SPATIAL AND TEMPORAL DETAILS OF SPONTANEOUS CORTICAL ACTIVITY PROVIDE INSIGHTS INTO FUNCTIONS IN THE ADULT AND DEVELOPING BRAIN

by

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ABSTRACT

While the body rests, the mind remains active. In fact, the brain exhibits a rich pattern of structured activity despite having few immediate sensory or motor tasks. During infancy, this brain activity appears tailored to assist in the maturation of neural systems. In the adult, it influences memory consolidation and maintenance of synaptic connections. In this thesis, I address these differences by using voltage-sensitive dye imaging to record spontaneous cortical activity in rodents during development and adulthood.

In the adult, I examine slow-wave activity, a key form of spontaneous activity. I show that functionally related regions of the cortex activate synchronously, forming a core set of structures that underlie spontaneous activation. I also show that sensory connections shape the patterns of this activity. These effects hold true in the quietly awake mouse, to a lesser extent. These findings are consistent with an active role for slow-wave activity in the maintenance of cortical connections.

In the infant, I examine a dominant form of brain activity, the spindle burst. This pattern of activity follows spontaneous sensory inputs generated by the developing sensory systems, including small twitches in the limbs and tail. It is generally thought to remain localized with the appropriate cortical sensory system, but using wide-field imaging, I show it spreads medially across the cortex. This suggests a potential role in the maturation of connections between sensory and motor regions.
I explored this possibility more closely by recording activity in early life from the whisker system of the rat. In the adult, connections exist between the sensory and motor regions of the whisker system. To gain insight into whether the spontaneous activation of these systems contributed to their development, I compared the activity evoked by stimulation to the spontaneous activation of these systems. I found synchronized spontaneous activation of motor and sensory areas that were not yet functionally connected. This suggests that other structures synchronize these areas to promote the maturation of connections between them.

Overall, this work reveals details about spontaneous activity that provide clues to why the brain devotes time and energy to activity disconnected from the outside world.
PREFACE

All experiments in this thesis were conducted with the supervision and approval of the University of British Columbia Animal Care Committee. Certificates of approval are A10-0150, A10-0140, and A09-0665.

Portions of this thesis have been published.

Chapter 2 is published in part in:

Chapter 2 uses the same data as the above paper, which examined interhemispheric correlations between isolated VSD signals. It did not examine intrahemispheric relationships between VSD signals, or make use of correlation maps. The first three authors of this paper (Majid Mohajerani, Matthew Fingas, and myself) collected the data collaboratively. The methods, which were prepared collaboratively by the co-authors, are reproduced in Chapter 2 (as section 2.2). Figure 3 from the above paper, which I prepared, is reproduced in Chapter 2 (as Figure 2.12). The remaining writing, analysis, and Figure preparation in Chapter 2 was done by myself.

Chapter 3 has been reproduced largely from:

Data collection, analysis, Figure preparation, and writing for the above paper was done by myself, with the advice and assistance of the co-authors.
# TABLE OF CONTENTS

ABSTRACT ........................................................................................................................... ii
PREFACE .............................................................................................................................. iv
TABLE OF CONTENTS ........................................................................................................ v
LIST OF TABLES ................................................................................................................... vii
LIST OF FIGURES .............................................................................................................. viii
LIST OF ABBREVIATIONS ................................................................................................. ix
ACKNOWLEDGMENTS ........................................................................................................ x
DEDICATION ......................................................................................................................... xi

## CHAPTER 1. INTRODUCTION ......................................................................................... 1
1.1 What does the brain do? ................................................................................................. 1
1.2 Spontaneous activity in the adult brain ........................................................................ 6
1.3 Spontaneous activity in the developing brain .............................................................. 31
1.4 Large-scale spatial organization of spontaneous activity .............................................. 48
1.5 Studying the spatial detail of spontaneous activity in infancy and maturity ............... 59
1.6 Figures .......................................................................................................................... 64

## CHAPTER 2. SLOW-WAVE ACTIVITY IN THE ADULT MOUSE REFLECTS
FUNCTIONAL CORTICAL SYSTEMS ................................................................................ 74
2.1 Introduction .................................................................................................................... 74
2.2 Methods ......................................................................................................................... 76
2.3 Results ........................................................................................................................... 82
2.4 Discussion ..................................................................................................................... 92
2.5 Figures .......................................................................................................................... 102

## CHAPTER 3. VOLTAGE-SENSITIVE DYE IMAGING REVEALS DYNAMIC
SPATIOTEMPORAL PROPERTIES OF CORTICAL ACTIVITY FOLLOWING
SPONTANEOUS MUSCLE TWITCHES IN THE NEWBORN RAT ............................... 114
3.1 Introduction .................................................................................................................... 114
3.2 Methods ......................................................................................................................... 116
3.3 Results ........................................................................................................................... 118
3.4 Discussion ..................................................................................................................... 130
3.5 Figures .......................................................................................................................... 137

## CHAPTER 4. SPONTANEOUS ACTIVITY SYNCHRONIZES WHISKER-
RELATED SENSORIMOTOR NETWORKS PRIOR TO THEIR MATURATION
IN THE DEVELOPING RAT CORTEX .............................................................................. 146
4.1 Introduction .................................................................................................................... 146
4.2 Methods ......................................................................................................................... 148
4.3 Results ........................................................................................................................... 152
4.4 Discussion ..................................................................................................................... 158
4.5 Figures .......................................................................................................................... 163

## CHAPTER 5. CONCLUSIONS ...................................................................................... 170
5.1 Aims and results .......................................................................................................... 170
5.2 Strengths and weaknesses ........................................................................................... 171
5.3 Ongoing activity in the adult and infant ...................................................................... 173
5.4 Activity in the motor cortex.................................................................179
5.5 Studying spontaneous activity to generate hypotheses .....................183
5.6 Concluding remarks.............................................................................185

