, I. 2002a. Brain imaging studies of the functional organization of human olfaction. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry*, 8, 204-11.
- SAVIC, I. 2002b. Imaging of brain activation by odorants in humans. *Curr Opin Neurobiol*, 12, 455-461.
- SAVIC, I., GULYAS, B., LARSSON, M. & ROLAND, P. 2000. Olfactory functions are mediated by parallel and hierarchical processing. *Neuron*, 26, 735-45.
- SCHAAL, B. & MARLIER, L. 1998. Maternal and paternal perception of individual odor signatures in human amniotic fluid--potential role in early bonding? *Biology of the neonate*, 74, 266-73.
- SCHÄFER, K., BLANKENBURG, F., KUPERS, R., GRÜNER, J. M., LAW, I., LAURITZEN, M. & LARSSON, H. B. W. 2012. Negative BOLD signal changes in ipsilateral primary somatosensory cortex are associated with perfusion decreases and behavioral evidence for functional inhibition. *NeuroImage*, 59, 3119-27.
- SCHENCK, J. F. 1998. MR safety at high magnetic fields. *Magn Reson Imaging Clin N Am*, 6, 715-30.
- SCHENCK, J. F. 2000. Safety of strong, static magnetic fields. *Journal of Magnetic Resonance Imaging*, 12, 2-19.
- SCHNEIDER, G. E. 1979. Is It Really Better to Have Your Brain Lesion Early - Revision of the Kennard Principle. *Neuropsychologia*, 17, 557-&.
- SCHNEIDER, J. F. & FLOEMER, F. 2009. Maturation of the olfactory bulbs: MR imaging findings. *AJNR Am J Neuroradiol*, 30, 1149-1152.
- SCHOUENBORG, J. 2008. Action-based sensory encoding in spinal sensorimotor circuits. *Brain Research Reviews*, 57, 111-7.
- SEELKE, A. M., DOOLEY, J. C. & KRUBITZER, L. A. 2012. The emergence of somatotopic maps of the body in S1 in rats: the correspondence between functional and anatomical organization. *PLoS One*, 7, e32322.
- SEGHER, M. L. & HÜPPI, P. S. 2010. The role of functional magnetic resonance imaging in the study of brain development, injury, and recovery in the newborn. *Seminars in perinatology*, 34, 79-86.

- SEGHIER, M. L., LAZEYRAS, F. & HUPPI, P. S. 2006. Functional MRI of the newborn. *Semin Fetal Neonatal Med*, 11, 479-88.
- SEGHIER, M. L., LAZEYRAS, F., ZIMINE, S., MAIER, S. E., HANQUINET, S., DELAVELLE, J., VOLPE, J. J. & HUPPI, P. S. 2004. Combination of event-related fMRI and diffusion tensor imaging in an infant with perinatal stroke. *Neuroimage*, 21, 463-72.
- SEHGAL, A. & STACK, J. 2006. Developmentally supportive care and NIDCAP. *Indian J Pediatr*, 73, 1007-10.
- SERAG, A., ALJABAR, P., BALL, G., COUNSELL, S. J., BOARDMAN, J. P., RUTHERFORD, M. A., EDWARDS, A. D., HAJNAL, J. V. & RUECKERT, D. 2012. Construction of a consistent high-definition spatio-temporal atlas of the developing brain using adaptive kernel regression. *NeuroImage*, 59, 2255-65.
- SEUBERT, J., FREIHERR, J., DJORDJEVIC, J. & LUNDSTROM, J. N. 2013. Statistical localization of human olfactory cortex. *Neuroimage*, 66C, 333-342.
- SEZILLE, C., MESSAOUDI, B., BERTRAND, A., JOUSSAIN, P., THEVENET, M. & BENSAFI, M. 2013. A portable experimental apparatus for human olfactory fMRI experiments. *J Neurosci Methods*, 218, 29-38.
- SHELLOCK, F. G. 2000. Radiofrequency energy-induced heating during MR procedures: a review. *J Magn Reson Imaging*, 12, 30-6.
- SHELLOCK, F. G. & CRUES, J. V. 2004. MR procedures: biologic effects, safety, and patient care. *Radiology*, 232, 635-52.
- SILVENNOINEN, M. J., CLINGMAN, C. S., GOLAY, X., KAUPPINEN, R. A. & VAN ZIJL, P. C. M. 2003. Comparison of the dependence of blood R2 and R2* on oxygen saturation at 1.5 and 4.7 Tesla. *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*, 49, 47-60.
