EARLY PAIN/STRESS AND MATH SKILLS IN RELATION TO FMRI DURING VISUOSPATIAL PROCESSING AT AGE 8 YEARS IN CHILDREN BORN VERY PRETERM

by

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BSc, Monash University, 2013

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

The Faculty of Graduate and Postdoctoral Studies

(Neuroscience)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)

August 2022

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Early Pain/Stress and Math Skills in Relation to fMRI During Visuospatial Processing at Age 8 Years in Children Born Very Preterm

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Abstract

Children born very preterm exhibit neurodevelopmental problems, compared to term-born peers. Math underperformance is particularly evident and is associated with poorer visuospatial skills. Neonatal exposure to repeated invasive procedures during neonatal intensive care unit (NICU) stay that induce pain and stress has been identified as one contributing factor to poorer cognitive performance, but relationships with mathematics achievement have not been studied.

Partial least squares correlation (PLSC) analyses were performed to investigate whether:

1. Neonatal pain-related stress and clinical factors are related to math skills at age 8 years, and whether: a) maternal education, b) white matter injury, and c) child sex, contribute to the relationship.

2. Neonatal pain-related stress and clinical factors are related to functional network activation during visuospatial processing.

3. Functional network activation during visuospatial processing is related to math skills.

In an ongoing prospective longitudinal cohort study, N=118 (63 male) children born very preterm at 8 years underwent neuropsychological and math skills assessment, and N=75 children also completed a visuospatial mental rotation of hands task. Prospective daily clinical chart review conducted across the NICU stay recorded pain/stress (number of invasive procedures) and clinical factors. No child with major brain injury and/or severe motor, sensory, or cognitive impairments was included. I found that:

1. Greater pain/stress exposure was related to poorer math skills, along with morphine, gestational age, ventilation, and infection ($\tau=80\%, p<.001$). None of maternal education, white matter injury, or child sex contributed to the relationship.
2. Greater neonatal pain/stress, morphine, ventilation, SNAP-II, infection, and lower gestational age were then related to increased brain network activation that included the right superior parietal, right lateral occipital, and left inferior temporal cortices ($\tau=65\%, p<.001$).

3. Poorer scores in all math domains were related to increased activation including in the superior parietal and lateral occipital cortices bilaterally and with concomitant decreased activation that included the right inferior parietal cortex ($\tau=93\%, p<.001$).

Overall this study suggests a link via visuospatial processing between math and pain/stress and supports the importance of visuospatial abilities in relation to math performance for children born very preterm.
Lay Summary

At school-age, children born very preterm are found to underperform in mathematics compared to their peers born at term. Math is supported by various foundational skills such as the ability to visualise and manipulate imagined representations of objects in space, referred to as visuospatial processing. Previous research establishes that early-life stress can have long-term effects on cognitive abilities. Infants born very preterm are hospitalised in the neonatal intensive care unit (NICU), and exposed to invasive procedures which can induce pain and stress (pain/stress). The goal of this research is to identify activity in the brain during visuospatial processing that relates to school-age math skills, and to examine whether neonatal pain/stress and other clinical factors may contribute to the development of these problems. This research helps to bridge the gap in understanding of neonatal experience in relation to functional brain networks and math skills at school-age in children born very preterm.
Preface

All work presented in this thesis was conducted under the supervision of Dr Ruth E Grunau and Dr Steven Miller. Input was also provided by committee members Dr Alex MacKay and Dr Lynne Williams. All chapters in this thesis were written by me, and the presented analyses were carried out by me. As part of an ongoing longitudinal cohort study, participant data collection was conducted by a team of researchers and staff.

The magnetic resonance imaging was conducted at BCCH MRI Research Facility by facility staff. Dr Bruce Bjornson and team developed and administered the MR scans, including the in-scanner task-based fMRI. Dr Steven Miller’s team, including Dr Jessie Guo, performed brain extraction for the T1 weighted structural images. Kenny Fok assisted in the collation of in-scanner behavioural data under my guidance, that was otherwise collated and further processed by me. Dr Lynne Williams performed pre-processing using AFNI 3dDespike where necessary. I performed all other pre-processing of the MR scans required for analyses.

During neonatal intensive care unit (NICU) stay, chart review was conducted by neonatal research nurses. For the follow-up at 8 years, assessment was performed by highly experienced psychology staff in the Neonatal Follow-Up Program at BC Women’s Hospital.

The study was approved by the University of British Columbia Research Ethics Board and BC Children’s & Women’s Hospitals review committee (certificate number H05-70579; Stress, Brain and Neurodevelopment in Children Born Preterm), and performed with CIHR funding (MOP79262, MOP86489, PJT159795).
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List of Abbreviations

AFNI: Analysis of Functional NeuroImages
ANOVA: analysis of variance
ATP: adenosine triphosphate
BC: British Columbia
BET: Brain Extraction Tool
BOLD: blood oxygenation level dependent
BSR: bootstrap ratio(s)
CI: confidence interval
CP: cerebral palsy
ELGA: extremely low gestational age
EPT: extremely preterm
FEAT: FMRI Expert Analysis Tool
fMRI: functional magnetic resonance imaging
FSIQ: Full Scale IQ
FSL: FMRIB’s Software Library
GA: gestational age
IASP: International Association for the Study of Pain
ICA: independent component analysis
ICA-AROMA: ICA-based Automatic Removal of Motion Artifacts
IQ: intelligence quotient
IVH: intraventricular haemorrhage
kg: kilogram(s)
KTEA: Kaufman Test for Educational Achievement
M: mean
MAGeT: multiple automatically generated templates
MEG: magnetoencephalography
mg: milligram(s)
mm: millimetre(s)
MNI: Montreal Neurological Institute
MR: magnetic resonance
MRI: magnetic resonance imaging
NICU: neonatal intensive care unit
NMD: Neurodevelopmental MRI Database
NMDA: N-methyl-D-aspartate
PLSC: partial least squares correlation
PMA: post menstrual age
PT: preterm
PVIH: periventricular-intraventricular haemorrhages
PVL: periventricular leukomalacia
ROI: regions of interest
s: second(s)
SD: standard deviation
SES: socioeconomic status
SNAP: Score for Neonatal Acute Physiology
STEM: science, technology, engineering, and mathematics
T: term
TE: echo time
TR: repetition time
VLGA: very low gestational age
VPT: very preterm
WASI: Wechsler Abbreviated Scale of Intelligence
WISC: Wechsler Intelligence Scale for Children
WMI: white matter injury
Acknowledgements

I would like to acknowledge the role of the greater research community, in particular for the development of tools and resources for graduate students such as myself, that makes the production of meaningful scientific work possible. I owe specific thanks to my supervisors Dr Ruth E Grunau and Dr Steven Miller. I appreciate the opportunity given to me to work and learn alongside Dr Grunau and her research team at the BC Children’s Hospital Research Institute as part of the Brain, Behaviour & Development Theme. I have received much guidance on the complexity and considerations of longitudinal studies and the importance of crafting specific research questions. My supervisors have shared with me a depth of knowledge in the experience of neonates born very preterm. Thank you for being attentive and available to discuss the thesis topic as it developed.

I would further like to thank my supervisory committee members, Dr Alex MacKay and Dr Lynne Williams. I am greatly appreciative for the guidance in developing a project of scope and depth appropriate to a Master’s thesis. In particular, Dr Lynne Williams’s guidance was essential in my journey to learn to work with neuroimaging data. Thank you as well to Cecil Chau and Dr Mia Mclean for your support as part of Dr Grunau’s research team.

I’d like to acknowledge the parents who provide consent to include their children in this study, and for all the time those families allocate to the completion of a longitudinal study like this. I further acknowledge the funding provided to support my research. I have received travel award funds from the International Society for Developmental Psychobiology (ISDP) and from the Brain, Behaviour & Development theme at BC Children’s Hospital Research Institute.

Finally, a sincere thank you to all my friends and family who have supported me. For my spouse, there are no words sufficient to acknowledge your belief in me.
1. Introduction

Preterm birth (before 37 weeks gestational age [GA]) represents approximately 11% of all live births worldwide (Blencowe et al., 2013; Chawanpaiboon et al., 2019; Walani, 2020). In Canada, about 1 in 12 babies were born preterm in 2020 (Statistics Canada, 2021).

Following very preterm birth (before 33 weeks GA), infants spend weeks to months in the neonatal intensive care unit (NICU). Neurodevelopmental problems (cognitive, motor, language, behavioural, academic) are prevalent among children born very preterm compared to full-term. Even without major disabilities (e.g. IQ<70, cerebral palsy) children born very preterm perform more poorly than their full-term peers, and difficulties are evident from infancy to young adulthood (e.g. Aarnoudse-Moens et al., 2009; Doyle & Anderson, 2010; Grunau et al., 2004; Joseph et al., 2016; Nosarti et al., 2010). Neurodevelopmental problems increase with degree of prematurity (lower GA) (Johnson & Marlow, 2011; Moore et al., 2013).

From early skepticism toward infant pain, the capacity for infants to perceive pain (Anand, Sippell, & Aynsley-Green, 1987; Fitzgerald, Millard, & Macintosh, 1988; Grunau & Craig, 1987) and the importance of pain and stress related to routine procedures during NICU care has been increasingly recognized (DiLorenzo, Pillai Riddell, & Holsti, 2016; Grunau, Miller, & Chau, 2021; Rodkey & Pillai Riddell, 2013; Vinall & Grunau, 2014). NICU care involves invasive procedures that can induce pain and stress (pain/stress), ranging from 8 to 17 average procedures per day in the first 14 days (Cruz, Fernandes, & Oliveira, 2016). For children born very preterm, early pain/stress from birth to term equivalent age or NICU discharge has been related to neurodevelopment (e.g. Grunau et al., 2009), as well as brain structure (e.g. Duerden et al., 2018; Ranger et al., 2013, 2015) and function (e.g. Doesburg et al., 2013; Schneider et al., 2018).
Neurodevelopmental problems and academic underachievement are common in children born very preterm (Aarnoudse-Moens et al., 2009; Burnett et al., 2019; Butcher et al., 2012; Grunau et al., 2004; Hille et al., 2007; Johnson et al., 2018; Joseph et al., 2016; van Gils et al., 2020). Academic underachievement is particularly pronounced in mathematics (e.g. Aarnoudse-Moens et al., 2009; Grunau et al., 2004; McBryde et al., 2020). By early school age and beyond, very preterm born children underperform in mathematics compared to full-term born peers (Aarnoudse-Moens et al., 2009; Grunau et al., 2004; McBryde et al., 2020; Simms, Cragg, Gilmore, Marlow, & Johnson, 2013; Taylor, 2010; Twilhaar, de Kieviet, Aarnoudse-Moens, van Elburg, & Oosterlaan, 2018). This math underperformance may be related to poorer visuospatial skills which are highly prevalent in this population (Grunau, Whitfield, & Davis, 2002; Simms et al., 2015; van Veen, van Wassenaer-Leemhuis, van Kaam, Oosterlaan, & Aarnoudse-Moens, 2019). Exposure to pain/stress in additional to other neonatal clinical factors has been related to several areas of neurodevelopment as well as altered brain structure and function, however, whether pain/stress contributes specifically to visuospatial processing and mathematics skills has not yet been addressed to my knowledge.

The present study aimed to examine whether:

1. Neonatal pain-related stress and clinical factors are related to math skills at age 8 years, and whether: a) maternal education, b) white matter injury, and c) child sex, contribute to the relationship.

2. Neonatal pain-related stress and clinical factors are related to functional network activation during visuospatial processing.

3. Functional network activation during visuospatial processing is related to math skills.
1.1 Brain development during the critical neonatal period

Brain development proceeds rapidly during the third trimester of intrauterine development. Infants born very preterm spend all or part of the late second and third trimesters in the NICU, exposed to developmentally unexpected stimulation and a varying clinical course. Knowledge of the processes of brain development informs the study of the impact of pain/stress, as well as other neonatal factors, on maturation of the immature brain.

The mature brain is an interconnected structure that includes not only the neurons that are most often the focus of functional signalling in cognition and behaviour, but also the glial cells, cerebral vasculature, and extracellular milieu. The brain is structurally categorised into white matter and grey matter. In the white matter, neurons’ axons are sheathed in myelin from oligodendrocytes, a type of aforementioned glial cell. This myelin facilitates signal transduction in the neuron. The grey matter is then the site of extensive dendritic arborisation; the ‘end-point’ for the transduced signal, dendrites form synapses to pass signals from neuron to neuron via the complementary system of neurotransmitter release and receptor activation. The cerebral cortex, a folded grey matter structure with gyri peaks and sulci grooves, is further conceptually organised into four major lobes: occipital, temporal, parietal, and frontal. Subcortical structures include the cerebellum and thalamus.

The majority of neurons have formed by 25–28 weeks post menstrual age (PMA, age in weeks as measured from the first day of the mother’s last menstrual period), a process that begins in the fifth week PMA (Bystron, Blakemore, & Rakic, 2008; Hadders-Algra, 2018). Neurons migrate from the germinal layers radially out to form the eventual mature cortical and subcortical network structure, with migration guided by radial glial cells. The process of overall neuronal migration has overlapping periods of migration unique to particular regions, such that,
for instance, a peak in migration is first seen toward occipital and temporal regions at 20 weeks PMA, parietal cortex at 23 weeks PMA, then frontal cortex at 26 weeks PMA (Lagercrantz, Hanson, Ment, & Peebles, 2010; Trivedi et al., 2009). Neuronal migration is largely complete before term age, but evidence suggests migration of select groups of interneurons continues into infancy (Hadders-Algra, 2018; Paredes et al., 2016). The formation of long-range connections across the brain begins with thalamocortical projections: from the thalamus, the projections arrive in the subplate zone around week 26, and have formed mostly complete connections around 32 weeks (Vanhatalo & Kaila, 2010). Meanwhile, long-range corticocortical connections begin contact formation with their targets in weeks 29–30 (Hüppi & Dubois, 2006; Judaš et al., 2005; Vanhatalo & Kaila, 2010).