REFERENCES.................................................................................................186
LIST OF TABLES

Table 1.1 Characteristics of sleep and sensation .................................................. 3
Table 1.2. Sleep stages and their characteristics .................................................. 9
Table 2.1. Cortical regions and positions ............................................................... 80
LIST OF FIGURES

Figure 1.1 Sleep stages and their characteristics.......................................................... 64
Figure 1.2. Slow oscillation as recorded from the somatosensory cortex.......................... 65
Figure 1.3. The cortical slow oscillation can group thalamic rhythms............................ 66
Figure 1.4. Generation of UP and DOWN states in cortical cells.................................... 67
Figure 1.5. Possible learning related functions to slow-wave sleep (SWS)......................... 68
Figure 1.6. Waking, quiet sleep, and active sleep in an eight-day old rat.......................... 69
Figure 1.7. Movements in human or rodent infants generate bursts of cortical activity........ 70
Figure 1.8. Organization of the rodent whisker system................................................. 71
Figure 1.9. Spontaneous BOLD oscillations within functionally related regions are correlated... 72
Figure 1.10. VSD images provides high spatial and temporal resolution of activity.............. 73

Figure 2.1 Voltage-sensitive dye (VSD) imaging in the adult anesthetized mouse............ 101
Figure 2.2: VSD imaging in the awake adult mouse. ..................................................... 102
Figure 2.3: Frequency of VSD bursts in the awake (black) and anesthetized (blue) state .... 103
Figure 2.4: Homotopic and interhemispheric correlations of VSD signals.................... 104
Figure 2.5: Pair-wise correlations between VSD signals from brain regions in the awake and anesthetized mouse........................................................................... 105

Figure 2.6: Correlation maps show precise spatial layout of correlation of VSD signals from all pixels with VSD signal from each brain region..................................................... 106
Figure 2.7: Pair-wise correlations between correlation maps generated from each cortical region..... 107
Figure 2.8: Correlation maps from medical cortical areas are highly correlated with maps from homotopic regions as well as maps from other medial areas.................................. 108
Figure 2.9: Slow-wave activity can reflect underlying sensory architecture...................... 109
Figure 2.10: Role of long-range cortical connections in slow-wave activity revealed in whisker sensorimotor circuits. .......................................................... 110
Figure 2.11: Cross-correlations reveal consistent anterior-posterior spread of slow-wave activity.... 111
Figure 2.12: Unilateral imaging region (of right hemisphere) reveals that slow-wave activity is composed of complex local events.................................................. 112

Figure 3.1. VSD imaging provides high-resolution images of large region of the developing rat cortex.............................................................. 136
Figure 3.2: Activity spreads asymmetrically following hindlimb stimulation..................... 137
Figure 3.3: Burst of VSD signal in sensory cortex lead to correlation with the overall cortical pattern evoked by stimulating the limbs/tail. ........................................... 138
Figure 3.4: Cortical bursts and twitches have a variable relationship.............................. 139
Figure 3.5: Changes in VSD signal are correlated across sensory regions....................... 140
Figure 3.6: Cortical activation is dynamic...................................................................... 141
Figure 3.7: Cortical activation of sensory regions spreads medially............................... 142
Figure 3.8: Limb twitches initiate dynamic activity across the cortex............................ 143
Figure 3.9: Bursts in the absence of twitches may occur in non- somatosensory regions of cortex... 144

Figure 4.1. Experimental approach............................................................................ 162
Figure 4.2. VSD imaging reveals patterns of cortical activation following sensory stimulation............................................................................................................ 163
Figure 4.3: VSD imaging reveals prominent changes of spatial and temporal properties of whisker responses with age.......................................................... 164
Figure 4.4: Frequency of spontaneous activity increases with age but can synchronize barrel and motor cortices at early ages. ..................................................... 165
Figure 4.5: Synchronous activation can exist during spontaneous activity that is not present during stimulation. .......................................................... 166
Figure 4.6: Sensory-evoked activity in M1 is not required for synchronized activity in barrel and motor cortex during spontaneous activity........................................ 167
Figure 4.7. Bursts of activity in barrel cortex coincide with bursts in motor cortex.............. 168
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>AC</td>
<td>Anterior cingulate</td>
</tr>
<tr>
<td>BC</td>
<td>Barrel cortex</td>
</tr>
<tr>
<td>BDNF</td>
<td>Brain-derived neurotrophic factor</td>
</tr>
<tr>
<td>BOLD</td>
<td>Blood oxygen level dependent</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
</tr>
<tr>
<td>FL</td>
<td>Forelimb</td>
</tr>
<tr>
<td>GABA</td>
<td>γ-aminobutyric acid</td>
</tr>
<tr>
<td>HL</td>
<td>Hindlimb</td>
</tr>
<tr>
<td>HSD</td>
<td>Honestly significant difference</td>
</tr>
<tr>
<td>LED</td>
<td>Light emitting diode</td>
</tr>
<tr>
<td>LFP</td>
<td>Local field potential</td>
</tr>
<tr>
<td>M1</td>
<td>Primary motor</td>
</tr>
<tr>
<td>M2</td>
<td>Secondary motor</td>
</tr>
<tr>
<td>NREM</td>
<td>Non-rapid eye movement</td>
</tr>
<tr>
<td>NMDA</td>
<td>n-methyl-d-aspartate</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PT</td>
<td>Parietal</td>
</tr>
<tr>
<td>REM</td>
<td>Rapid eye movement</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>RS</td>
<td>Retrosplenial</td>
</tr>
<tr>
<td>S1</td>
<td>Primary somatosensory</td>
</tr>
<tr>
<td>S2</td>
<td>Secondary somatosensory</td>
</tr>
<tr>
<td>SAT</td>
<td>Slow activity transient</td>
</tr>
<tr>
<td>SPW-R</td>
<td>Sharp wave ripples</td>
</tr>
<tr>
<td>SWA</td>
<td>Slow-wave activity</td>
</tr>
<tr>
<td>TL</td>
<td>Tail</td>
</tr>
<tr>
<td>V1</td>
<td>Primary visual</td>
</tr>
<tr>
<td>V2</td>
<td>Secondary visual</td>
</tr>
<tr>
<td>VSD</td>
<td>Voltage sensitive dye</td>
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I would like to thank first of all my supervisor, Dr. Tim Murphy, for his support and encouragement through my program. His enthusiasm for finding (or building!) new ways to explore the brain is unmatched and I hope to carry that enthusiasm forward.