- SKIOLD, B., VOLLMER, B., BOHM, B., HALLBERG, B., HORSCH, S., MOSSKIN, M., LAGERCRANTZ, H., ADEN, U. & BLENNOW, M. 2012. Neonatal Magnetic Resonance Imaging and Outcome at Age 30 Months in Extremely Preterm Infants. *Journal of Pediatrics*, 160, 559-+.
- SMITH, S. M. 2001a. Overview of fMRI analysis. In: JEZZARD, P., MATTHEWS, P. M. & SMITH, S. M. (eds.) *Functional MRI an introduction to methods*. New York, USA: Oxford University Press.
- SMITH, S. M. 2001b. Preparing fMRI data for statistical analysis. In: JEZZARD, P., MATTHEWS, P. M. & SMITH, S. M. (eds.) *Functional MRI an introduction to methods*. New York, USA: Oxford University Press.
- SMITH, S. M., FOX, P. T., MILLER, K. L., GLAHN, D. C., FOX, P. M., MACKAY, C. E., FILIPPINI, N., WATKINS, K. E., TORO, R., LAIRD, A. R. & BECKMANN, C. F. 2009. Correspondence of the brain's functional architecture during activation and rest. *Proceedings of the National Academy of Sciences*, 106, 13040-13045.
- SMITH, S. M., JENKINSON, M., JOHANSEN-BERG, H., RUECKERT, D., NICHOLS, T. E., MACKAY, C. E., WATKINS, K. E., CICCARELLI, O., CADER, M. Z., MATTHEWS, P. M. & BEHRENS, T. E. J. 2006. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. *NeuroImage*, 31, 1487-505.

- SMITH, S. M., JENKINSON, M., WOOLRICH, M. W., BECKMANN, C. F., BEHRENS, T. E., JOHANSEN-BERG, H., BANNISTER, P. R., DE LUCA, M., DROBNJAK, I., FLITNEY, D. E., NIAZY, R. K., SAUNDERS, J., VICKERS, J., ZHANG, Y., DE STEFANO, N., BRADY, J. M. & MATTHEWS, P. M. 2004a. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage*, 23 Suppl 1, S208-19.
- SMITH, S. M., JENKINSON, M., WOOLRICH, M. W., BECKMANN, C. F., BEHRENS, T. E. J., JOHANSEN-BERG, H., BANNISTER, P. R., DE LUCA, M., DROBNJAK, I., FLITNEY, D. E., NIAZY, R. K., SAUNDERS, J., VICKERS, J., ZHANG, Y., DE STEFANO, N., BRADY, J. M. & MATTHEWS, P. M. 2004b. Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage*, 23 Suppl 1, S208-19.
- SMITH, S. M. & NICHOLS, T. E. 2009. Threshold-free cluster enhancement: Addressing problems of smoothing, threshold dependence and localisation in cluster inference. *NeuroImage*, 44, 83-98.
- SMYSER, C. D., INDER, T. E., SHIMONY, J. S., HILL, J. E., DEGNAN, A. J., SNYDER, A. Z. & NEIL, J. J. 2010. Longitudinal analysis of neural network development in preterm infants. *Cereb Cortex*, 20, 2852-62.
- SMYSER, C. D., SNYDER, A. Z. & NEIL, J. J. 2011. Functional connectivity MRI in infants: exploration of the functional organization of the developing brain. *NeuroImage*, 56, 1437-52.
- SMYSER, C. D., SNYDER, A. Z., SHIMONY, J. S., BLAZEY, T. M., INDER, T. E. & NEIL, J. J. 2013. Effects of white matter injury on resting state fMRI measures in prematurely born infants. *PLoS One*, 8, e68098.
- SOBEL, N., PRABHAKARAN, V., DESMOND, J. E., GLOVER, G. H., SULLIVAN, E. V. & GABRIELI, J. D. 1997. A method for functional magnetic resonance imaging of olfaction. *J Neurosci Methods*, 78, 115-23.
- SOMMER, J. U., MABOSHE, W., GRIEBE, M., HEISER, C., HÖRMANN, K., STUCK, B. A. & HUMMEL, T. 2012. A mobile olfactometer for fMRI-studies. *Journal of neuroscience methods*, 209, 189-94.