A key structure in the process of not only neuronal migration but also differentiation and synaptogenesis is the cortical subplate, a temporary structure that exists during brain development (Hadders-Algra, 2018; Lagercrantz et al., 2010). Before neurons can migrate to the cortical plate, which is a precursor to and the location for the eventual mature cerebral cortex, they first migrate into the cortical subplate. The subplate is a transient, neuronally dense zone, that contains functional networks that reflect, but exist prior to, the mature functional organisation of the brain in primary motor, somatosensory, and visual cortices. Synaptic activity develops in subplate neurons from 9–10 weeks PMA, being the thickest at 28–34 weeks PMA, with neuronal activity diminished in the subplate and continuous in the cortical plate by 3 months post-term (Hadders-Algra, 2018; Judaš, Sedmak, & Kostović, 2013; Kostović, Sedmak, Vukšić, & Judaš, 2015; Marín-Padilla, 2014; Vasung et al., 2016). Besides the ongoing migration of neurons, there are ongoing synaptogenesis and synaptic pruning processes, as well as ongoing myelination of axons.
The myelination of neuronal axons during brain development relies on the maturation of oligodendrocytes. Reflecting the chronological course of maturation, the oligodendroglial population is divided into four types: the oligodendroglial progenitor becomes the pre-oligodendrocyte, which becomes an immature oligodendrocyte, before finally becoming a mature myelin-producing oligodendrocyte. The immature oligodendrocytes and pre-oligodendrocytes are still responsible for ensheathing axons prior to their maturation into myelin-producing form. The presence of myelin-producing oligodendrocytes can be detected by myelin basic protein labelling; in parietal white matter, this method was used to detect the presence of myelin at approximately 30 weeks GA in select white matter around the ventricles of the brain (ventricles form the brain’s network of cerebrospinal fluid), and by 40 weeks GA evidence of myelin presence extended into white matter regions further from the ventricles (Back et al., 2001). Immature oligodendrocytes undergo an approximately threefold increase when comparing 18–27 weeks GA and 28–41 weeks GA (Back et al., 2001).

All of these interconnected processes of neuronal brain development, as well as vasculature development concurrently, are sensitive to possible perturbation. Synaptogenesis, which encompasses all of the organisation, proliferation, and pruning of synapses, key to the creation of overall functional networks in the brain, in particular is highly sensitive to environmental conditions (Bourgeois, 2010). Synaptogenesis occurring before and after birth has markedly different stimuli driving development. In the case of children born very preterm, this change in environment at birth occurs earlier in development than for full-term born infants and is developmentally unexpected. The developing brain of children born very preterm is thus vulnerable to perturbation given the immaturity of the nervous system.
1.2 Early pain/stress in children born very preterm

The International Association for the Study of Pain (IASP) defines pain as “an unpleasant and emotional experience associated with, or resembling that associated with, actual or potential tissue damage”, with a specific clarification that this definition was revised to ensure that pain does not exclude infants due to their inability to verbally articulate pain (IASP Task Force on Taxonomy, Merskey (ed) & Bogduk (ed), 1994). Pain in the mature brain encompasses both an emotional-cognitive processing component and a response of the nervous system, from the peripheral nervous system, including nociceptive receptors, to the central nervous system, including nerve fibres (also known as axon fibres) in the spinal cord and the brain. Noxious or potentially noxious stimuli include sources of inflammation, excessive heat or cold, high threshold touch, and skin breakage. In the context of children born very preterm, the relative immaturity of nociceptive and cortical processes associated with pain are critical.

Importantly, even low threshold touch can become noxious stimuli due to sensitisation (Fitzgerald, 2005; Hathway, Greenspon, & Baccei, 2021). Sensitisation is the change of threshold sufficient for stimuli to activate nociceptors. Sensitisation can be due to inflammation as well as repeated stimuli, among others. Hyperalgesia describes sensitisation where there is an increased pain response to already painful stimuli, while allodynia describes sensitisation where there is now a pain response to what was not previously a painful stimuli (previously ‘innocuous’ stimuli) (Hathway et al., 2021). For neonates born very preterm, this can result in a far greater response to something as simple as a diaper change if performed after a routine heel lance. In a study of infants at 32 weeks GA, all infants had heightened facial, body and heart rate responses when ‘clustered care’ including diaper change was performed after a painful procedure, compared to after a prolonged period undisturbed (Holsti, Grunau, Oberlander, & Whitfield,
Infants at 30 weeks GA and above have also showed evidence of sensitisation, with facial response during blood collection indicative of heightened pain sensitivity when the blood collection came after routine nursing interventions (Holsti, Grunau, Whitfield, & Lindh, 2006). Sensitisation, whereby inherently non-noxious stimuli become aversive due to a lowering of tactile threshold, remains a crucial consideration when identifying aspects of care that may present as potentially noxious stimuli for neonates born very preterm (Holsti et al., 2006).

Even where a stimulus may or may not be considered a source of pain, disturbance of an infant’s comfort may yet be a source of stress. Particularly in the context of children born very preterm, even non-skin breaking routine tactile nursing actions like diaper changes, especially when clustered with other routine procedures, impact responses to skin-breaking care in blood collection (Holsti et al., 2005, 2006). Due to sensitisation, reactivity is not only a function of extent of tissue damage, but reflects what has occurred in the past hour (Holsti et al., 2006), or the past 24 hours (Grunau, Holsti, Whitfield, & Ling, 2000). Moreover, pain reactivity cannot be distinguished from stress response, physiologically or behaviourally, in these immature neonates (e.g. Grunau, 2013; Grunau et al., 2021; Ranger, Johnston, & Anand, 2007). Therefore the term pain-related stress (pain/stress) is often used to denote the responses evoked by invasive procedures in very preterm infants.

1.2.1 The development of pain perception and nociceptive processing

Nociceptors are sensory receptors that signal to sensory afferent fibres the presence of damaging or potentially damaging stimuli. Three types of sensory afferent fibres are key to the development of both mechanoreception and nociceptive processing: myelinated A-beta and A-delta fibres, and unmyelinated C-fibres (Hathway et al., 2021). In the mature pain processing system, A-beta fibres are typically associated with ‘low-threshold’ mechanoreception. A-delta
fibres are then ‘high-threshold’ receptive, with free nerve endings, and also respond to heat and cold. C-fibres also respond under a ‘high-threshold’, to heat and cold, and additionally to chemical itch stimuli; they also have free nerve endings. Of note, some C-fibres are only responsive following an inflammatory event (Hathway et al., 2021; Todd, 2010). Inflammatory events are characterised by a cascade of molecular and cellular responses, and some of these inflammation-related molecules are detected by and responded to via pain pathways. Potentially painful events, such as skin breakage, also often result in the triggering of inflammatory cascades through tissue damage (Hathway et al., 2021)—thus, inflammation and pain are closely related, bidirectionally (Ji, Chamessian, & Zhang, 2016).

Animal rodent models, particularly rats, are frequently utilised to study the development of sensory afferent fibres that would not be possible in humans. Rats are born neurologically immature, where the first postnatal week in rats corresponds to approximately the duration from preterm to full-term (24 to around 40 weeks) in humans (Hathway et al., 2021). Such models indicate that sensory afferent fibre organisation and function differs from newborn to adult systems (Fitzgerald, 2005). For instance, the location of the termination of immature A-beta fibres results in the ability to access and activate A-delta and C-fibres which would not be accessed or activated in the mature system, and which have different roles in the processing of noxious and non-noxious stimuli (Beggs, Torsney, Drew, & Fitzgerald, 2002; DiLorenzo et al., 2016; Fitzgerald, Butcher, & Shortland, 1994). For neonates born very preterm, this results in an inability to discriminate between noxious and non-noxious stimuli until approximately 35 weeks GA; specifically, where electrical activity of the brain in response to tactile stimuli or heel lance are initially evoked in widespread regions and only later in development become specific responses localised in the somatosensory cortex (Fabrizi et al., 2011). This directly impacts how
stimuli are conceptualised in the context of infants born very preterm as potentially noxious, particularly for those undergoing care in the NICU.

While the excitatory signalling ascending pathway originating at nociceptors is active, inhibitory and modulatory signalling of the reciprocal descending pathway in nociceptive circuits is immature (Fitzgerald, 2005). While descending fibres are present, the functional inhibition exhibited is altered in ways that may be considered weak, lacking precision, or even absent, compared with the mature system; inhibition is known to have a prolonged postnatal maturation in rodent models (Fitzgerald, 2005).

The development of thalamocortical connections is critical to the development of pain perception and effects of nociceptive stimulation. The thalamus acts as an essential hub for all sensory processing, relaying and integrating sensory input from the periphery to the brain. Development of connectivity from the thalamus to the cortex reflects a time period (26–32 weeks; Vanhatalo & Kaila, 2010) particularly relevant to somatosensory processing in infants born very preterm. The thalamus receives sensory information from the peripheral nervous system, including from the aforementioned sensory afferent fibres via spinothalamic tract neurons. Sensory afferents project to the thalamus where specific regions of the thalamus, termed thalamic nuclei, project to the cortices. Functional magnetic resonance imaging (fMRI) studies enable identification of brain regions associated with specific processes or events. Such studies of both adult and infant pain have revealed many shared regions of cortical and subcortical activation including the thalamus, insula, parietal lobe, and precuneus (Goksan et al., 2015; Gursul, Hartley, & Slater, 2019). One key difference between the adult and infant response to noxious stimulation is in the hemispheric localisation of responses, likely due to the immaturity of long-range hemispheric connections in the case of infants (Goksan et al., 2015).
In summary, the development of mature pain perception involves extensive nociceptive pathways from sensory afferent receptors to spinal interneurons, as well as cortical and subcortical brain regions and cognitive pain processing networks. Within this, the vulnerability of pre-oligodendrocytes and subplate neurons to pain/stress is known (e.g. Anand & Scalzo, 2000; Back et al., 2001; Buntinx, Gielen, et al., 2004; Buntinx, Moreels, et al., 2004; Fern & Möller, 2000), and details are provided below.

1.2.2 Specific vulnerabilities of the central nervous system to repeated pain/stress

The roles of subplate neurons, particularly for development of thalamocortical connections, and pre-oligodendrocytes in development of myelination have been summarised in earlier sections. Their specific vulnerability in the context of pain/stress in neonates born very preterm is highlighted in this section.

Previously I summarised the close relationship between pain and inflammation; the inflammatory role of cytokines in particular is known to impact other cells in the environment. Cytokines are released by microglia recruited as part of the inflammation response. Oligodendrocytes cultured in the presence of proinflammatory cytokines become more vulnerable to cell death (Buntinx, Gielen, et al., 2004; Buntinx, Moreels, et al., 2004). Thus, cytokine abundance as a result of repeated potentially painful procedures and its associated inflammation can impact the integrity of white matter in the brain, and in turn overall brain network function.

Oligodendroglial populations are also vulnerable to oxidative stress. There is a significant relationship between oxidative stress and procedural pain in premature neonates (Slater et al., 2012). Painful procedures bring about increased energy requirements and reduced oxygenation; the enhanced ATP (adenosine triphosphate) degradation that results increases production of
reactive oxygen species. Even in a single heel lance, an increase in markers of oxidative stress is seen in the plasma of premature neonates (Bellieni et al., 2009). Even within wide ranging impacts of oxidative stress on a newborn, the oligodendroglial cell type lineage has been found to be less resistant to oxidative damage than other neuronal and glial cell types (Back et al., 2005). Within the oligodendroglial population, immature oligodendrocytes in rodent models have also been found to be more susceptible to oxidative stress than more mature oligodendrocytes (Fern & Möller, 2000). Given that immature oligodendrocytes make up approximately 31% of the total oligodendroglial population at 28–41 weeks postconceptual age (Back et al., 2001), this highlights the particular vulnerability of those born very preterm to the oxidative stress resulting from pain/stress.

Given the effects of sensitisation, immature inhibitory signalling, and enhanced excitatory signalling for neonates, excitotoxicity is a concern for neonates born very preterm, particularly where repeated invasive procedures during care in the NICU are evidenced as resulting in sensitised responses in neonates (Holsti et al., 2005, 2006). Excitatory signalling results in excitotoxicity when there is excessive intracellular calcium. Excitatory signalling involves glutamate transmission and NMDA (N-methyl-D-aspartate) receptor ion channel activation (Anand & Scalzo, 2000). Glutamate is a neurotransmitter common throughout the central nervous system. The NMDA receptor ion channel on the neuronal cell membrane opens in response to abundant glutamate, allowing the influx of calcium to the neuron, which allows for a depolarisation of the neuron and the beginning of signal transduction down the axon. Expression of specific glutamate NMDA receptor subunits changes over development, and this alters how sensitive the receptor is to the presence of glutamate. Specifically, the higher proportion of NR2B subunits to NR2A subunits early in life results in a more easily excitable neuron (e.g.
Monyer et al., 1992; Sheng et al., 1994). Repetitive pain/stress stimulation may lead to neuronal ‘excitotoxicity’—literally, an intracellular environment that is ‘toxic’ (i.e. leading to cell damage or cell death) to the neurons due to excitation (Anand & Scalzo, 2000). While calcium influx is a necessary part of synaptic function, excessive intracellular calcium results in cell death (Anand & Scalzo, 2000). Subplate neurons have been highlighted for their particular vulnerability to excitotoxicity due to the aforementioned timing of the subplate development. Cell damage or death in developing networks in the brain for those born very preterm may impact both structure and function therein, and so their behavioural and cognitive neurodevelopment.