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My family is unfailingly supportive. I want to thank my parents in particular for guiding me with “the voice of a teacher and the hands of an engineer.”

My best friend needs recognition for always reminding me to Chase my dreams.

Finally, my spouse Jodie is a constant source of laughter and joy. I would like to extend a very special thanks to her and I hope I will be able to return the favour.
DEDICATION

To my family,
near and far,
here and gone.
CHAPTER 1. INTRODUCTION

1.1 What does the brain do?

“What does the brain do?”

Every elementary school student knows the answer to this question. It is learned along with the function of other parts of the body, maybe taught between the sounds of farm animals and the multiplication tables. “The lungs breathe, the heart pumps, and the brain thinks.” Later, in high-school or university, the same student might expand: “The brain is the generator of our thoughts, and through it we perceive and interact with the outside world.”

It seems difficult to disagree with either the elementary or the high-school student. Introspection tells us that we perceive a richly detailed moment-to-moment experience of the world, and that we are in control of our actions in this world. Although we contemplate the future and remember the past, we do so as a traveler might look forward or backward along his path, remaining anchored at our current position in time.

We also know that this experience of the world depends on the physical condition of our brain. Brain injuries limit our abilities to perceive and act in the world (Stuss and Levine, 2002; Kennedy and Coelho, 2005; Flanagan et al., 2009; McAllister, 2011) and can result in changes in our personality and interests (Neylan, 1999; Miller and Hou, 2004). Manipulating our brains via drugs can change (de Oliveira and Juruena, 2006; Krishnan and Nestler, 2008; Alfonso-Loeches and Guerri, 2011) or even eliminate (Orser, 2007; Alkire et al., 2008) our experience of the world. What else to conclude
except that the purpose of the brain is to build an internal representation of the world and allow us to act within it?

Nevertheless, even the earliest investigators noted that the brain is active even in the absence of any ongoing sensory or motor tasks (Berger, 1929; Adrian and Matthews, 1934a; Jasper and Carmichael, 1935). The elementary student we met earlier, despite his conviction about the brain’s role in building a world for his conscious mind, would be intimately familiar with at least one example of this; dreaming, in which the brain’s activity is minimally connected to the immediate environment (Nir and Tononi, 2010).

This activity is not trivial. In fact, it has been known for many decades that mental tasks only minimally increase energy usage by the brain (Sokoloff et al., 1955). More recent qualitative work estimates that only 5% of the energy consumed by the brain is used in response to ongoing sensory or motor tasks (Raichle and Mintun, 2006). Furthermore, the large majority (~75%) of the overall energy consumed by the brain is used for active signaling as opposed to passive processes such as maintenance of the resting potential or transmitter recycling (Attwell and Laughlin, 2001; Howarth et al., 2012). Thus ongoing, spontaneous brain activity extracts a substantial energy cost on the body and on this basis alone would be expected to contribute to brain function in some significant fashion.

Like dreams themselves, though, spontaneous activity in the brain is difficult to study and the challenges of recording and interpreting it have caused it to remain generally unappreciated (Raichle, 2011). In contrast to events related to sensory inputs or motor outputs, ongoing spontaneous activity has no fixed point to which it can be
Factors that influence it are not clear and thus harder to control. Most challenging, though, has been the lack of a clear and consistent framework through which to examine it. Referring in particular to these differences between sleep and waking sensation (as summarized in Table 1.1), Steriade and Hobson commented, “… these differences … account for the fact that the cellular study of sensation has progressed steadily while the cellular study of sleep has tended to be confusing and inconclusive and is only now beginning to find its way.” (Steriade and Hobson, 1976).

The study of spontaneous brain activity truly began to ‘find its way’ roughly twenty years ago, with the application of imaging to the study of brain activity. These techniques, particularly functional magnetic resonance imaging (fMRI) but also positron emission tomography (PET) collect signals of brain activity from a wide expanse of cortical and subcortical areas. By collecting signals from the entire brain, two important observations could be made: first, seemingly random fluctuations in brain activity are