- SONG, D., JEGATHEESAN, P., WEISS, S., GOVINDASWAMI, B., WANG, J., LEE, J., ODER, A. & BARLOW, S. M. 2014. Modulation of EEG spectral edge frequency during patterned pneumatic oral stimulation in preterm infants. *Pediatr Res*, 75, 85-92.
- SPITTLE, A. J., DOYLE, L. W. & BOYD, R. N. 2008. A systematic review of the clinimetric properties of neuromotor assessments for preterm infants during the first year of life. *Dev Med Child Neurol*, 50, 254-66.
- STANDLEY, J. M. 2003. The effect of music-reinforced nonnutritive sucking on feeding rate of premature infants. *J Pediatr Nurs*, 18, 169-73.
- STAUDT, M. 2010. Brain plasticity following early life brain injury: insights from neuroimaging. *Semin Perinatol*, 34, 87-92.
- STAUDT, M., BRAUN, C., GERLOFF, C., ERB, M., GRODD, W. & KRAGELOH-MANN, I. 2006. Developing somatosensory projections bypass periventricular brain lesions. *Neurology*, 67, 522-5.
- STEFANACCI, L., REBER, P., COSTANZA, J., WONG, E., BUXTON, R., ZOLA, S., SQUIRE, L. & ALBRIGHT, T. 1998. fMRI of monkey visual cortex. *Neuron*, 20, 1051-7.

- STICKROD, G., KIMBLE, D. P. & SMOTHERMAN, W. P. 1982. In utero taste/odor aversion conditioning in the rat. *Physiol Behav*, 28, 5-7.
- STIPPICH, C., FREITAG, P., KASSUBEK, J., SOROS, P., KAMADA, K., KOBER, H., SCHEFFLER, K., HOPFENGARTNER, R., BILECEN, D., RADU, E. W. & VIETH, J. B. 1998. Motor, somatosensory and auditory cortex localization by fMRI and MEG. *Neuroreport*, 9, 1953-7.
- STOIANOVICI, D., PATRICIU, A., PETRISOR, D., MAZILU, D. & KAVOUSSI, L. 2007. A New Type of Motor: Pneumatic Step Motor. *IEEE ASME Trans Mechatron*, 12, 98-106.
- STUMM, S., BARLOW, S. M., ESTEP, M., LEE, J., CANNON, S., CARLSON, J. & FINAN, D. 2008. Respiratory Distress Syndrome Degrades the Fine Structure of the Non-Nutritive Suck In Preterm Infants. *J Neonatal Nurs*, 14, 9-16.
- SURMAN, G., HEMMING, K., PLATT, M. J., PARKES, J., GREEN, A., HUTTON, J. & KURINCZUK, J. J. 2009. Children with cerebral palsy: severity and trends over time. *Paediatric and perinatal epidemiology*, 23, 513-21.
- TANAKA, S., KOGO, M., CHANDLER, S. H. & MATSUYA, T. 1999. Localization of oral-motor rhythmogenic circuits in the isolated rat brainstem preparation. *Brain Res*, 821, 190-9.
- TAO, J. D. & NEIL, J. J. 2014. Advanced magnetic resonance imaging techniques in the preterm brain: methods and applications. *Current pediatric reviews*, 10, 56-64.
- TAWIA, S. 1992. When is the Capacity for Sentience Acquired During Human Fetal Development? *Journal of Maternal-Fetal and Neonatal Medicine*, 1, 153-165.
- TAYLOR, M. J., BOOR, R. & EKERT, P. G. 1996. Preterm maturation of the somatosensory evoked potential. *Electroencephalography and clinical neurophysiology*, 100, 448-52.
- TEUBER, H. & RUDEL, R. G. 1962. Behaviour after cerebral lesions in children and adults. *Dev Med Child Neurol*, 4, 3-20.
- THAYYIL, S., CHANDRASEKARAN, M., TAYLOR, A., BAINBRIDGE, A., CADY, E. B., CHONG, W. K. K., MURAD, S., OMAR, R. Z. & ROBERTSON, N. J. 2010. Cerebral Magnetic Resonance Biomarkers in Neonatal Encephalopathy: A Meta-analysis. *Pediatrics*.
- THULBORN, K. R., WATERTON, J. C., MATTHEWS, P. M. & RADDA, G. K. 1982. Oxygenation dependence of the transverse relaxation time of water protons in whole blood at high field. *Biochimica et biophysica acta*, 714, 265-70.