A rodent model further supports the significance of cell death resulting from over-excitation on visual-spatial processing and memory function specifically. In the rodent model, the increased neuronal excitation and associated increased cell death from repetitive neonatal pain further relates to a decrease in visual-spatial clue task performance, as well as a decrease in short-term and long-term memory (Anand et al., 2007). Such evidence is relevant to children born very preterm—as highlighted previously, when compared to their term-born peers, their performance is poorer in areas including visuospatial processing.

This section briefly summarised key known mechanisms for pain/stress to affect the developing brain. While causal relationships between pain/stress stimulation and altered brain development cannot be established in the human preterm neonate, these mechanisms of fetal brain development and sensitivity to excitotoxicity provide biological plausible links to support the associations between pain/stress, brain and neurobehavioral development in the following section. No matter the mechanism at play, a relationship between pain/stress (after accounting for or together with known clinical factors) and both brain structure and function is seen for children born very preterm.
1.2.3 Pain/stress in relation to brain structure, function and neurodevelopment in children born very preterm

Greater exposure to neonatal pain/stress has been found to predict poorer cognitive development in children born very preterm at both 8 and 18 months corrected chronological age, after controlling for early illness severity, morphine exposure, and days on dexamethasone (Grunau et al., 2009). The measurement used for cognitive function in that study included language function and nonverbal abilities such as block stacking and problem solving tasks. Moreover, pain/stress, after accounting for known clinical risk factors, has been found to relate to altered brain structure and function, and then in turn to cognition (Chau et al., 2019; Doesburg et al., 2013; Duerden et al., 2018; Kozhemiako et al., 2019; Ranger et al., 2015; Tortora et al., 2019). Even where the relationship to cognition was not specifically examined in studies of pain/stress in relation to altered brain structure, the implications of reduced cortical thickness in frontoparietal regions (Ranger et al., 2013) may be significant to considerations of both math skills and visuospatial processing.

For children born very preterm, greater pain/stress has been found to relate to slower thalamic growth during the neonatal period in somatosensory regions which have further been related to cognitive and motor scores at 3 years corrected age (Duerden et al., 2018). Specifically, slower thalamic macrostructural growth for children born very preterm during the neonatal period was associated with greater early pain, controlling for confounders such as GA at birth. This slower thalamic growth was mostly in somatosensory regions, and the somatosensory cortex, to which these regions of the thalamus project, is located in the postcentral gyrus of the parietal lobe, which is implicated in normative visuospatial processing of the mature brain (Hawes, Sokolowski, Ononye, & Ansari, 2019). This slower thalamic growth was further related
to lower cognitive and motor scores at 3 years corrected age, adjusting for GA and sex (Duerden et al., 2018). Greater early pain/stress has also been found to relate to decreased functional connectivity between the right thalamus and ipsilateral somatosensory cortex from neonatal MRI at term equivalent age, where weaker functional connectivity of the right thalamocortical pathway was further related to lower locomotor scores at 24 months of age (Tortora et al., 2019). At 8 years of age, pain/stress was also found to relate to cerebellar volume for children born very preterm in an analysis of 30 cerebellar subregions and multiple neonatal factors, including pain/stress, neonatal infection, and gestational age (Ranger et al., 2015). Ranger et al. (2015) concluded that a higher number of invasive procedures was associated particularly with smaller volumes in the bilateral posterior VIII A and VIIB lobules of the cerebellum, i.e. sensorimotor mapping; regions of reduced volume in the cerebellum were further found to relate to poorer visual perception and working memory. Also at 8 years of age, pain/stress has been found to relate to perceptual reasoning, through analysis of brain activity using magnetoencephalography (MEG) activity recorded during viewing of a fixed ‘happy face’ visual stimulus, where MEG oscillatory profiles were characterised by the ratio of spontaneous gamma oscillations to alpha rhythms (gamma/alpha) (Doesburg et al., 2013). In those born at extremely low gestational age (ELGA, ≤28 weeks GA), greater gamma/alpha was related to higher cumulative neonatal pain, adjusted for confounders. In this same ELGA group, greater gamma/alpha was negatively correlated with perceptual reasoning; although those born at very low gestational age (VLGA, 28–32 weeks) did not meet significance, the relationship between MEG oscillatory profile and perceptual reasoning trended in the same direction. Together, these studies provide evidence to support relationships between neonatal pain/stress and cognitive domains including visual
perception and perceptual reasoning, suggesting the possibility of further relationships between pain/stress and other related cognitive domains, via the brain.

More generally, in children born very preterm at 8 years of age, reduced cortical thickness in regions found to be associated with greater pain/stress, controlling for neonatal factors, were concentrated in regions of the frontal and parietal lobes (Ranger et al., 2013). The frontal and parietal lobes are associated with both math and visuospatial processing in the normative mature brain (Hawes et al., 2019), as well as in fMRI studies of math skills and visuospatial working memory in children and adults born very preterm (Bäuml et al., 2017; Clark, Liu, Wright, Bedrick, & Edgin, 2017; Klein et al., 2018, 2014; Tokariev et al., 2019). Moreover, higher procedural pain has been found to be negatively associated with functional connectivity; specifically, this was found in component networks of the thalamus and sensorimotor cortices, when adjusting for PMA at scan and glucose dose during NICU stay (Schneider et al., 2018). This study also found that more invasive procedures were associated with reduced growth up to term equivalent age (40 weeks PMA) in brain volumes, in the thalamus, basal ganglia, and whole brain, but for their analysis of fMRI recorded at term equivalent age, the study a priori selected only networks that involved the thalamus and sensorimotor cortices, motivating further studies that consider the relationship of pain/stress with brain-wide activity during fMRI. Analysis using visuospatial processing during fMRI may further maximise the brain activity related to frontoparietal networks and math skills.

In summary, pain/stress in those born very preterm has been found to relate to early neurodevelopment, as well as via brain to infant cognition, school-age visual perception and school-age perceptual reasoning. While research has not yet examined the relationship between pain/stress and math, in the context of visuospatial processing in children born very preterm the
relationship between math and visuospatial skills in both those born very preterm and the general population has been examined; the following section details the motivation for the consideration of these related domains in this study.

1.3 Math skills & visuospatial processing in children born very preterm

Poorer academic performance compared to children born full-term is particularly evident in math skills (McBryde et al., 2020; Simms et al., 2013; Twilhaar et al., 2018). The gap in achievement compared to peers is both greater in math than reading skills (Grunau et al., 2004; McBryde et al., 2020) and more significant to adult wealth than reading skills or general IQ (Basten, Jaekel, Johnson, Gilmore, & Wolke, 2015).

The learning and utilisation of math skills and concepts reflects both a core academic outcome and a broader cognitive skill. As an academic outcome, math performance as gauged with school assessments may also be related to general academic performance and classroom behaviour of the individual. As a cognitive skill, the ability to conceptualise and manipulate quantitative relationships may be reflected beyond skills-based norm testing in schools. To assess the gap in achievement for children born very preterm compared to their term-born peers, both McBryde et al. (2020) and Simms et al. (2013) highlight the need to consider math as composed of skills and subskills, using ideas like general and domain specific skills that relate to math, or lower order and higher order math skills. In this way, studies may identify underlying processing that drives the underperformance seen in children born very preterm.

One such possible related area to math skills is visuospatial processing. Visuospatial processing is the ability to gauge or represent where objects are located in physical space, including distal objects as well as your own body parts. Visuospatial processing has been linked
to math skills in normative populations (Hawes & Ansari, 2020), and some studies have supported this link in children born very preterm (Simms et al., 2015; van Veen et al., 2019). Specifically, Simms et al. (2015) found that among 8–10-year-old children born very preterm, math difficulties were associated with lower scores in visuospatial processing, while van Veen et al. (2019) found that the significantly lower, compared to their term-born peers, mathematics scores for 5-year-old children born below 30 weeks GA were significantly mediated by their visuospatial performance. If this relationship continues to be substantiated, it suggests that interventions that target visuospatial abilities may also benefit math skills (Simms et al., 2015).

Some evidence indicates that visuospatial processing has been found as a deficit even where other motor, visual, or cognitive domains have not, especially for otherwise healthy participants born very preterm (e.g. Berndt et al., 2019; Tokariev et al., 2019; van Veen et al., 2019). For instance, van Veen et al. (2019) found the difference in scores of 5-year-old children born below 30 weeks GA to term-born peers was unique to visuospatial processing, as they did not find a significant difference for children born very preterm in visual-motor integration or motor coordination. Tokariev et al. (2019) found that tests of visuospatial processing revealed a comparative deficit not seen in their tests of visual attention or visual memory in 7-year-old children born extremely preterm (<28 weeks GA), compared to term-born peers. Although in adults 27 years of age, Berndt et al. (2019) did similarly find that, compared to term-born peers, those born very preterm differed only in performance IQ and visuospatial processing, not visual attention or visual memory. Together, these findings motivate the consideration of visuospatial processing with a task that better isolates the visuospatial processing component over the visual, attentional, or memory components.
More strikingly, Berndt et al. (2019), Tokariev et al. (2019), and van Veen et al. (2019) all reported that their samples had either otherwise normal global cognitive ability, were without major disabilities, or, despite the difference apparent for children born either very or extremely preterm when compared with term-born controls, had visuospatial performance scores within the age-scaled normal range. Does this enable children born very preterm to fall through the cracks, where their relative underperformance to children born term is not addressed since they remain within the ‘normal’ range? When children born very preterm enter school alongside term-born peers, undiagnosed with neurological or cognitive deficits, by the time they receive classroom assistance for their math abilities, they have already fallen behind. Further, although their math performance will be evident from school-age, their visuospatial processing skills may never be assessed. Given that in normative populations visuospatial abilities predict entry into STEM disciplines (science, technology, engineering, and mathematics) as well as innovation in the workplace (Wai, Lubinski, & Benbow, 2009), this effect should not be underestimated. Analysis techniques that consider functional activity in the brain provide an ideal opportunity to consider these possibly subtle but significant functional relationships between math skills and visuospatial processing.

1.4 Brain structure & function in relation to math skills & visuospatial processing in children born very preterm

Mental rotation, a type of visuospatial processing, has received specific attention in relation to math skills in the context of both normative populations and those with math giftedness, particularly in fMRI task studies (e.g. Hawes et al., 2019; Hawes & Ansari, 2020; Hoppe et al., 2012; O’Boyle et al., 2005; Prescott et al., 2010). Mental rotation is the ability to
rotate mental representations of objects. These may be puzzle-solving or comparison tasks and may involve two-dimensional or complex three-dimensional shapes. The intraparietal sulcus is the most consistently activated brain region associated with mental rotation, with the right intraparietal sulcus the largest region of activation, and more broadly meta-analysis studies have found neural activation across bilateral parietal and frontal regions (Hawes et al., 2019; Zacks, 2008). In fact, activation in regions in and around the intraparietal sulcus, such as the inferior and superior parietal lobes, are common to studies of both mental rotation and math skills (Hawes et al., 2019); shared neural correlates is a prominent line of evidence considered when discussing the connection between visuospatial processing and mathematics (Hawes & Ansari, 2020).

Broadly, group comparison studies of those born very preterm compared to their term-born peers find structural and morphological changes in the brain that include, although often extend beyond, frontoparietal regions (e.g. Bjuland, Løhaugen, Martinussen, & Skranes, 2013; Frye, Malmberg, Swank, Smith, & Landry, 2010; Nosarti et al., 2008, 2014). Briefly, for instance, smaller white matter volumes in parietal and frontal regions have been found for those born preterm compared to those born at term, and the smaller volumes were further related to lower gestational age and lower full-scale IQ score respectively, within those born preterm (Nosarti et al., 2014). Beyond implicated regions structurally, mental rotation has not yet been considered during fMRI for children born very preterm.

Although mental rotation has not yet been considered during fMRI for those born very preterm, studies have utilised a visuospatial working memory task (Tokariev et al., 2019) and non-task resting state fMRI (Bäuml et al., 2017). In a visuospatial working memory task during fMRI, 8-year-old children born extremely preterm (<28 weeks GA) found activation largely in prefrontal areas, as did term-born controls, but compared to term-born controls, children born
extremely preterm exhibited weaker prefrontal activation, which was related to poorer task performance. Moreover, poorer visuospatial test scores outside the scanner for children born extremely preterm were associated with higher fractional anisotropy for the posterior cerebellum (Tokariev et al., 2019). The 8-year childhood math abilities of those born preterm have also been related to later altered frontoparietal intrinsic functional connectivity of the adult brain (at 26 years) during resting state fMRI (Bäuml et al., 2017). While working memory, particularly visuospatial working memory, is also considered of interest with respect to academic difficulties in children born very preterm (e.g. Twilhaar et al., 2020), it is unclear how visuospatial processing during fMRI, separate to working memory, relates to deficits in math; compared to resting state fMRI, task-based fMRI affords the opportunity to consider specific activations related to relevant tasks of interest that inform on specific processing types’ relationships with math.