<table>
<thead>
<tr>
<th>Behavioral features:</th>
<th>Sensation</th>
<th>Sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>State dependent, i.e. precludes sleep. Behaviorally partial</td>
<td>Stimuli exogenous</td>
<td>State determinant, i.e. precludes sensation. Behaviorally global</td>
</tr>
<tr>
<td>Stimuli known, effective features to be determined</td>
<td>Stimuli endogenous</td>
<td>Stimuli unknown, unnecessary, or precluded</td>
</tr>
<tr>
<td>Subject to experimental control</td>
<td>Not subject to experimental control</td>
<td></td>
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<thead>
<tr>
<th>Structural features:</th>
<th>Sensation</th>
<th>Sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptors, pathways, and relays known and well studied</td>
<td>Critical structures suspected but relatively little-studied</td>
<td></td>
</tr>
<tr>
<td>Effected structures localized</td>
<td>Effected structures diffuse</td>
<td></td>
</tr>
<tr>
<td>Parallel paths make sampling of unit reasonable</td>
<td>Divergent paths make sampling of units difficult</td>
<td></td>
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<th>Functional features:</th>
<th>Sensation</th>
<th>Sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feature extraction to be determined</td>
<td>Central control mechanisms to be determined</td>
<td></td>
</tr>
<tr>
<td>Sequential processing indicated by structural features</td>
<td>Transform of internally generated information of unknown significance</td>
<td></td>
</tr>
<tr>
<td>Information processing: clearly predicates interpretations</td>
<td>? Rest and recovery: makes interpretation doubtful or erroneous</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1.1:** Contrasting features of sleep and sensation. From Steriade and Hobson, 1976. Used with permission.
correlated between functionally and anatomically related regions (Fox and Raichle, 2007). Using these correlations as a measure of connectivity, brain regions can be seen to be organized into networks that strike an optimal balance between minimizing axon length and maximizing connectivity (Bassett and Bullmore, 2006; Bullmore and Sporns, 2009). A second key observation is that there exists a set of brain regions, termed the default mode network, that are consistently less active during performance of a task than during quiet resting (Raichle and Snyder, 2007).

Taken together, these observations have helped frame a hypothesis in which spontaneous brain activity reflects the communication of brain regions to process past experiences, create predictions of the future, and mediate learning and memory.

This framework has spurred a great deal of research into the potential role of spontaneous brain activity in health (Fox and Greicius, 2010), cognition (Sadaghiani et al., 2010), and even consciousness (He and Raichle, 2009a). Within this framework, the study of other forms of spontaneous brain activity, particularly those of the sleeping brain, have also progressed rapidly (Tononi and Cirelli, 2006; Palva and Palva, 2007; Hobson, 2009; Nir and Tononi, 2010; Luczak and MacLean, 2012).

In broad support of this framework is the role of spontaneous activity in the development of the brain and nervous system in early life. While the first-grade student from earlier in this introduction might be baffled by the question, “What did the brain do before you were born?”, the brain and nervous system are in fact active before they are functional (Blankenship and Feller, 2010). This activity, like the adult brain activity discussed above, is not reflective of current sensory or motor processes involving the external world. Studying this activity has the important benefit that its function can be
more easily tested, by disrupting it and examining the subsequent deficits in resulting adult brain. Doing so has revealed crucial roles for this activity in measurable processes such as the formation of ocular dominance columns in the visual cortex (Torborg and Feller, 2005), refinement of corticospinal projections (Martin et al., 2007), and maturation of spinal reflex circuits (Grillner, 2004). Furthermore, there is substantial overlap between the processes that underlie developmental plasticity and that plasticity which continues through life in response to learning or injury (Singer, 1995; Rønn et al., 2000; Caleo and Maffei, 2002; Wolpaw, 2002; Turrigiano and Nelson, 2004), suggesting that lessons learned via the study of spontaneous activity during development are likely to prove useful in understanding the adult brain as well (Constantine-Paton et al., 1990).

Among these lessons will likely be insights related to synaptic plasticity. Since being formalized by Hebb (Hebb, 1949), a framework describing plasticity that strengthens connections between neurons that are active together has been a driving force in the study of learning and memory (Sejnowski, 1999; Lynch, 2004; Miles et al., 2005). In the intervening years, a great deal of progress has been made towards understanding how coincident activity in adjacent cells can drive modifications in their synaptic connections via glutamatergic N-methyl-D-aspartate (NMDA) responsive synapses (Kandel, 2001; Nakazawa et al., 2004; Hawkins et al., 2006; Feldman, 2009). With such activity-dependent plasticity in mind, spontaneous activity, which continually activates neurons within related circuits, stands out as an important factor in promoting changes that establish memory formation and other learning.

Many lines of evidence show that the answer to the question, “What does the brain do?” will have to consider what the brain does in the absence of sensory or motor
inputs and outputs, both in the adult and the developing brain. In this dissertation I use imaging techniques to examine activity in the adult and newborn rodent brain with the goal of providing new insights into these topics. This introduction is divided into two broad sections, the first providing an overview of spontaneous activity in the inactive adult brain and the second, an overview of such activity in the developing brain. I then provide an introduction to the techniques I use in this thesis and the specific topics explored in the following chapters.

### 1.2 Spontaneous activity in the adult brain

The first reports of electrical activity in the human brain were made by Berger (Berger, 1929). Subsequent investigations by Adrian and Matthews confirmed their existence and brought them into widespread acceptance (Bishop, 1933; Adrian and Matthews, 1934b; Adrian and Yamagiwa, 1935; Gibbs et al., 1935; Jasper and Carmichael, 1935; Loomis et al., 1936). These studies, as well as countless since, noted that oscillatory patterns are a prominent feature of activity in the brain (Gray, 1994; Singer, 1999; Buzsaki and Draguhn, 2004; Haider and McCormick, 2009).

#### 1.2.1 Spontaneous activity of NREM sleep

One of the most intriguing features noted by the early investigators is that the patterns of oscillations recorded from the brain shifts depending on the current behavioural state. The most dramatic shift in behavior occurs when falling asleep, when the brain becomes much less responsive to the external world and focuses inwards on an
internal one; it was understandably an attractive target for early EEG researchers (Berger, 1929; Adrian and Matthews, 1934b; Adrian and Yamagiwa, 1935; Gibbs et al., 1935; Loomis et al., 1935). Despite its disengagement from the environment, the brain remains active with a wide repertoire of patterns, and thus sleep is one of the most obvious examples of spontaneous brain activity detached from immediate behaviours. Therefore it remains an attractive subject for those interested in understating the purpose of spontaneous brain activity.