- TOFT, P. B., LETH, H., LOU, H. C., PRYDS, O., PEITERSEN, B. & HENRIKSEN, O. 1995. Local vascular CO2 reactivity in the infant brain assessed by functional MRI. *Pediatr Radiol*, 25, 420-4.
- TOMBINI, M., PASQUALETTI, P., RIZZO, C., ZAPPASODI, F., DINATALE, A., SEMINARA, M., ERCOLANI, M., ROSSINI, P. M. & AGOSTINO, R. 2009. Extrauterine maturation of somatosensory pathways in preterm infants: a somatosensory evoked potential study. *Clin Neurophysiol*, 120, 783-9.
- TSEKOS, N. V., KHANICHEH, A., CHRISTOFOROU, E. & MAVROIDIS, C. 2007. Magnetic resonance-compatible robotic and mechatronics systems for image-guided interventions and rehabilitation: a review study. *Annu Rev Biomed Eng*, 9, 351-387.
- TUSOR, N., ARICHI, T., COUNSELL, S. J. & EDWARDS, A. D. 2014. Brain development in preterm infants assessed using advanced MRI techniques. *Clin Perinatol*, 41, 25-45.

- VAFABEE, M. S. & GJEDDE, A. 2000. Model of blood-brain transfer of oxygen explains nonlinear flow-metabolism coupling during stimulation of visual cortex. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*, 20, 747-54.
- VAN DER AA, N. E., LEEMANS, A., NORTHINGTON, F. J., VAN STRAATEN, H. L., VAN HAASTERT, I. C., GROENENDAAL, F., BENDERS, M. J. N. L. & DE VRIES, L. S. 2011. Does Diffusion Tensor Imaging-Based Tractography at 3 Months of Age Contribute to the Prediction of Motor Outcome After Perinatal Arterial Ischemic Stroke? *Stroke*, 42, 3410-3414.
- VAN DIJK, K. R., SABUNCU, M. R. & BUCKNER, R. L. 2012a. The influence of head motion on intrinsic functional connectivity MRI. *Neuroimage*, 59, 431-8.
- VAN DIJK, K. R. A., SABUNCU, M. R. & BUCKNER, R. L. 2012b. The influence of head motion on intrinsic functional connectivity MRI. *NeuroImage*, 59, 431-8.
- VAN HAASTERT, I. C., GROENENDAAL, F., UITERWAAL, C. S., TERMOTE, J. U., VAN DER HEIDE-JALVING, M., EIJSERMANS, M. J., GORTER, J. W., HELDERS, P. J., JONGMANS, M. J. & DE VRIES, L. S. 2011. Decreasing incidence and severity of cerebral palsy in prematurely born children. *J Pediatr*, 159, 86-91 e1.
- VAN ZIJL, P. C. M., HUA, J. & LU, H. 2012. The BOLD post-stimulus undershoot, one of the most debated issues in fMRI. *NeuroImage*, 62, 1092-102.
- VANDERHAECHEN, P., LU, Q., PRAKASH, N., FRISEN, J., WALSH, C. A., FROSTIG, R. D. & FLANAGAN, J. G. 2000. A mapping label required for normal scale of body representation in the cortex. *Nat Neurosci*, 3, 358-65.
- VANHATALO, S. & KAILA, K. 2010. Emergence of spontaneous and evoked electroencephalographic activity in the human brain. In: LAGERCRANTZ, H., HANSON, M. A., MENT, L. R. & PEEBLES, D. M. (eds.) *The Newborn Brain: Neuroscience and Clinical Applications*. Cambridge: Cambridge University Press.
- VANHATALO, S. & LAURONEN, L. 2006. Neonatal SEP - back to bedside with basic science. *Semin Fetal Neonatal Med*, 11, 464-70.
- VARENDI, H., PORTER, R. H. & WINBERG, J. 1996. Attractiveness of amniotic fluid odor: evidence of prenatal olfactory learning? *Acta Paediatr*, 85, 1223-7.
- VIGOUROUX, M., BERTRAND, B., FARGET, V., PLAILLY, J. & ROYET, J. P. 2005. A stimulation method using odors suitable for PET and fMRI studies with recording of physiological and behavioral signals. *J Neurosci Methods*, 142, 35-44.
- VINCER, M. J., ALLEN, A. C., JOSEPH, K. S., STINSON, D. A., SCOTT, H. & WOOD, E. 2006. Increasing prevalence of cerebral palsy among very preterm infants: a population-based study. *Pediatrics*, 118, e1621-6.