Similar to visuospatial skills supporting early math acquisition, foundational or lower level math skills or subskills of number processing may then be related to higher level math acquisition such as arithmetic calculation (Hawes & Ansari, 2020). Such areas include nonsymbolic and symbolic magnitude comparison, and intentional and automatic number processing. For 6–7-year-olds born very preterm (<32 weeks GA), greater activation in frontoparietal regions, including bilateral parietal regions, during intentional and automatic number magnitude processing, was related to lower math proficiency (Klein et al., 2014). Moreover, gestational age modulated the activations during intentional number processing, but not during automatic number processing (Klein et al., 2018). Although in the older population of young adults born preterm (20 years old), there is further evidence of increased activations in the right inferior frontal and parietal regions, compared to term born young adults, during a
nonsymbolic magnitude comparison task (Clark et al., 2017). These increased activations during
task in preterm-born young adults were then associated with poorer calculation task performance
outside the scanner (Clark et al., 2017). However, all three of these studies had only a small
sample size (ranging from N=15–20), and so larger studies remain necessary. Further, while
some attempts are made to incorporate early-life factors like gestational age, there remains the
opportunity to explore the relationship between neonatal factors like pain/stress and later
functional processing within those born very preterm.

Given a sparsity of studies looking specifically at fMRI with math skills in those born very
preterm, aspects of brain structure from magnetic resonance imaging (MRI) related to math skills
remain relevant. For instance, for 6–7-year-olds born very preterm (<32 weeks GA), as semantic
number knowledge increased, there was greater grey matter and decreased white matter in
regions including the right anterior inferior parietal cortex (Starke et al., 2013), which is also a
shared region of importance for studies in the general population looking at either math or
visuospatial processing during fMRI (Hawes et al., 2019). Ullman et al. (2015) also found, for
children born very preterm (<30 weeks GA), greater volume in regions around the insula and
putamen in neonatal MRI were associated with math performance at 5 and 7 years of age; though
Hawes et al. (2019) did not find that the insula provided significant activation in their meta-
analysis of mental rotation during fMRI in the general population, they did find significant
activation for the insula for symbolic number processing and mental arithmetic. It remains
unclear, then, if these areas are key also to the relationship between math skills and visuospatial
processing in children born very preterm.

Similarly, studies considering aspects of brain structure from MRI for those born preterm
as relates to visuospatial ability also inform the study of visuospatial processing in fMRI.
Although in the mature brain, for adults born very preterm, at roughly 26 years of age, Berndt et al. (2019) found impaired dorsal attention network to pulvinar (of the thalamus) connectivity partly mediated the effect of being born preterm on the poorer visuospatial ability seen when compared to those born full-term. As mentioned in previous sections, thalamocortical connectivity is a clear target for structural connectivity research given their ongoing subplate organisation during the critical pre-32-week GA period (Vanhatalo & Kaila, 2010), and the dorsal attention network overlaps posterior parietal regions as well as the intraparietal sulcus. It remains unclear, however, what relationship aspects of being born very preterm have with this finding, such as pain/stress.

In these aforementioned fMRI and MRI studies of those born very preterm, while the ratio of male to female child sex has been reported, group differences in terms of child sex have not been considered as a variable of interest (Clark et al., 2017; Starke et al., 2013; Tokariev et al., 2019; Ullman et al., 2015). Where sex was controlled for in the analyses for the outcome of interest, a sex-effect was not separately reported (Bäuml et al., 2017; Berndt et al., 2019), and in some cases the sample size was too small and unbalanced to consider a sex effect (Klein et al., 2018, 2014). Within the general population, sex differences do not regularly occur on measures of numerical reasoning (Hawes & Ansari, 2020), but this should be confirmed for those born very preterm. Briefly, while sex differences have been reported in visuospatial processing in the general population, with males tending to outperform females specifically for mental rotation (Hawes & Ansari, 2020; Hoppe et al., 2012), there has also been some evidence that effect size diminishes to non-significant below the age of about 10 years (Hawes & Ansari, 2020; Titze, Jansen, & Heil, 2010).
In sum, complex neural systems are utilised for developing math skills and completing math tasks, and these neural systems are likely to be related to subskill domains including visuospatial processing for those born very preterm. Mental rotation is a type of visuospatial processing that has been used during fMRI to consider math skills in non-preterm born populations. While frontoparietal regions are likely to be of interest, disrupted neural activity allows for a functionally distributed impact to brain networks, and no specific studies have considered mental rotation during fMRI in children born very preterm in order to inform definite regions of interest (ROI). Moreover, where sufficiently powered, the extent of distributed network impacts are more likely to be revealed in brain-wide analyses. It is my hypothesis that neonatal factors including pain/stress and also math skills will be related to visuospatial processing during fMRI. These relationships in turn have the potential to inform the understanding of the neural correlates during visuospatial processing underlying poor math ability in very preterm born individuals.

1.5 Research aims

The current research aims to add to the above literature by examining the relationship between pain/stress and math skills for 8-year-olds born very preterm, as well as how each relates to functional network activation during visuospatial processing. Pain/stress is known to be closely related to other neonatal factors such as gestational age. Therefore, studies of pain/stress incorporate such factors into their statistical models (Doesburg et al., 2013; Duerden et al., 2018; Ranger et al., 2013, 2015; Schneider et al., 2018). Careful model building to include clinical factors is essential to address confounding by indication.

I aim to investigate in children born very preterm whether:
1. Neonatal pain-related stress and clinical factors are related to math skills at age 8 years, and whether: a) maternal education, b) white matter injury, and c) child sex, contribute to the relationship. I hypothesise that neonatal pain-related stress and clinical factors will be related to math skills.

2. Neonatal pain-related stress and clinical factors are related to functional network activation during visuospatial processing. I hypothesise that neonatal pain-related stress and clinical factors will be related to functional network activation during visuospatial processing.

3. Functional network activation during visuospatial processing is related to math skills. I hypothesise that math skills will be related to functional network activation during visuospatial processing.
2. Methods

2.1 Participants

As part of an ongoing prospective longitudinal cohort study, 126 children born very preterm (24–32 weeks gestation) recruited after admission to the level III neonatal intensive care unit (NICU) of BC Women’s Hospital, returned for 8-year follow-up between September 2014 and July 2018. As shown in the participant flow chart (see Figure 1), children were excluded from this study for the following reasons: sensori-neural hearing impairment, visual impairment (legally blind), non-ambulatory cerebral palsy (CP), cognitive impairment (IQ<70), and/or severe brain injury evident on neonatal ultrasound (cystic periventricular leukomalacia [PVL] and/or either Grade 3 intraventricular haemorrhage [IVH] or periventricular-intraventricular haemorrhages [PVIH]). In total, N=75 participants who met criteria for inclusion completed neuropsychological assessment and a mental rotation task during fMRI.
Figure 1. Flow diagram of participants, showing the number of participants included in each of the aims for the study. a Kaufmann Test for Educational Achievement, 3rd Edition (KTEA-3)
2.2 Procedures

2.2.1 Neonatal factors

Prospective daily clinical chart review was conducted by experienced neonatal research nurses across the NICU stay up to discharge or term equivalent age, whichever came first. From the clinical data, variables included for the present study were gestational age (GA) at birth, illness severity on day 1 (Score for Neonatal Acute Physiology [SNAP-II]; Richardson et al., 2001), postnatal infection (confirmed by positive culture), days of mechanical ventilation, cumulative morphine (mg/kg), and neonatal pain/stress exposure. For the cumulative morphine measure, the average daily intravenous and converted oral dose, adjusted for body weight, multiplied by number of days administered was used. Morphine is administered as an analgesic agent to suppress neuronal activation and so reduce extracellular concentrations of excitatory neurotransmitters (Grunau, Holsti, & Peters, 2006). Neonatal pain/stress was quantified as the number of invasive procedures, e.g. heel lance, peripheral intravenous or central line insertion, chest-tube insertion, tape removal; each attempt at the procedure was included. SNAP-II is a score for neonatal acute physiology, a scoring system that quantifies the initial risk within the first 12 hours of admission of the neonate to the NICU, roughly equivalent to the first day of life for a very preterm baby admitted after delivery. The higher the score, the higher the risk for adverse outcome. Mechanical ventilation provides lifesaving respiratory support; for this study, I used days on mechanical ventilation.

2.2.2 Maternal education

As an index of socioeconomic status (SES), mother’s years and level of education were collected from parent questionnaire. Education is a supported proxy measure for SES (e.g. Cirino et al., 2002). Parent education, especially maternal education, is a known predictor for childhood
neurodevelopmental outcomes (e.g. Noble et al., 2015), and has been used previously in studies of brain development with neurodevelopmental outcomes at earlier ages (e.g. Benavente-Fernández et al., 2019; Miller et al., 2022).

### 2.2.3 White matter injury

Neonatal white matter injury (WMI) volume and location were calculated as described previously (Cayam-Rand et al., 2019; Guo et al., 2017), with detailed imaging methods also described previously (Chau et al., 2012, 2009). In brief, newborns underwent specialised neonatal MRI, without pharmacologic sedation, within weeks of birth. Manual WMI segmentation was performed and then reviewed by experienced neonatal neurologists, from which total WMI volume was calculated. The location of white matter injury, if present, was also characterised as either anterior or posterior to the midventricle line. This yielded two distinct measures to consider severity of neonatal white matter injury, as either volume or location of injury.

### 2.2.4 Neuropsychological and math skills measures at 8-year follow-up

Assessment was performed by highly experienced psychology staff in the Neonatal Follow-Up Program at BC Women’s Hospital. Cognitive ability was assessed using the Wechsler Abbreviated Scale of Intelligence (WASI-II; Vocabulary and Matrix Reasoning subtests yielding Full Scale IQ [FSIQ]; Wechsler, 2011). Mathematics skills were assessed using the three math subtests of the Kaufman Test for Educational Achievement, 3rd Edition (KTEA-3; Kaufman et al., 2014): Math Computation, Math Fluency, and Math Concepts and Applications. Math Computation assessed the child’s skill in calculation problems. Math Fluency taps into speed of processing, answering as many mixed operation problems as possible in 60 seconds. Math Concepts and Applications requires the application of math principles to concepts.
like time and money. Higher scores indicate better math skills, and each is a standard score with a mean of 100 and standard deviation of 15.

2.2.5 Mental rotation of hands task

Before scanning, each child was familiarised with the task and acclimatised to the noise and feeling of undergoing MRI in an MRI simulator. The mental rotation task (based on Kosslyn et al., 1998) was presented in an alternating block design using MatLab Psychtoolbox with five baseline blocks and five activation blocks. Each block lasted 36s; the total duration of the task was 6 minutes. The baseline condition presented scrambled images with response required when a tone was presented. For the active task condition, participants were presented with an image of a pair of hands (Figure 2). If the participant determined that the hands ‘matched’ (i.e. were both right or left hands) but were rotated, then a ‘matching’ response was recorded via button-press. Mirrored, non-matching hands required no response. Only the volumes of the fMRI scan corresponding to when the participant performed the active condition of the task, not during the baseline condition, were used in analyses. Task difficulty was adjusted for optimised engagement in the task for each child; task performance was not an outcome of interest in this study.

Figure 2. Stimuli for the active condition in the mental rotation of hands task, based on Kosslyn et al., 1998.
2.2.6 MR Image Acquisition

MRI data were acquired on a 3T 32 channel head coil GE Discovery MR750 scanner with TR=3s, field of view 96x96x52, TE=0.03s, flip angle=90 degrees, 3x3x3mm voxels, resulting in 120 volumes of blood oxygenation level dependent (BOLD) fMRI data for each subject. The same scanner was used in the same imaging session to acquire a high-resolution anatomical (T1w structural) image for each subject with TR=0.008s, TE=0.003s, flip angle=12 degrees, slice thickness=0.9mm. A semi-automatic segmentation protocol based on the MAGeT (Multiple Automatically Generated Templates) Brain algorithm was used to remove non-brain tissue from the structural image (e.g. used in Chakravarty et al., 2013; Guo et al., 2015).

2.2.7 MR Image Processing

Functional MRI data were preprocessed using FSL 6.0 (FMRIB’s Software Library, Oxford, UK), including FSL’s FEAT (FMRI Expert Analysis Tool; Woolrich et al., 2001) and ICA-AROMA (ICA-based Automatic Removal of Motion Artifacts; see Pruim, Mennes, Buitelaar, et al., 2015; Pruim, Mennes, van Rooij, et al., 2015). Where necessary, AFNI (Analysis of Functional NeuroImages; Cox, 1997; Cox, 1996) 3dDespike was also used. During FEAT preprocessing, I applied MCFLIRT motion correction, slice-timing correction, non-brain tissue removal using BET (Brain Extraction Tool), spatial smoothing with a Gaussian kernel of 5mm full-width half-maximum, intensity normalisation, and boundary-based registration to that participants’ structural T1w image as well as registration to the standard MNI (Montreal Neurological Institute) template (nonlinear registration with warp resolution 10mm). Finally, after I applied ICA-AROMA, I performed high-pass temporal filtering and registration to the 8-year NMD template (Neurodevelopmental MRI Database; Richards et al., 2016). I used MRIQC (version 0.14.2; Esteban et al., 2017) to assess the signal-to-noise ratio and evaluate movement-
related denoising; ultimately, 7 participants exceeded the post-preprocessing mean DVARS (a measure for the root mean square variance over voxels between successive images) threshold of 13 and their data were not included in the fMRI analyses.

2.3 Data Analysis

I undertook statistical analyses using R (version 4.0.3; R Core Team, 2021). To examine relationships between math skills and participant demographics of maternal education and sex, I used analyses of variance (ANOVA), t-tests, and Pearson’s correlations. To examine study aims, I used the R package TExPosition (Beaton, Chin Fatt, & Abdi, 2014) to perform partial least squares correlation (PLSC) analyses (Krishnan, Williams, McIntosh, & Abdi, 2011; McIntosh & Lobaugh, 2004; McIntosh, Bookstein, Haxby, & Grady, 1996). In PLSC, data tables of X and Y are cross-correlated. A singular value decomposition (SVD) is then applied which results in latent variables: orthogonal dimensions that maximally explain the covariance between X and Y. Dimension significance was determined using permutation. Salience values reflect the inputs of X and Y, from which bootstrap ratios (BSR) are calculated (as salience/bootstrap error). BSR provides a measure of reliability of contribution to the model, which is proportional to a z-score. This analysis approach allows for brain-wide analyses for which correction of multiple comparisons is not required, due to the use of permutation in significance testing.