The research I have performed in adult animals relates to one component of sleep, non-rapid eye movement (NREM) sleep. It is helpful to provide a brief overview of the components of sleep to place this in an appropriate context, before describing the genesis, function, and outstanding issues related to this form of spontaneous activity.

1.2.1.1 Sleep stages

Early studies of brain activity in sleep noted primarily the replacement of the main rhythm of the alert but unoccupied brain, the alpha rhythm, with random and unpredictable activity during sleep which engendered a belief that sleep represented a passive, homogenous state of slowed cortical activity (Dement, 2005; Shepard et al., 2005). Later studies reported some structure to the EEGs of sleep, with distinctly slower and larger EEG activity that corresponded to increased arousal difficulty as sleep progressed (Davis et al., 1937; Loomis et al., 1937).

A seminal observation in the 1950’s resulted in the concept of sleep as a homogenous passive state being put to bed for good. Aserinsky and Kleitman reported previously unrecognized periods of rapid eye movements throughout sleep, the
occurrence of which was correlated with reports of dreaming (Aserinsky and Kleitman, 1953). They fittingly termed this stage of sleep *rapid eye movement* (REM) (Aserinsky and Kleitman, 1955). Similar events were shown in cats (Dement, 1958). Shortly after, William Dement and Nathaniel Kleitman proposed a classification of sleep into 4 stages plus REM (Dement and Kleitman, 1957). Although a similar classification of sleep into progressive stages had been proposed earlier (Loomis et al., 1937), this later scheme was widely accepted and incorporated into guidelines that formed a common framework for both clinical and research-oriented sleep work for many years (Hirshkowitz, 2000) before being updated recently into a three-stage + REM scheme (Silber, 2009). This current staging system depends on the occurrence of 3 key features in addition to REM;

1) *Delta waves*

The term *delta wave* was first used to describe large, slow (1-4 Hz) oscillations that were seen pathologically during waking in patients with brain tumours (Walter, 1936). They were reported during sleep and incorporated into the sleep literature by shortly after (Loomis et al., 1937).

2) *Sleep spindles*

Also first described by Loomis in 1937 (Loomis et al., 1937), spindles are oscillations of between 7-15 Hz whose power increases and decreases smoothly over a few seconds, giving them their eponymous shape. They occur every ~10 seconds and synchronize wide regions of cortex (Contreras et al., 1997).

3) *K-Complexes*
First described by Loomis in 1938 (Loomis et al., 1938), but the origin of the term is unknown (Colrain, 2005). They are events characterized by a large, slow, biphasic wave, often followed by a sleep spindle (Amzica and Steriade, 2002). They are generated simultaneously across large areas of the cortex in the upper layers (Cash et al., 2009).

The current sleep staging system is divided into four stages, shown in Table 1.2 (Silber, 2009). In many cases, researchers still use the older 4 stage system, in which N3 is subdivided into two stages based on the size and frequency of the delta waves, as illustrated in Figure 1.1. Stages N1-N3 are often grouped together as non-REM (NREM) sleep as they share a neuronal substrate as well as behavioural features. They are initiated by a withdrawal of ascending aminergic inputs from the locus coeruleus and the raphe nucleus to thalamocortical networks (Steriade et al., 1990a), while REM is initiated by the activation of ascending cholinergic inputs from the laterodorsal tegmental and pedunculopontine nuclei (Hobson et al., 2000; Hobson and Pace-Schott, 2002). These two groups of nuclei reciprocally inhibit one another, leading to the alternation of REM

<table>
<thead>
<tr>
<th>Sleep stage</th>
<th>Characteristic</th>
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<tbody>
<tr>
<td>N1</td>
<td>Alpha activity is reduced and replaced with low amplitude, slower bands of activity (4-7 Hz).</td>
</tr>
<tr>
<td>N2</td>
<td>Appearance of k-complexes and spindles</td>
</tr>
<tr>
<td>N3</td>
<td>Appearance of delta oscillations</td>
</tr>
<tr>
<td>REM</td>
<td>Low amplitude, mixed frequency EEG with rapid eye movements and atonia</td>
</tr>
</tbody>
</table>

Table 1.2. Sleep stages and their characteristics.
and NREM stages of sleep (Hobson et al., 1975).

1.2.1.2 Neural substrate of delta rhythms and spindle bursts

Thus, while seen in the early years of research as an unstructured state resulting from the loss of cortical activity, sleep is in fact an actively generated and structured state (Steriade and Hobson, 1976). What are the neuronal structures that underlie the generation of these patterns? Thalamocortical interactions are essential (Steriade and Llinás, 1988; Steriade et al., 1990b; Llinás and Steriade, 2006; Crunelli and Hughes, 2010; Hughes et al., 2011), and it is appropriate to briefly review the features of the thalamus which will be referenced often.

Thalamic circuitry and channel properties

There are three key cells types within the thalamus (Steriade and Deschenes, 1984; Jones, 2002; Pinault, 2004; Huguenard and McCormick, 2007): relay cells that project excitatory fibers to the cortex as part of sensorimotor pathways; reticular neurons that project local GABAergic inhibitory neurons onto relay neurons; and local GABAergic interneurons. Local interneurons are primarily involved in shaping sensory responses and have little role in oscillatory or spontaneous activity (Huguenard and McCormick, 2007) and will not be further discussed. Relay cells are grouped into spatially confined nuclei that receive ascending afferents related to a single modality and project to a related cortical region. With very few exceptions, thalamocortical relay cells do not project collaterals outside of their nuclei (Yen and Jones, 1983; Steriade and Deschenes, 1984). In contrast, reticular neurons, found in a thin shell over the dorsal and lateral thalamus, project diffusely to thalamic relay neurons but not to the cortex (Jones,
1975; Steriade et al., 1984). They are almost entirely inhibitory (Houser et al., 1980), and linked via dendro-dendritic and axono-axonic synapses (Jones, 2002). Both thalamic relay and reticular neurons receive extensive feedback from the cortex via cortico-thalamic fibers (Jones, 2002), but these fibers are more extensive to, and have greater impact on, reticular neurons (Golshani et al., 2001).