- VOLPE, J. J. 2008. *Neurology of the newborn*, Philadelphia, Saunders/Elsevier.
- VOLPE, J. J. 2009a. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. *Lancet neurology*, 8, 110-24.
- VOLPE, J. J. 2009b. The encephalopathy of prematurity--brain injury and impaired brain development inextricably intertwined. *Semin Pediatr Neurol*, 16, 167-78.
- WANG, C. L., ZHANG, L., ZHOU, Y., ZHOU, J., YANG, X. J., DUAN, S. M., XIONG, Z. Q. & DING, Y. Q. 2007. Activity-dependent development of callosal projections in the somatosensory cortex. *J Neurosci*, 27, 11334-42.

- WEISMANN, M., YOUSRY, I., HEUBERGER, E., NOLTE, A., ILMBERGER, J., KOBAL, G., YOUSRY, T. A., KETTENMANN, B. & NAIDICH, T. P. 2001. Functional magnetic resonance imaging of human olfaction. *Neuroimaging clinics of North America*, 11, 237-50, viii.
- WESTBROOK, C., ROTH, C. K. & TALBOT, J. 2005. *MRI in practice*, Oxford, Blackwell Publishing.
- WHITE, C. P. & COOKE, R. W. 1994. Somatosensory evoked potentials following posterior tibial nerve stimulation predict later motor outcome. *Dev Med Child Neurol*, 36, 34-40.
- WHITEUS, C., FREITAS, C. & GRUTZENDLER, J. 2014. Perturbed neural activity disrupts cerebral angiogenesis during a postnatal critical period. *Nature*, 505, 407-+.
- WILSON, D. A., KADOHISA, M. & FLETCHER, M. L. 2006. Cortical contributions to olfaction: plasticity and perception. *Semin Cell Dev Biol*, 17, 462-470.
- WILSON, D. A. & SULLIVAN, R. M. 2011. Cortical processing of odor objects. *Neuron*, 72, 506-19.
- WINBERG, J. & PORTER, R. H. 1998. Olfaction and human neonatal behaviour: clinical implications. *Acta paediatrica (Oslo, Norway : 1992)*, 87, 6-10.
- WINKLER, A. M., RIDGWAY, G. R., WEBSTER, M. A., SMITH, S. M. & NICHOLS, T. E. 2014. Permutation inference for the general linear model. *Neuroimage*, 92, 381-397.
- WOLFF, P. H. 1968. The serial organization of sucking in the young infant. *Pediatrics*, 42, 943-56.
- WOOD, E. 2006. The child with cerebral palsy: diagnosis and beyond. *Semin Pediatr Neurol*, 13, 286-96.
- WOOLRICH, M. W., BEHRENS, T. E. J. & SMITH, S. M. 2004. Constrained linear basis sets for HRF modelling using Variational Bayes. *Neuroimage*, 21, 1748-1761.
- WOOLRICH, M. W., RIPLEY, B. D., BRADY, M. & SMITH, S. M. 2001. Temporal autocorrelation in univariate linear modeling of FMRI data. *Neuroimage*, 14, 1370-86.
- WORSLEY, K. J. & FRISTON, K. J. 1995. Analysis of fMRI time-series revisited--again. *NeuroImage*, 2, 173-81.
- YACoub, E. & HU, X. 2001. Detection of the early decrease in fMRI signal in the motor area. *Magn Reson Med*, 45, 184-90.
- YAMADA, H., SADATO, N., KONISHI, Y., KIMURA, K., TANAKA, M., YONEKURA, Y. & ISHII, Y. 1997. A rapid brain metabolic change in infants detected by fMRI. *Neuroreport*, 8, 3775-8.
- YAMADA, H., SADATO, N., KONISHI, Y., MURAMOTO, S., KIMURA, K., TANAKA, M., YONEKURA, Y., ISHII, Y. & ITOH, H. 2000. A milestone for normal development of the infantile brain detected by functional MRI. *Neurology*, 55, 218-23.
- YESILYURT, B., UGURBIL, K. & ULUDAG, K. 2008. Dynamics and nonlinearities of the BOLD response at very short stimulus durations. *Magn Reson Imaging*, 26, 853-862.
- ZHANG, N., GORE, J. C., CHEN, L. M. & AVISON, M. J. 2007. Dependence of BOLD signal change on tactile stimulus intensity in SI of primates. *Magn Reson Imaging*, 25, 784-94.