Three separate models were considered: 1) neonatal factors and math subtest scores, 2) active-task mental rotation fMRI with neonatal factors, and 3) active-task mental rotation fMRI with math subtest scores. For brain network analyses, each participant’s MRI data were entered across all voxels. For the first model, supplementary analyses were also performed to consider the contributions of maternal education, WMI, and sex. For supplementary analyses, additional
variable saliences for years of maternal education and WMI volume were calculated as projected onto the correlation sets for an existing latent variable. Using bootstrap resampling, mean centres with 95% confidence interval (CI) ellipses for group categorisations of participants were also determined for sex, level of maternal education and WMI location, in order to assess the presence of a distinguishable contribution to pairs of latent variables from the PLSC model for separate groupings.
3. Results

3.1 Demographics

Participant demographics are displayed in Table 1. Notably, maternal education of the children in this study has a relatively high mean of 16 years, which reflects post-high school studies. Considered as levels of ‘up to high school’, ‘partial or complete college or university’, and ‘post-graduate university’, 83.9% of all participants reported above high school level education. Children who did not complete the mental rotation fMRI had a lower gestational age (p<0.05), fewer days on ventilation (p<0.05), and scored lower in all three math subtests (Math Concepts & Applications p<0.001, Math Computation p<0.001, Math Fluency p<0.001) than those who did complete the mental rotation fMRI.
Table 1. Participant demographics, neonatal factors and 8-year math skills for all participants and participants included in fMRI analyses.

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<tr>
<th>Demographics</th>
<th>All participants, N=118</th>
<th>Participants with fMRI, N=75</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at follow-up (years)</td>
<td>8.4 0.4</td>
<td>8.4 0.4</td>
</tr>
<tr>
<td>Maternal education (years)</td>
<td>16.0 2.8</td>
<td>16.1 2.7</td>
</tr>
<tr>
<td>Up to high school graduation</td>
<td>16.1</td>
<td>12.0</td>
</tr>
<tr>
<td>Partial/complete university/college</td>
<td>64.4</td>
<td>65.3</td>
</tr>
<tr>
<td>Postgraduate university</td>
<td>19.5</td>
<td>22.7</td>
</tr>
<tr>
<td>IQ at follow-up (FSIQ)</td>
<td>102.4 14.8</td>
<td>104.0 14.3</td>
</tr>
<tr>
<td>Sex, male</td>
<td>53.4</td>
<td>54.7</td>
</tr>
<tr>
<td>Handedness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambidextrous</td>
<td>4.2</td>
<td>2.7</td>
</tr>
<tr>
<td>Left</td>
<td>16.1</td>
<td>12.0</td>
</tr>
<tr>
<td>Right</td>
<td>79.7</td>
<td>85.3</td>
</tr>
<tr>
<td>Neonatal Factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age at birth (weeks)</td>
<td>27.9  2.3</td>
<td>28.3 2.4</td>
</tr>
<tr>
<td>Illness severity day 1 (SNAP-II)</td>
<td>12.7  13.7</td>
<td>12.9 14.2</td>
</tr>
<tr>
<td>Mechanical ventilation (days)†</td>
<td>50.3  37.8</td>
<td>44.3 38.2</td>
</tr>
<tr>
<td>Morphine (cumulative mg/kg)††</td>
<td>5.3  11.9</td>
<td>4.8  11.4</td>
</tr>
<tr>
<td>Number of invasive procedures†</td>
<td>125.5 84.7</td>
<td>119.8 89.2</td>
</tr>
<tr>
<td>Presence of infection</td>
<td>- 46.6</td>
<td>- 41.3</td>
</tr>
<tr>
<td>White matter injury (volume, mm$^3$) †††</td>
<td>73.9 287.1</td>
<td>71.8 232.8</td>
</tr>
<tr>
<td>No white matter injury †††</td>
<td>- 65.3</td>
<td>- 62.7</td>
</tr>
<tr>
<td>Anterior ††</td>
<td>- 3.4</td>
<td>- 2.7</td>
</tr>
<tr>
<td>Posterior †††</td>
<td>- 26.3</td>
<td>- 33.3</td>
</tr>
<tr>
<td>IVH Grade †††</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No IVH †††</td>
<td>- 50.0</td>
<td>- 56.0</td>
</tr>
<tr>
<td>IVH Grade 1 †††</td>
<td>- 11.0</td>
<td>- 10.7</td>
</tr>
<tr>
<td>IVH Grade 2 †††</td>
<td>- 32.2</td>
<td>- 30.7</td>
</tr>
<tr>
<td>IVH Grade 3 †††</td>
<td>- 0</td>
<td>- 0</td>
</tr>
<tr>
<td>PVIH †††</td>
<td>- 1.7</td>
<td>- 1.3</td>
</tr>
<tr>
<td>Presence of cerebellar haemorrhage †††</td>
<td>- 19.5</td>
<td>- 18.7</td>
</tr>
<tr>
<td>Math skills (KTEA-3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Math Computation</td>
<td>89.8 12.4</td>
<td>93.6 10.8</td>
</tr>
<tr>
<td>Math Concepts &amp; Applications</td>
<td>91.6 14.0</td>
<td>95.0 13.2</td>
</tr>
<tr>
<td>Math Fluency</td>
<td>85.3 16.1</td>
<td>90.4 14.7</td>
</tr>
</tbody>
</table>
Note: SD: standard deviation. For full sample of all participants N=118 (except where noted: † n=117, †† n=116), for sample of participants with fMRI N=75 (except where noted: †† n=74). For one participant where WASI-II FSIQ was not available it was estimated from WISC-V (Wechsler Intelligence Scale for Children, Fifth Edition; Wechsler, 2014). ††† For measures taken at early neonatal MRI: for full sample of participants, n=112 (5.1% no scan); for sample of participants with fMRI, n=74 (1.3% no scan). Note that although participants were excluded for grade 3 IVH or PVIH from neonatal ultrasound, the IVH grading from early neonatal MRI differs such that some participants were graded with PVIH.
3.2 Neonatal clinical factors relate to math skills

Better math skill performance on each subtest at age 8 years was significantly correlated with lower exposure to neonatal pain/stress, morphine, fewer days on mechanical ventilation, and higher GA (Table 2). All of the neonatal factors were significantly correlated with each other (Table 3). The three math subtests also were substantially intercorrelated (Table 4). I used PLSC to examine the neonatal factors in relation to math skills.

**Table 2. Correlations between math skills and neonatal factors.**

<table>
<thead>
<tr>
<th></th>
<th>Math Computation</th>
<th>Math Concepts &amp; Applications</th>
<th>Math Fluency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at birth (weeks)</td>
<td>0.22*</td>
<td>0.20*</td>
<td>0.27**</td>
</tr>
<tr>
<td>Number of invasive procedures†</td>
<td>-0.23*</td>
<td>-0.22*</td>
<td>-0.25**</td>
</tr>
<tr>
<td>Cumulative morphine (log, mg/kg) ††</td>
<td>-0.25**</td>
<td>-0.21*</td>
<td>-0.24**</td>
</tr>
<tr>
<td>Mechanical ventilation (days) ††</td>
<td>-0.22*</td>
<td>-0.21*</td>
<td>-0.25**</td>
</tr>
<tr>
<td>Illness severity day 1 (SNAP-II)</td>
<td>-0.06</td>
<td>-0.09</td>
<td>-0.08</td>
</tr>
</tbody>
</table>

** ** <p .01, * <p .05, ^ <p .1; N=118, † n=117, †† n=116
Table 3. Inter-correlations among neonatal factors.

<table>
<thead>
<tr>
<th></th>
<th>Gestational age at birth (weeks)</th>
<th>Number of invasive procedures †</th>
<th>Cumulative morphine (log, mg/kg) ††</th>
<th>Mechanical ventilation (days) ††</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of invasive procedures †</td>
<td></td>
<td>-0.72****</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative morphine (log, mg/kg) ††</td>
<td></td>
<td>-0.61****</td>
<td>0.84****</td>
<td></td>
</tr>
<tr>
<td>Mechanical ventilation (days) ††</td>
<td></td>
<td>-0.86****</td>
<td>0.84****</td>
<td>0.73****</td>
</tr>
<tr>
<td>Illness severity day 1 (SNAP-II)</td>
<td>-0.34***</td>
<td>0.28**</td>
<td>0.25**</td>
<td>0.30**</td>
</tr>
</tbody>
</table>

**** p<.0001, *** p<.001, ** p<.01; N=118, † n=117, †† n=116

Table 4. Inter-correlations among math skills.

<table>
<thead>
<tr>
<th></th>
<th>Math Concepts &amp; Applications</th>
<th>Math Computation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Math Concepts &amp; Applications</td>
<td>0.80****</td>
<td></td>
</tr>
<tr>
<td>Math Fluency</td>
<td>0.81****</td>
<td>0.68****</td>
</tr>
</tbody>
</table>

**** p<.0001, N=118

One statistically significant dimension emerged from the PLSC analysis, and this first dimension accounted for 80% of the covariance among the neonatal factors and math skills (N=115, λ=0.64, τ=80%, p<.001). Scores for all math subtests decreased as neonatal factors of pain/stress and days of mechanical ventilation increased, as GA decreased, and also with presence of postnatal infection. Neither cumulative morphine exposure nor SNAP-II contributed significantly to the dimension (Figure 3).
3.2.1 Maternal education

There were no statistically significant relationships between years of maternal education and math skills ($r=0.17$ for Math Computation, $r=0.15$ for Math Concepts & Applications, $r=0.04$ for Math Fluency; for all $p>0.05$). Projected as supplementary to the PLSC relationship between mathematics skills and neonatal factors, years of maternal education did not contribute to latent variables (small saliences as seen suggest no significant contribution to the relationship) (Figure 4). Maternal education was then considered grouped into levels ‘up to high school’, ‘partial or complete college or university’, and ‘post-graduate university’. ANOVAs for level of maternal education with math skills were non-significant – $F(2,115)=1.5$, $p=0.2$ for Math Concepts & Applications; $F(2,115)=1.6$, $p=0.2$ for Math Fluency; $F(2,115)=2.8$, $p=0.06$ for Math Computation. The PLSC latent variable for the relationship between mathematics skills and neonatal factors did not differ by maternal education level: when maternal education level mean
centres were evaluated with 95% confidence ellipses determined with bootstrap resampling, the ellipse intervals were found to overlap, suggesting that there is no significant contribution of maternal education to this dimension (Figure 5).

**Figure 4.** Projected saliences of years of maternal education on PLSC of neonatal factors and math skills first dimension latent variables, N=115.
3.2.2 White matter injury

There were no statistically significant relationships between white matter injury volume and math skills. Table 1 includes details for white matter injury information for participants. The PLSC latent variable for the relationship between mathematics skills and neonatal factors did not differ by white matter injury location, and, projected as supplementary, white matter injury volume also did not contribute to the latent variables (Figure 6).
Figure 6. Neonatal factors with math skills PLSC first dimension latent variables and: a) white matter injury location mean centres with 95%CI ellipses (N=115, $\lambda=0.64$, $\tau=80\%$, $p<.001$), b) projected salience of white matter injury volume with other neonatal factors (n=109, $\lambda=0.80$, $\tau=89\%$, $p<.001$; reduced n due to 6 participants without early neonatal MRI scan for WMI).
3.2.3 Child sex

There was no sex difference in the relationship between neonatal pain/stress and math skills as revealed by the latent variables of PLSC, where 95% confidence intervals for the mean centres of male and female sex overlapped (Figure 7). However, specifically in the group that did complete mental rotation fMRI, males had greater pain/stress exposure (p<0.05) and greater morphine administration (p<0.05) than females. No other differences by sex were statistically significant for the group that completed the mental rotation fMRI, nor were there sex differences when considering all participants.

![PLSC: First Pair of Latent Variables, with 95% CI sex means](image)

**Figure 7.** Neonatal factors with math skills PLSC: mean centres for males (green) and females (purple) shown on the first dimension latent variables plot, with 95% confidence interval ellipses for those means.

3.3 Neonatal clinical factors relate to functional networks during visuospatial processing

The PLSC analysis conducted to examine the relationship between neonatal factors in relation to functional activation during visuospatial processing at age 8 years (N=73) yielded two statistically significant dimensions.
GA, mechanical ventilation, presence of infection, pain/stress, and SNAP-II in relation to task-active fMRI was captured in the first dimension ($\tau=65\%$, $\lambda=36.66$, $p<.001$; Figure 8). All neonatal factors considered were significant, except morphine exposure. As expected, GA was in the opposite direction to the clinical factors, reflecting birth at higher gestational age and lower exposure to adverse clinical factors.

**Figure 8.** Clusters of activation during visuospatial processing fMRI relating to neonatal factors, PLSC first dimension ($N=73$, $\lambda=36.66$, $\tau=65\%$, $p<.001$). Clusters have minimum size of 60 voxels and $|\text{BSR}|>4$; all network activation clusters load positively for this dimension. For neonatal factors, coloured (green) bars indicate $|\text{BSR}|>4$.

The first dimension explained 65% of the variance in the model. Higher pain/stress and more days of mechanical ventilation, lower GA, greater illness severity on day 1, and presence of infection were positively related to greater brain activity in a network captured predominantly by activation in the right hemisphere superior parietal cortex, right hemisphere lateral occipital, and left hemisphere inferior temporal. A summary of cluster size and location is provided in Table 5.
Table 5. Clusters of activation during visuospatial processing fMRI relating to neonatal factors, PLSC first dimension (N=73, \( \lambda=36.66, \tau=65\%, \ p<.001 \)).