There are a large variety of channels within thalamic neurons (Llinás and Steriade, 2006), but the two most important to the discussion at hand are 1) the low-threshold, slowly-inactivating T-type calcium channel (Crunelli et al., 2006) ($I_T$) that must be de-inactivated by hyperpolarization (Llinás and Jahnsen, 1982; Llinás, 1988; Coulter et al., 1989) and 2) a hyperpolarization-activated, inward-rectifying cation channel ($I_h$) (Steriade et al., 1993b).

The internal circuitry of the thalamus and the intrinsic membrane properties of individual neurons work together to generate many of the patterns of brain activity in sleep. Their interactions vary depending on background membrane potential and neuromodulators, as described below.

*Generation of spindle bursts*

Spindle bursts were first shown to be generated in the thalamus by decortication (Morison and Bassett, 1945); in addition, transection of cortico-cortical fibers does not impair their long-range synchrony across the cortex which implies a subcortical origin (Contreras et al., 1996a). Specifically, they are generated in the GABAergic cells of the reticular nucleus of the thalamus (Steriade et al., 1987; Buzsaki et al., 1988; Marini et al., 1992) via the slowly-inactivating calcium currents $I_T$ (Huguenard and Prince, 1992; Fuentealba et al.,
This has recently been confirmed by directly stimulating the cells using an optogenetic technique (Halassa et al., 2011). This activity is synchronized within reticular neurons via dendrodendritic (Deschenes et al., 1985) and axoaxonic (Pinault et al., 1997) synapses. Interestingly, rodents predisposed to absence seizures, which also involve inhibitory projections from the thalamus (Steriade, 2005), have a selective increase of $I_T$ in thalamic reticular cells (Tsakiridou et al., 1995).

Coordinated inhibitory activity is subsequently spread to sensory relay nuclei (Steriade et al., 1985; Paré et al., 1987) by wide-ranging projections (Scheibel and Scheibel, 1967; Steriade et al., 1984). These arrive as rhythmic IPSPs in thalamic relay nuclei (Bal et al., 1995), which de-inactivate the low-threshold Ca$^{2+}$ channels (Crunelli et al., 1989); high-frequency Na$^+$ spikes are superimposed upon the resulting slow Ca$^{2+}$ (Deschenes et al., 1982; Llinás and Jahnsen, 1982; Krosigk et al., 1993). These spikes are projected to the cortex via thalamo-cortical fibres. Spikes are also projected back to reticular cells, exciting them and prompting another volley of IPSPs arriving at relay neurons (Destexhe et al., 1996) (see (Steriade, 1995; Llinás and Steriade, 2006) for review).

*Generation of delta waves*

Oscillations of cortical cells within delta frequencies survive removal of the thalamus (Steriade et al., 1990b) and current source density analysis seemed to demonstrate their genesis in layers II and III of the cortex (Petsche et al., 1984). However, oscillations in the delta range were also recorded from neurons of sensory nuclei (Steriade et al., 1971) and the reticular nucleus (Steriade et al.,
1986) of the thalamus, leading to uncertainty about their origin. They were later
demonstrated to be a product of thalamic cells, which can oscillate at the delta
rhythm in the decorticate state (Dossi et al., 1992). Unlike spindles, which emerge
from network properties of the thalamocortical circuit, delta rhythms arise from
intrinsic properties of the thalamic neurons, namely alternation between a) the
inward rectifying cation currents ($I_h$) activated by hyperpolarization (Pape and
McCormick, 1989; McCormick and Pape, 1990a; 1990b) and b) the low-threshold
Ca$^{2+}$ current ($I_T$) (Jahnsen and Llinás, 1984; Suzuki and Rogawski, 1989) giving
rise to fast Na spikes. The role of these two currents was demonstrated almost
concurrently in cat (McCormick and Pape, 1990b) and rat (Leresche et al., 1991;
Soltesz et al., 1991) by abolition of the rhythm by application of Ni$^{2+}$ or Cs$^+$
which blocks calcium and sodium/potassium currents, respectively.

Transition from spindle bursting to delta activity

It is worth noting that spindles and delta oscillations are both generated within the
thalamus, and yet rarely overlap (Uchida et al., 1991; Lancel et al., 1992). In fact, as
described above, the replacement of spindles with delta oscillations is a criterion of the
arrival of stage N3 sleep. Spindles oscillations predominate at higher resting membrane
potentials (Dossi et al., 1992; Nuñez et al., 1992) because $I_h$ is larger, and the delay
between each calcium burst is smaller, preventing the Ca$^{2+}$ current from de-inactivating
and ultimately stopping the oscillation after a few cycles (Soltesz et al., 1991). Support of
this conclusion comes from injections of NMDA antagonists into the thalamus, which
hyperpolarizes thalamocortical neurons and results in a switch from spindles to delta
rhythms (Buzsaki, 1991). During normal sleep, then, as cortical and brainstem activating
influences are withdrawn further between the N2 and N3 sleep stages, the resting potential of thalamic cells also decreases further, allowing $I_h$ and $I_T$ currents to balance into the nearly continuous and ongoing delta oscillations of N3 sleep (Buzsaki, 1991; Steriade et al., 1993a).