<table>
<thead>
<tr>
<th>Brain regions</th>
<th>Cluster size (voxels)</th>
<th>Max BSR</th>
<th>Max BSR co-ordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td><strong>Right hemisphere</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior parietal</td>
<td>561</td>
<td>5.60</td>
<td>39</td>
</tr>
<tr>
<td>Superior parietal</td>
<td>351</td>
<td>6.72</td>
<td>51</td>
</tr>
<tr>
<td>Lateral occipital</td>
<td>272</td>
<td>5.28</td>
<td>27</td>
</tr>
<tr>
<td>Lateral occipital</td>
<td>61</td>
<td>4.42</td>
<td>41</td>
</tr>
<tr>
<td><strong>Left hemisphere</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inferior temporal</td>
<td>149</td>
<td>4.65</td>
<td>119</td>
</tr>
</tbody>
</table>

Clusters have minimum size of 60 voxels and |BSR|>4; all network activation clusters load positively for this dimension. Co-ordinates for maximum BSR location for the cluster correspond to 8-year NMD template space.

As the dimension that explains as much variance as possible orthogonal to the first dimension, the second and significant dimension explained 19\% of the variance in the model, and was driven only by SNAP-II, a measure of illness severity in the first day of life (\( \tau=19\%, \ \lambda=10.53, \ p<0.001; \) Figure 9). The network of functional activation that positively correlated with increasing SNAP-II was evident mainly in the cerebellum in both hemispheres, in the right hemisphere precuneus, and left hemisphere precentral gyrus and fusiform; see Table 6 for a full list of activation clusters for this network.
Figure 9. Clusters of activation during visuospatial processing fMRI relating to neonatal factors, PLSC second dimension (N=73, λ=10.53, τ=19%, p<.001). Clusters have minimum size of 60 voxels and |BSR|>4; all network activation clusters load positively for this dimension. For neonatal factors, coloured (green) bars indicate |BSR|>3.

Table 6. Clusters of activation during visuospatial processing fMRI relating to neonatal factors, PLSC second dimension (N=73, λ=10.53, τ=19%, p<.001).

<table>
<thead>
<tr>
<th>Brain regions</th>
<th>Cluster size (voxels)</th>
<th>Max BSR</th>
<th>Max BSR co-ordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>Right hemisphere</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precuneus</td>
<td>643</td>
<td>5.09</td>
<td>69</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>189</td>
<td>5.19</td>
<td>50</td>
</tr>
<tr>
<td>Left hemisphere</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>723</td>
<td>5.20</td>
<td>97</td>
</tr>
<tr>
<td>Precentral</td>
<td>171</td>
<td>5.48</td>
<td>112</td>
</tr>
<tr>
<td>Fusiform</td>
<td>72</td>
<td>4.59</td>
<td>111</td>
</tr>
</tbody>
</table>

Clusters have minimum size of 60 voxels and |BSR|>4; all network activation clusters load positively for this dimension. Co-ordinates for maximum BSR location for the cluster correspond to 8-year NMD template space.

3.4 Math skills relate to functional networks during visuospatial processing

The PLSC analysis conducted to examine the relationship between functional activation during visuospatial processing and the math skills subtests yielded one significant dimension that explained 93% of the variance in the model (N=75, λ=44.40, τ=93%, p<.001) (Figure 10). All
three of the math skill subtests contributed to this dimension, and in the same direction. Network activity during visuospatial processing that positively correlated with math skills included increased activation in the inferior parietal cortex, precuneus, and posterior cingulate cortex, all in the right hemisphere. Network activity which was negatively correlated with math skills—that is, where increased activation in these areas related to lower scores on the math skills subtests—included activation in the superior parietal, fusiform, and precentral gyrus of the right hemisphere, and in the lateral occipital cortices of both hemispheres. Table 7 shows all clusters and the direction of their relationship with math skills.

**Figure 10.** Clusters of activation during visuospatial processing fMRI relating to math skills, PLSC first dimension (N=75, λ=44.40, τ=93%, p<.001). Clusters have minimum size of 60 voxels and |BSR|>4. For math skills, coloured (red) bars indicate |BSR|>4.
Table 7. Clusters of activation during visuospatial processing fMRI relating to math skills, PLSC first dimension (N=75, λ=44.40, τ=93%, p<.001).

<table>
<thead>
<tr>
<th>Brain regions</th>
<th>Cluster size (voxels)</th>
<th>Max BSR</th>
<th>Max BSR co-ordinates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>x</td>
</tr>
<tr>
<td><strong>Positive loading</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Right hemisphere</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inferior parietal</td>
<td>810</td>
<td>5.98</td>
<td>29</td>
</tr>
<tr>
<td>Precuneus</td>
<td>555</td>
<td>5.03</td>
<td>65</td>
</tr>
<tr>
<td>Posterior cingulate</td>
<td>267</td>
<td>5.01</td>
<td>62</td>
</tr>
<tr>
<td>Precuneus</td>
<td>210</td>
<td>5.31</td>
<td>58</td>
</tr>
<tr>
<td>Lingual</td>
<td>146</td>
<td>5.34</td>
<td>59</td>
</tr>
<tr>
<td>Middle temporal</td>
<td>112</td>
<td>5.56</td>
<td>16</td>
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<tr>
<td>Supramarginal</td>
<td>76</td>
<td>4.91</td>
<td>18</td>
</tr>
<tr>
<td>Precuneus</td>
<td>70</td>
<td>4.42</td>
<td>59</td>
</tr>
<tr>
<td><strong>Left hemisphere</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Precuneus</td>
<td>64</td>
<td>4.70</td>
<td>86</td>
</tr>
<tr>
<td><strong>Negative loading</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Right hemisphere</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior parietal</td>
<td>760</td>
<td>6.35</td>
<td>39</td>
</tr>
<tr>
<td>Superior parietal</td>
<td>489</td>
<td>6.60</td>
<td>48</td>
</tr>
<tr>
<td>Fusiform</td>
<td>475</td>
<td>6.21</td>
<td>31</td>
</tr>
<tr>
<td>Precentral</td>
<td>473</td>
<td>6.31</td>
<td>41</td>
</tr>
<tr>
<td>Lateral occipital</td>
<td>352</td>
<td>5.38</td>
<td>28</td>
</tr>
<tr>
<td>Superior parietal</td>
<td>209</td>
<td>5.43</td>
<td>46</td>
</tr>
<tr>
<td>Superior parietal</td>
<td>84</td>
<td>4.99</td>
<td>41</td>
</tr>
<tr>
<td><strong>Left hemisphere</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral occipital</td>
<td>209</td>
<td>5.16</td>
<td>113</td>
</tr>
</tbody>
</table>

Clusters have minimum size of 60 voxels and |BSR|>4. Co-ordinates for maximum BSR location for the cluster correspond to 8-year NMD template space.

The results revealed partial overlap in network activation clusters between the PLSC models. Therefore, I calculated the cluster overlap (Table 8, Figure 11). Specifically, where poorer math skills were related to greater activation in the right hemisphere superior parietal, right hemisphere fusiform and left hemisphere lateral occipital as part of a network of function
during visuospatial processing (seen as negatively loaded clusters in Table 7), greater pain/stress, lower gestational age, more days on mechanical ventilation, presence of postnatal infection, and higher illness severity on day 1 were also related to increased activation in the right hemisphere superior parietal, right hemisphere fusiform, and left hemisphere lateral occipital (seen for the first dimension of the neonatal factors and visuospatial processing PLSC as positively loaded clusters in Table 5). Similarly, where better math skills were related to greater activation in the right hemisphere precuneus as part of the network of function during visuospatial processing (seen as positively loaded clusters in Table 7), greater SNAP-II was also related to increased activation in the right hemisphere precuneus (seen for the second dimension of the neonatal factors and visuospatial processing PLSC as positively loaded clusters in Table 6). The percent of overlap for the clusters of each PLSC model are included to help inform the relative extent of overlap in each case, along with the original cluster size and overlap size in voxels (Table 8).
<table>
<thead>
<tr>
<th>Brain region</th>
<th>% overlap w/ original cluster</th>
<th>Original cluster size (voxels)</th>
<th>% overlap w/ original cluster</th>
<th>Original cluster size (voxels)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Right hemisphere</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior parietal</td>
<td>78%</td>
<td>561</td>
<td>Superior parietal</td>
<td>57%</td>
</tr>
<tr>
<td>Lateral occipital</td>
<td>83%</td>
<td>272</td>
<td>Fusiform</td>
<td>47%</td>
</tr>
<tr>
<td>Superior parietal</td>
<td>26%</td>
<td>351</td>
<td>Superior parietal</td>
<td>44%</td>
</tr>
<tr>
<td><strong>Left hemisphere</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inferior temporal</td>
<td>19%</td>
<td>149</td>
<td>Lateral occipital</td>
<td>13%</td>
</tr>
</tbody>
</table>

**Table 8.** Overlap of clusters with the brain region labels determined for the original clusters from each PLSC.
**Figure 11.** Cluster overlap between the first and second dimensions of the PLSC examining the relationship between neonatal factors and visuospatial processing, and the first dimension of the PLSC examining the relationship between math skills and visuospatial processing.
4. Discussion

In a prospective longitudinal cohort study of children born very preterm, this thesis utilised a multivariate statistical approach to examine relationships between neonatal factors, including neonatal pain/stress, math skills, and visuospatial processing during fMRI at 8 years. Better math skills were related to higher gestational age at birth, lower neonatal pain/stress, fewer days on mechanical ventilation, and no postnatal infection. I identified functional brain networks underlying visuospatial processing in very preterm children at school-age which were related to neonatal clinical factors and to math skills at age 8 years. Shared regions of activation were identified in the right hemisphere precuneus, superior parietal, lateral occipital, fusiform, and left hemisphere inferior temporal and lateral occipital. A brief summary of the regions and functional associations for those regions is given in Table 9.
Table 9. Summary of brain regions, their functional associations in literature and relationships found during mental rotation fMRI for those born very preterm.

<table>
<thead>
<tr>
<th>Region</th>
<th>Functional association in literature</th>
<th>Relationship found during mental rotation</th>
</tr>
</thead>
</table>
| Superior parietal | • Activation related to number manipulation (Dehaene, Piazza, Pinel, & Cohen, 2003)  
• Greater pain/stress related to reduced cortical thickness in VPT *(RH)* (Ranger et al., 2013)  
• Activation related to mental rotation (reference frames) *(RH)* (Hawes et al., 2019; Zacks, 2008) | • Greater activity related to greater pain/stress, SNAP-II, ventilation, lower GA, infection *(RH)*  
• Greater activity related to poorer math skills *(RH)* |
| Inferior parietal | • Activation related to mental rotation and arithmetic *(RH)* (Hawes et al., 2019)  
• Greater grey matter and decreased white matter related to better semantic number knowledge in PT *(RH)* (Starke et al., 2013)  
• Greater activation during non-symbolic comparison task in PT vs T *(RH)* (Clark et al., 2017) | • Greater activity related to better math skills *(RH)* |
| Inferior temporal | • Greater pain, morphine, ventilation, SNAP-II, lower GA, surgery related to decreased cortical thickness in VPT *(LH)* (Ranger et al., 2013)  
• Lower fractional anisotropy in VPT *(LH)* (Rogers, Lean, Wheelock, & Smyser, 2018; Rogers et al., 2016; Thompson et al., 2012)  
• Activation related to mental rotation (extent of rotation) *(LH)* (Zacks, 2008) | • Greater activity related to greater pain/stress, SNAP-II, ventilation, lower GA, infection *(LH)* |
<table>
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<tr>
<th>Region</th>
<th>Functional association in literature</th>
<th>Relationship found during mental rotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral occipital</td>
<td>• Activation related to visuospatial selective attention (stimulus anticipation) (Hahn, Ross, &amp; Stein, 2006)   • Activation related to change in viewpoint (RH) (Large, Aldcroft, &amp; Vilis, 2007)   • Activation related to mental rotation (LH) (Zacks, 2008)   • Increased connectivity to right frontoparietal network related to better math scores in PT (LH) (Bäuml et al., 2017)</td>
<td>• Greater activity related to poorer math skills   • Greater activity related to greater pain/stress, SNAP-II, ventilation, lower GA, infection (RH)</td>
</tr>
<tr>
<td>Precuneus</td>
<td>• Activation related to mental rotation (Zacks, 2008)   • Activation related to directing spatial and goal-directed attention (Cavanna &amp; Trimble, 2006; Hawes et al., 2019; Kawashima, Roland, &amp; O’Sullivan, 1995)   • Activation related to number manipulation (Dehaene et al., 2003)</td>
<td>• Greater activity related to better math skills   • Greater activity related to greater SNAP-II (RH)</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>• Activation related to mental rotation (Hoppe et al., 2012; O’Boyle et al., 2005; Prescott et al., 2010)   • Activation related to motor and cerebral function (Brisenden &amp; Somers, 2019; Buckner, 2013)   • Smaller volumes related to higher SNAP-II in VPT (Ranger et al., 2015)   • Higher fractional anisotropy related to poorer visuospatial scores in EPT (Tokariev et al., 2019)</td>
<td>• Greater activity related to greater SNAP-II</td>
</tr>
<tr>
<td></td>
<td>• Activation related to motor response inhibition in VPT (Lawrence et al., 2009)   • Activation related to visual paired associates task in VPT (Narberhaus et al., 2009)</td>
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<tr>
<td>Region</td>
<td>Functional association in literature</td>
<td>Relationship found during mental rotation</td>
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</tbody>
</table>
| Precentral       | • Activation related to mental rotation (Zacks, 2008)  
• Activation related to motor planning, simulation and execution (Michelon, Vettel, & Zacks, 2006; Zacks, 2008)  
• Activation related to calculation (Dehaene et al., 2003)                                                                                                                 | • Greater activity related to poorer math skills (RH)  
• Greater activity related to greater SNAP-II (LH)                                                                                                                                   |
| Fusiform         | • Activation related to shape discrimination (Hahn et al., 2006)  
• Activation related to visuospatial number processing (Clark et al., 2017; Menon, Boyett-Anderson, & Reiss, 2005)                                                                | • Greater activity related to poorer math skills (RH)  
• Greater activity related to greater SNAP-II (LH)                                                                                                                                       |
| Posterior cingulate | • Activation related to arithmetic processing (RH) (Hawes et al., 2019)                                                                                                                      | • Greater activity related to better math skills (RH)                                                                                                             |
| Middle temporal  | • Increased connectivity to left frontoparietal network related to better math scores in PT (RH) (Bäuml et al., 2017)                                                                       | • Greater activity related to better math skills (RH)                                                                                                             |
| Supramarginal    | • Activation related to arithmetic and symbolic number processing (Hawes et al., 2019)  
• Activation related to mental rotation (Hoppe et al., 2012)                                                                                                               | • Greater activity related to better math skills (RH)                                                                                                             |