1.2.1.3 The slow oscillation

Evidence for a cortical factor in thalamic rhythms

We have seen that spindles and delta oscillations, two of the important rhythms of NREM sleep, are generated in the thalamus. This presented something of a mystery for some time (Amzica and Steriade, 1998).

In the case of delta oscillations, they arise from intrinsic cellular properties, but with few exceptions (dorsal lateral geniculate nucleus, (Friedlander et al., 1981; Van Horn et al., 1986)), thalamocortical sensory relay cells do not extend collaterals outside of their nuclei (Yen and Jones, 1983; Steriade and Deschenes, 1984). In the absence of an external synchronizing influence, therefore, delta rhythms projected from the thalamus to the cortex would not be expected to be synchronized, and in fact synchrony between thalamic nuclei was not demonstrated in the isolated thalamus (Dossi et al., 1992). Asynchronous oscillations within thalamic nuclei would not project widely synchronized activity to the cortex, meaning large-scale cortical EEG delta oscillations would not be expected (Amzica and Steriade, 1998).

In the case of spindle bursts, while their thalamic origin was clear, they occurred with a regular rhythm every 10-15 seconds (Deschenes et al., 1984; Steriade et al., 1986), possibly due to afferent excitatory connections to reticular neurons, the thalamic source
of spindle bursts (Steriade et al., 1987; Steriade, 1995). Furthermore, spindle bursts were not synchronized in disparate nuclei, despite their genesis from network effects of interactions between reticular and thalamocortical cells (Amzica and Steriade, 1998). The cortical manifestation of these recurring thalamic oscillations as large, coherent EEG signals therefore necessitates an additional extra-thalamic influence.

The origin of this influence was suspected as cortical in origin based on the ability of cortical excitation to augment spindle burst frequency (Morison and Dempsey, 1943), and reliability elicit a series of oscillatory spindle-like bursts in thalamic nuclei (Steriade and Deschenes, 1984). Cortico-thalamic cells also have strong influences on the behaviour of thalamic delta activity; in particular they can reset or pace (Steriade et al., 1991), suppress (Dossi et al., 1992), and synchronize (Dossi et al., 1992) oscillatory activity of thalamic cells.

Description of the cortical slow rhythm

Understanding the nature of this influence took a substantial step forward with the demonstration of a new cortical rhythm in 1993. In as series of three papers, Steriade and his colleagues described a slow (under 1 Hz) oscillation that would provide the link between rhythms generated in the thalamus and their synchronous projection to the cortex (Steriade et al., 1993c; 1993d; 1993a). Intracellularly, this oscillation was seen as a relatively rapid switch between a hyperpolarized (by 7-10 mV), silent state and a depolarized state lasting for ~1 s and characterized by spiking at 5-30 Hz in neurons of all cortical regions examined (Steriade et al., 1993c). Both thalamocortical and reticular cells also displayed similar intracellular oscillations (Steriade et al., 1993a). The two states are referred to as UP and DOWN states, and the alternation between them is generally
referred to as the slow oscillation (Van Someren et al., 2011). An example of this oscillation is shown in Figure 1.2.

It had been recognized previously that spiking of single cortical units increased and decreased in concert with the slow activity of sleep as seen by EEG (Hobson and McCarley, 1971; Calvet et al., 1973), but the finding that cells from multiple regions of the cortex, along with subcortical structures, were synchronized by a single rhythm brought the newly described slow oscillation a good deal of attention. Soon after its description in anesthetized cats, it was shown during natural sleep in cats (Steriade et al., 1996; Amzica and Steriade, 1998) and in humans (Achermann and Borbély, 1997; Simon et al., 2000) and it has since been demonstrated in rodents (Cowan and Wilson, 1994; Petersen et al., 2003; Doi et al., 2007; Ruiz-Mejias et al., 2011). Subsequent investigations have shown it exists in nearly all sensory, motor and association areas of the cortex (Steriade et al., 1993c; Amzica and Steriade, 1995a; Arieli et al., 1996; Kenet et al., 2003; Petersen et al., 2003; Luczak et al., 2007; 2009; Nir et al., 2011) and synchronizes the membrane potential of cells in different functional regions far removed from each other (Destexhe et al., 1999; Volgushev et al., 2006).

*The slow oscillation during waking*

Although the slow oscillation is primarily described as a rhythm of sleep, cortical neurons can undergo synchronized shifts in membrane potential during waking also, although at a faster frequency (Haider and McCormick, 2009). A number of studies have shown oscillating shifts in membrane potential in the rodent brain during quiet rest, using voltage-sensitive dye imaging (Petersen et al., 2003), membrane potential (Poulet and Petersen, 2008), and field potential recordings (Poulet and Petersen, 2008; Poulet et al.,
The slow oscillation groups other cortical rhythms

A key observation from the initial set of papers was that the slow oscillation was reflected in both spindle and delta activity at the level of the cortex (Steriade et al., 1993d). The depolarizing envelope of the slow oscillation can group delta waves events into sequences occurring every ~ 3-5 s, presumably by rhythmically depolarizing thalamic cells; this, in turn, moves the cells in and out of the range of resting potential that engages the alternating currents that generate delta activity (see above). Spindles also are grouped by the slow oscillation and predominantly follow the transition to the UP stage in cortical cells (Steriade et al., 1993d). Interactions between the slow oscillation and other rhythms of NREM sleep are shown in Figure 1.3. Although K-complexes received less study due to their transient existence in early sleep stages (Amzica and Steriade, 2002) they too were shown to be grouped by the slow oscillation (Amzica and Steriade, 1997) and more recently have been shown to represent localized manifestations of the slow oscillation in the early stages of sleep (Cash et al., 2009; Crunelli and Hughes, 2010).