Note: VPT: very preterm; PT: preterm; EPT: extremely preterm; T: term; RH: right hemisphere; LH: left hemisphere.
4.1 Neonatal pain/stress and clinical factors were related to math skills at age 8 years

First, I considered the relationship between neonatal factors and math skills. I found that lower gestational age, greater pain/stress exposure, more days on mechanical ventilation, and presence of postnatal infection were related to poorer math skills at age 8 years, while neither cumulative morphine exposure nor illness severity on day 1 were significant. The underachievement in math of children born very preterm, compared to their term-born peers, is well established (Aarnoudse-Moens et al., 2009; Grunau et al., 2004; McBryde et al., 2020; Simms et al., 2013; Taylor, 2010; Twilhaar et al., 2018). Relatively greater pain/stress exposure has previously been found to predict lower cognition in children born very preterm at 8 and 18 months corrected age (Grunau et al., 2009), and at school age (Kozhemiako et al., 2020; Ranger et al., 2015; Vinall et al., 2014) after accounting for clinical confounders. The present study extends the relationship of neonatal pain/stress to examine math skills and visuospatial processing during fMRI. Importantly, postnatal infection and time spent on a mechanical ventilator may act cumulatively with pain/stress to involve inflammatory factors that may impact brain development while neonates are in the NICU. Prior evidence of a gradient of increasing risk for mathematics deficits with decreasing gestational age is supported by this study (McBryde et al., 2020). The exact extent of how a neonate’s response to invasive procedure may be altered by postnatal infection, gestational age at birth, or time spent on mechanical ventilation is unknown. It is clear, though, that the relative immaturity of the neonate’s nociceptive system (Fitzgerald, 2005; Gursul et al., 2019; Hathway et al., 2021; Lagercrantz et al., 2010), further complicated by sensitisation (Holsti et al., 2005, 2006), leaves those born very preterm vulnerable to mechanisms of brain injury and brain dysmaturation that can impact neurodevelopment. These mechanisms include oxidative stress (Back et al., 2001, 2005; Back &
Miller, 2014; Buntinx, Gielen, et al., 2004; Buntinx, Moreels, et al., 2004; Fern & Möller, 2000) and excitotoxicity (Anand & Scalzo, 2000).

Moreover, maternal education, white matter injury, and child sex were not related significantly to either math skills directly or the relationship between neonatal factors and math skills. It is surprising to note that neither maternal education nor socioeconomic status are often reported in studies of math deficits in children born very preterm (Twilhaar et al., 2018), and because of this a comparison to literature is difficult, although more broadly maternal education has been a stronger indicator of verbal reasoning than nonverbal reasoning (Joseph, O’Shea, Allred, Heeren, & Kuban, 2018). To further clarify the role of SES beyond maternal education, a composite index that incorporates, for instance, parental income and parental occupation together with parental education (Sirin, 2005) may be desirable in the future. For white matter injury, 77 participants (64% of participants with available early neonatal MR scan data) had no white matter injury at all, and this skew toward a lack of white matter injury may have reduced the ability to detect an effect on math skills when injury is present. Math performance may also be more generally less related to white matter injury than other outcomes. Greater volume of white matter injury, widespread in the brain, has been found to predict poor motor function rather than cognitive or language function (Guo et al., 2017). Instead, adverse cognitive development has been found to be predicted by lesions located in the frontal lobe (Guo et al., 2017); frontal lobe lesions would be those located anteriorly, and, in this study, only 4 participants had anterior injury. However, in turn, this strengthens the conclusions of this study when considering otherwise healthy children born preterm who nonetheless show math underperformance. Despite sex differences often being reported in children born very preterm previously in neurodevelopmental and brain morphology (DiPietro & Voegtline, 2017; Grunau et al., 2004;
Månsson, Fellman, Stjernqvist, et al., 2015; Skiöld et al., 2014), sex differences in academic performance is not seen (Grunau et al., 2004; Twilhaar et al., 2018), and we did not find a difference in math skills between males and females in this study. Although not found to contribute to math skills in this study, the relationship especially of maternal education and white matter injury to IQ in children born very preterm (Benavente-Fernández et al., 2019; Miller et al., 2022) may motivate further studies to consider those aspects specifically in other cognitive domains than math skills.

4.2 Neonatal pain/stress and clinical factors were related to functional network activation during visuospatial processing

Next, I considered the relationship between neonatal factors and visuospatial processing during fMRI. My primary finding was that greater pain/stress exposure, more days on mechanical ventilation, greater illness severity on day 1, lower gestational age, and presence of postnatal infection were related to greater activity in the superior parietal and lateral occipital of the right hemisphere, and inferior temporal of the left hemisphere. This aligns with previous findings where pain/stress has been related to cortical thickness in the right hemisphere superior parietal and left hemisphere inferior temporal (Ranger et al., 2013). Specifically, greater pain/stress has been found to be related to reduced cortical thickness at age 7 years in children born very preterm in the right hemisphere superior parietal, after controlling for clinical confounders (Ranger et al., 2013). In the same study of 7 year old children born very preterm, multivariate analyses found that the left hemisphere inferior temporal, together with other cortical regions, was found to relate to a cumulative model of pain, morphine, surgery, ventilation, SNAP-II, and GA (Ranger et al., 2013). The left inferior temporal lobe in very
preterm infants has also been found to have lower fractional anisotropy, which relates to neural tract development (Rogers et al., 2018, 2016; Thompson et al., 2012). Fractional anisotropy is measured in white matter, where fMRI signal typically originates from cortical grey matter, making a direct comparison difficult; the nature of the relationship between cortical thickness and BOLD signal is also an ongoing area of research, where, for instance, the overall standard deviation of BOLD signal across the brain has been found to relate specifically with cortical thickness but not grey matter volume or cortical area (Pur, Eagleson, de Ribaupierre, Mella, & de Ribaupierre, 2019), and only certain regions have shown increased brain activation with increased cortical thickness in a response inhibition task during fMRI (Hegarty et al., 2012). Still, the importance of these regions, the superior parietal right hemisphere and inferior temporal left hemisphere, to neonatal factors is broadly supported.

Moreover, prior research has acknowledged the partial overlap among brain alterations attributed to repetitive pain exposure and prematurity, with complexity introduced when very preterm newborns undergo a variety of treatments and surgeries for a diversity of conditions (Boggini et al., 2021). However, morphine remained non-significant, which appears to add to growing evidence that morphine administration does little to alter the brain’s trajectory, where pre-emptive administration of morphine does not benefit adverse neonatal sequelae (Grunau et al., 2006). Responses to neonatal pain may vary based on illness severity (Ranger et al., 2007; van Dijk & Tibboel, 2012; Vinall & Grunau, 2014). SNAP-II on day 1 captures a number of variables that reflect the physiological condition of the neonate that may complicate neonate response to pain/stress at the outset of NICU stay. Post-natal infection has also been found to contribute to altered brain maturation previously (Back & Miller, 2014; Manon Ranger et al., 2015); infection causes widespread inflammation, and inflammation forms a crucial part of the
pain/stress response in infants born very preterm. Given that biological mechanisms exist whereby preoligodendrocytes and subplate neurons may be particularly vulnerable to the oxidative stress from invasive procedures (Back et al., 2001, 2005; Back & Miller, 2014; Buntinx, Gielen, et al., 2004; Buntinx, Moreels, et al., 2004; Fern & Möller, 2000) and excitotoxicity from early propensity for greater activation (Anand & Scalzo, 2000) and immature inhibitory circuits (Fitzgerald, 2005), these potential impacts on white matter integrity and early neural organisation can be widespread, reflected in a distributed network of function in the brain by school age for those born very preterm (Back & Miller, 2014). Thalamocortical connections are ongoing between 26 and 32 weeks GA (Vanhatalo & Kaila, 2010), and the thalamus projects to many cortical areas including the parietal cortex; infant pain processing moreover recruits regions of the brain including the parietal lobe (Goksan et al., 2015; Gursul et al., 2019). In a rodent model, repetitive neonatal pain accentuated neuronal excitation and cell death, with cell death in cortical and subcortical regions including the parietal cortex (Anand et al., 2007). Further, adult rats that were neonatally exposed to repetitive pain demonstrated decreased ability to acquire visual-spatial cues (Anand et al., 2007), offering causal experimental support in an animal model for the correlational link found here between pain/stress, the superior parietal cortex, and visuospatial processing in children born very preterm.

The right hemisphere superior parietal and left hemisphere inferior temporal cortex involvement in mental rotation is supported by meta-analyses of mental rotation neuroimaging studies (Hawes et al., 2019; Zacks, 2008). While the superior parietal cortex has been consistently observed as important in visuospatial image transformations like mental rotation and specifically in implementing maps of space or ‘reference frames’ (Hawes et al., 2019; Zacks, 2008), the left hemisphere inferior temporal cortex has also been found to consistently activate in
tasks contrasting extent of transformation or rotation required (Zacks, 2008). Additionally, the lateral occipital sulcus has previously been found to be involved in stimulus anticipation, responding along with other regions as a function of endogenous attentional resource allocation during a study of visuospatial selective attention (Hahn et al., 2006). For the lateral occipital complex including the fusiform gyrus, evidence supports greater sensitivity to variations in the visual form and changes in viewpoint for the right hemisphere than the left (Large et al., 2007). The use of reference frames, transformation, stimulus anticipation, attention, and changes in viewpoint can all be understood as part of the visuospatial processing required during a mental rotation of hands task. Beyond a general relationship between neonatal factors and global visuospatial processing, the results of this PLSC suggest that greater neonatal pain/stress, lower gestational age, more days on mechanical ventilation, presence of postnatal infection and greater illness severity on day 1 relate to activations that implicate those specific aspects of visuospatial processing.

My secondary finding from the PLSC considering neonatal factors and visuospatial processing was a second dimension driven only by severity of illness on day 1 (SNAP-II). Measures incorporated into SNAP-II include blood pressure, temperature, oxygenation, blood pH level, and seizures (Richardson et al., 2001). It is therefore possible that the second dimension driven by SNAP-II captures different aspects of this measure than the first dimension, to which SNAP-II also contributed; specifically, some amount of the variance that is not as correlated with the other neonatal factors of pain/stress, mechanical ventilation, gestational age, or infection.

This second dimension revealed that greater illness severity on day 1 was related to greater activity in the right hemisphere precuneus, bilateral cerebellum, and left hemisphere precentral
and fusiform gyrus during visuospatial processing. The precentral cortex, bilaterally, has been related to mental rotation in meta-analysis (Zacks, 2008), as has the precuneus, also bilaterally (Hawes et al., 2019). Mental rotation has also revealed the involvement of the cerebellum in other studies (Hoppe et al., 2012; O’Boyle et al., 2005; Prescott et al., 2010), while the fusiform gyrus is recognised for a more general role in shape and higher order object recognition, in particular in discriminating shape stimuli (Hahn et al., 2006). The cerebellum is implicated in both motor and cerebral function (Brissenden & Somers, 2019; Buckner, 2013), the precentral cortex has been associated with motor planning and execution, and with motor simulation strategies (Michelon et al., 2006; Zacks, 2008), and the precuneus with directing attention in space and planning or imagining goal-directed movements (Cavanna & Trimble, 2006; Hawes et al., 2019; Kawashima et al., 1995). Together, this implies a network that captures the imagined or real movement involved in visuospatial processing during a mental rotation of hands task; indeed, the solving of the mental rotation of hands task may employ a movement simulation strategy of the stimuli hands or even imagining moving one’s own hands as proxy, but this must be interpreted conservatively when the task also required a button-press response. Moreover, though, smaller cerebellar subregion volumes have been found to relate to higher SNAP-II, among other neonatal factors, for 8 year old children born very preterm (Ranger et al., 2015), higher fractional anisotropy for the posterior cerebellum has been associated with poorer visuospatial scores for 7–8 year old children born extremely preterm (Tokariev et al., 2019), although interpretation of the relationship between volume and fractional anisotropy to fMRI signal remains, as mentioned, not straightforward. Still, the importance of the cerebellum in particular in task performance for children born very preterm has been found in motor response inhibition task (Lawrence et al., 2009) and in a visual paired associates task (Narberhaus et al.,
2009). Given that the cerebellar peak growth stage in the last trimester of gestation and first year of life (Barnes-Davis, Williamson, Merhar, Holland, & Kadis, 2020; Volpe, 2009) is highly relevant to the possible perturbed brain development in children born very preterm, there remains the possibility that a motor simulation network during visuospatial processing relates to early neonatal experience captured in part by SNAP-II, separately from the network during visuospatial processing that was captured by neonatal factors in the first dimension.

**4.3 Math skills were related to functional network activation during visuospatial processing**

I next considered the relationship between math skills and visuospatial processing during fMRI. Better math skills were related to an extended network of both increased and decreased activations for visuospatial processing during fMRI. All three of the math skill domains examined loaded onto this same dimension; that is, this network of activation during visuospatial processing relates to the shared correlation between mathematical performance on calculations, speed of operations, and the utilisation of concepts like time and money. Regions of increased activation were in the right hemisphere inferior parietal, posterior cingulate, lingual, middle temporal, and supramarginal, as well as the bilateral precuneus. Regions of decreased activation were in the right hemisphere superior parietal, fusiform, precentral, and bilaterally in the lateral occipital cortices. This complex and extensive network encompasses both where better math skills are related to increased activation, and where poorer math skills are related to increased activation, depending on the part of the network involved; the increased and decreased activations are also correlated with each other. Both directions of relationship can be found in the literature. Heightened activation of the anterior cingulate during mental rotation (O’Boyle et al., 2005) and, separately, increased activation in the left superior parietal lobule and right
postcentral gyrus during mental rotation (Hoppe et al., 2012) have been found for mathematically gifted subjects when compared to those with average math ability. Yet for children born very preterm, greater activation in frontoparietal regions during number processing tasks has been related to lower math proficiency (Klein et al., 2014), and similarly for young adults born preterm increased activation in the right inferior frontal and parietal regions during nonsymbolic magnitude comparison were associated with poorer calculation task performance (Clark et al., 2017). As it can be difficult to interpret increases or decreases in fMRI without relative reference, the strength of this study is that the network of activation during visuospatial processing reported here is maximally related to math skills, and so relative increase and decrease can be understood in the context of better or worse math performance.

The regions identified in the network during visuospatial processing that relates to better math skills are well supported by the notion of shared neural correlates between math skills and visuospatial processing (Hawes & Ansari, 2020), with reported regions involved in both math and visuospatial processing. The right hemisphere inferior parietal and bilateral precuneus are shared neural correlates for neuroimaging in both mental rotation and arithmetic (Hawes et al., 2019). The fusiform gyrus, along with the intraparietal sulcus, is considered an anchoring system for visuospatial number processing (Clark et al., 2017; Menon et al., 2005). As mentioned earlier, the right hemisphere superior parietal is implicated in mapping space and reference frames, and, alongside the right hemisphere precentral and left hemisphere lateral occipital, is supported in meta-analyses of mental rotation (Hawes et al., 2019; Zacks, 2008). Further, though, the superior parietal lobule, often together with the precuneus, is also found to activate in number manipulation tasks (Dehaene et al., 2003), and the precentral cortex similarly has been found to activate during calculation (Dehaene et al., 2003). Meanwhile, the supramarginal gyrus
is more commonly related in meta-analysis to both arithmetic and symbolic number processing rather than mental rotation (Hawes et al., 2019), although has elsewhere been found to relate to mental rotation task performance (Hoppe et al., 2012). The right hemisphere cingulate has been found in meta-analysis to relate specifically to arithmetic processing (Hawes et al., 2019). Given that this analysis calculates specifically the maximal covariance between math skills and brain activity during mental rotation, the predominant significance of regions that share a relationship with both math skills and visuospatial processing is unsurprising, such that some regions also may not be typically found for meta-analysis of mental rotation activation.

Meta-analyses of mental rotation and number processing during fMRI have not focused on those born very preterm (e.g. Hawes et al., 2019; Zacks, 2008). However, the right inferior parietal cortex, right middle temporal cortex, and left lateral occipital cortex have been previously identified as significant in studies of those born preterm or very preterm. In 6–7 year old children born preterm, a study of structural MRI identified the right inferior parietal cortex was related to mathematics performance: greater grey matter and decreased white matter in this region was related to increased semantic number knowledge (Starke et al., 2013). Also, group comparisons of adults born preterm to those born term indicated higher levels of activity in the inferior parietal lobule during non-symbolic comparison tasks (Clark et al., 2017). Math scores in childhood for those born preterm were also positively associated in adulthood with both the increased intra-network connectivity between a left frontoparietal network and the right middle temporal cortex, and the increased intra-network connectivity between a right frontoparietal network and the left lateral occipital cortex (Bäuml et al., 2017).

The exact nature of the neural overlap between number processing, arithmetic, and mental rotation is unknown, but several theories have been suggested, often centring on the shared
involvement of the parietal cortex during both number and visuospatial processing (Dehaene et al., 2003; Hawes & Ansari, 2020). For instance, math may be foundationally related to perceiving numbers as kinds of objects manipulated in abstract space, or both mental rotation and number processing may rely on the same underlying spatial and sensorimotor system (Hawes et al., 2019; Walsh, 2003). In the context of children born very preterm, the relationship between visuospatial processing and math skills is supported in the present study, and further informs the discussion about the importance of visuospatial testing and possibly for the future development of interventions for this vulnerable population (Simms et al., 2013). Overall, it is surprising that neither the relationship between neonatal factors and mental rotation during fMRI nor the relationship between math skills and mental rotation during fMRI revealed significant involvement of frontal brain regions. Meta-analyses indicate the significance of various bilateral frontal activations during mental rotation (Hawes et al., 2019; Zacks, 2008), and various frontal regions have been found to be involved in number processing and comparison tasks for children born very preterm and adults born preterm, and further were related to gestational age and calculation ability, respectively (Clark et al., 2017; Klein et al., 2018). This may suggest that the relationships between neonatal factors and visuospatial processing, and math skills and visuospatial processing, are less related to effortful attention and working memory, and are instead more related to the visual and spatial imagining, manipulation, and transformation of objects in the mind. Alternatively, this may in part be a feature of the study’s design, where the effect of mental rotation performance was minimised by adjusting the difficulty of the task for the participant where necessary.

The nature of the relationship between neonatal factors, visuospatial processing, and math skills is further informed by the common regions between functional networks during mental
rotation that related to neonatal factors, and the functional network that related to math skills. Indeed, the overlap suggests a functional connection between neonatal factors and math skills via brain. Specifically, greater pain/stress, lower gestational age, more days on mechanical ventilation, greater illness severity on day 1 and presence of infection relate to greater activation of a network that includes the right hemisphere superior parietal and lateral occipital and left hemisphere inferior temporal, while in turn a network that included greater activation in the right hemisphere superior parietal and fusiform and left hemisphere lateral occipital relate to poorer performance on math subtests. While the right hemisphere superior parietal is greatly implicated in both visuospatial processing and math skills, the additional regions in the fusiform/lateral occipital and inferior temporal/lateral occipital, discussed previously, suggest that the processing which underlies visual integration of information into imagined manipulation of an object is most affected. Importantly, the networks in each case expanded to regions beyond the specific overlap found.

Further, from the second dimension of the relationship between neonatal factors and visuospatial processing, greater illness severity related to increased activation in the right hemisphere precuneus, and in turn increased activation in the precuneus related to higher math scores—this may at first appear counterintuitive, but it is important to remember that this second dimension is orthogonal to the first, to which SNAP-II also contributed. That is, in the first dimension, greater SNAP-II, among other neonatal factors, was related to an increased activation in regions further related to poorer math skills; in the second dimension, higher SNAP-II alone was related to an increased activation in a region further related to better math skills. SNAP-II is a score that captures newborn illness severity by the degree of derangement from physiological norm in six items, including, as detailed earlier, blood pressure, temperature, oxygenation, blood
pH, and seizures (Richardson et al., 2001). Thus, the physiological states of illness severity that this score reflects may in turn impact treatment courses provided by physicians; that course of treatment, not examined in this study, may benefit visuospatial processing networks and thus math skills by school-age. This suggestion of a connection to math skills via visuospatial processing is in the right hemisphere precuneus, and the precuneus, as mentioned previously in the discussion, is associated with both mental rotation and arithmetic (Hawes et al., 2019), and moreover with visuospatial imagery, directing of attention, and imagining goal-directed movements (Cavanna & Trimble, 2006; Hawes et al., 2019; Kawashima et al., 1995). Although significant contribution for the precuneus to mental rotation is true bilaterally, the largest area of activation associated with mental rotation found in meta-analysis was the right precuneus (Hawes et al., 2019). This review did not offer commentary on why the right precuneus might contribute more than the left, although the right hemisphere’s importance more broadly in the posterior parietal cortex has also been recognised in a review of the functional anatomy of the precuneus (Cavanna & Trimble, 2006). At least in one study of adolescents, those born very preterm had greater volume of grey matter when compared to those born term in the right—but not left—precuneus, among other regions (Nosarti et al., 2008). This, together with the findings of the current study, suggests that while the precuneus bilaterally (together with other regions) is significant to the relationship between math skills and visuospatial processing for those born very preterm, the role of the right precuneus may be further complicated by preterm neonatal factors, despite the difficulty of interpretation for the SNAP-II driven dimension.
4.4 Limitations and strengths

This thesis is not without limitations. Unfortunately, 43 participants (34%) who were eligible after exclusions refused or were otherwise unable to complete the mental rotation task in the MR scanner. Since this study was designed to consider a task in an area of deficit for children born very preterm, the perceived difficulty of the task may further have been exacerbated by the intimidating nature of an MR scan. Given the relationship between visuospatial processing and math skills, it was not surprising that this resulted in group differences in math skills between those who did and did not complete the mental rotation task, and as a result this study did not capture the lowest performers in either math skills or visuospatial processing. Careful consideration should be made in designing future studies when assessing specifically areas of detriment in an MR task in a vulnerable population such as children born very preterm in order to successfully capture the widest variance possible.

A well-known limitation of functional neuroimaging itself is that the activation of certain brain regions under a given condition does not translate directly to treatment targets. Given, for instance, the possibility that neonatal pain/stress disrupts widespread neural organisation via excitotoxicity, the BOLD signal PLSC analyses will reveal only where the effects are most significant functionally. The activation seen may even be the result of compensation in one area due to the direct impact of neonatal pain/stress to another area entirely. Thus, treatment that aims at suppressing activation related to, for instance, lower math scores, could make the scores worse. While direct brain region treatment targets may not be determinable by this study, this research reveals a meaningful connection between visuospatial processing, math skills, and neonatal factors related to prematurity, which supports the exploration of further interventions.
and in particular the usefulness of earlier evaluative testing for both math skills and visuospatial processing in otherwise healthy children born very preterm.

Further, without a normative control group, it is not possible at this time to conclude whether the associations with math performance seen here are typical or if some activations are the result of an abnormal compensatory mechanism. In the examination of neonatal factors, however, a true control group is not possible since healthy full-term infants are in-utero to term, and not in the NICU during the specific vulnerable developmental period that those born very preterm are. Therefore, due to the timing and quality of variables considered, the relationship examined between neonatal factors and visuospatial processing is unique to children born very preterm. The suggestion of a relationship between clinical neonatal factors and school-age math skills via visuospatial processing from this study does not require control comparisons.
5. Conclusion

This thesis aimed to examine the relationship between neonatal factors and math skills, and further to identify functional brain networks underlying visuospatial processing in very preterm children at school-age as related to, first, neonatal factors including pain/stress, and, second, to math skills. Using multivariate analyses, common regions of activation were found in the right hemisphere precuneus, superior parietal, lateral occipital, fusiform, and left hemisphere inferior temporal and lateral occipital, offering insight into the possibility that early neonatal experience relates to visuospatial processing, which in turn relates to math skill at 8 years.

These findings support the need for early intervention in general cognitive domains like visuospatial processing, in order to benefit future academic and career trajectories. Spatial visualisation skills appear to be highly subject to practice and training effects in children, with the possibility of benefit to both trained and untrained tasks (Hawes, Moss, Caswell, & Poliszczuk, 2015; Uttal et al., 2013), including to mathematics (Cheng & Mix, 2014). Although Hawes et al. (2015) failed to replicate the transfer of mental rotation training beyond spatial performance to math skills, children born very preterm present a specific population for whom visuospatial and math underperformance are both seen and have been found to be related—others have already suggested that interventions that target visuospatial abilities may benefit math skills in children born very preterm (Simms et al., 2013), and proposed the inclusion of spatial orientation tasks in the typical neuropsychological evaluation for children born preterm (Fernandez-Baizan, Alcántara-Canabal, Solis, & Mendez, 2020), and my study further supports these assertions. Early interventions are often supported by early testing and detection of underperformance in the relevant skillset, highlighting the importance of follow-up and support for otherwise healthy children born very preterm, without major impairment.
Further research may also consider specific child sex differences that were not considered in this thesis. While sex differences were not found in math skills for this study, specific sex analyses may investigate the possibility of more subtle mediating relationships between sex, neonatal factors, and visuospatial processing.

Complex behaviours such as mental rotation require integrated function of multiple connected brain regions. Given that areas activated during mental rotation are unlikely to be involved in only visuospatial processing, this enabled the consideration of underlying relationships with another area, that of math skills, but the same principle could be used to relate visuospatial processing with other domains of cognition. In particular, global intelligence could be considered in future studies to determine whether the functional network during visuospatial processing that related to math skills remains unique to math skills or is also captured by IQ. Further characterising the nature of visuospatial processing in children born very preterm will only continue to inform interventions, in regard to cognition and academics, but as well in regard to understanding the impacts that their early life experiences may have on later brain and neurodevelopment.
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