Mechanisms of generation of the slow oscillation

Crucially, the cortical slow oscillation survived disconnection from the thalamus and developed in the cortex earlier in the sleep cycle than in the thalamus (Steriade et al., 1993c) – these observations suggested a direction of influence of cortex-to-thalamus, presumably via excitation during UP states of corticothalamic cells projecting to reticular
thalamic neurons, which subsequently send IPSPs to thalamic cells and trigger spindles in these cells (see section 1.2.1.2). This observation spurred a flurry of additional studies directly examining the role of the newly described cortical oscillation in synchronizing thalamic oscillations. Two important observations were that cortical stimulation could entrain thalamo-cortical and thalamic reticular neurons (Contreras and Steriade, 1997) and that synchrony between thalamic nuclei was lost following removal of the ipsilateral cortex (Contreras and Steriade, 1996; Timofeev and Steriade, 1996). It follows from these observations that the slow oscillation is generated in the cortex, and indeed isolated slabs of cortex can generate the pattern (Timofeev et al., 2000). However, a minimum size of cortical slab (approximately one gyrus) was necessary to sustain the oscillation, implying the oscillation relies heavily on network effects and not intrinsic neuronal properties.

*Generation of UP and DOWN states*

*A priori* one might assume that inhibitory interneurons, which make up ~ one-fifth of cortical neurons, project densely to neighboring pyramidal cell (Fino and Yuste, 2011; Packer and Yuste, 2011), and are involved in the generation of cortical oscillations (Haider and McCormick, 2009; Sohal et al., 2009) suppress activity during the DOWN state. However, these interneurons do not have activity synchronized with the hyperpolarized DOWN state (Steriade et al., 1994; Sanchez-Vives and McCormick, 2000). In addition, IPSPs triggered by such interneurons are relatively brief (~270 ms, compared to the DOWN state lasting ~0.5-1 s) (Connors et al., 1988). Instead, the DOWN state is triggered by the synchronized withdrawal of synaptic activity of cortical neurons (termed *disfacilitation*) as indicated by increased input resistance during the
DOWN state in anesthetized (Contreras et al., 1996b) and naturally sleeping states (Steriade et al., 2001; Timofeev et al., 2001), as well as via modeling of network behaviour under different conditions of background synaptic activity (Compte et al., 2003).

The UP state, in contrast, is characterized by pronounced excitatory and inhibitory activity and is impeded by both glutamatergic and GABAergic antagonists (Castro-Alamancos, 2000; Sanchez-Vives and McCormick, 2000; Compte et al., 2003; Sanchez-Vives et al., 2010). A number of studies have shown it to be characterized by large but balanced inhibitory and excitatory conductances and low input resistance (Paré et al., 1998; Destexhe and Paré, 1999; Sanchez-Vives and McCormick, 2000; Compte et al., 2003; McCormick et al., 2003; Shu et al., 2003b; Haider et al., 2006). This is consistent with general models of cortical function in which inhibitory and excitatory inputs are roughly balanced so as to maintain a reasonable dynamic range of spiking in the face of high connectivity (Shadlen and Newsome, 1994; 1998; Chance et al., 2002). However, other reports show the decreased input resistance of UP states is dominated by higher inhibitory conductances (Rudolph et al., 2005; 2007). (See also (Waters and Helmchen, 2006) who report sparse synaptic activity and high input resistance during UP states.) In any case, these conductances (and membrane potential) fluctuate rapidly, and this variance contributes to the high-frequency spiking during the UP states (Hô and Destexhe, 2000; Rudolph et al., 2007; Piwkowska et al., 2008) by occasionally bringing the membrane potential to threshold when placed on the background of a depolarized up state (McCormick et al., 2003). Spontaneous decreases in inhibitory conductances are particularly important (Haider and McCormick, 2009), often leading to spikes as shown
in vivo (Rudolph et al., 2007), in vitro (Piwkowska et al., 2008) and via modeling (Boustani et al., 2007). The high conductances and synaptic activity of the upstate reflect and depend on the highly interconnected nature of the cortex (Douglas et al., 1995; Douglas and Martin, 2007); in network models of the slow oscillation, reductions of local interconnections between model neurons of ~20% results in a loss of the slow oscillation (Compte et al., 2003).

Transitions between UP and DOWN states

The events that lead to transitions between UP and DOWN states are not confirmed. The transition to the DOWN state appears to be activity dependent, as progressive blockade of GABAergic inhibition leads to increased spiking activity during UP states, but shorter and less frequent UP states (Mann et al., 2009; Sanchez-Vives et al., 2010). One factor could be depletion of extracellular calcium by the sustained synaptic events (Massimini and Amzica, 2001), which has a pronounced impact on synaptic efficacy (Katz and Miledi, 1970; Bootman and Berridge, 1995; Mintz et al., 1995). A second factor that is likely to be important is activity dependent K⁺-channels which cause after-hyperpolarizations and decrease excitability following persistent activity (Schwindt et al., 1989; Sanchez-Vives et al., 2000). Consistent with this mechanism, neurons show a K⁺-mediated after-hyperpolarization following the UP state, whose duration approximates that of the DOWN state (Sanchez-Vives and McCormick, 2000). In models, a similar current (in this case, a sodium-activated potassium current) is crucial for the slow oscillation; without it, the model neurons fire tonically (Compte et al., 2003). In addition, blocking an activity-dependent ATP:ADP sensitive K⁺-channel prolongs the UP state (Cunningham et al., 2006). A third proposed mechanism is slowly
inactivating sodium-channels (Fleidervish et al., 1996) that reduce their depolarizing effects throughout the course of UP states (Steriade and Timofeev, 2003). Finally, there is evidence that metabotropic GABA-B receptors, though not involved in the excitation/inhibition balance of the UP state, are involved in its termination (Mann et al., 2009). As GABA-B receptors are generally found at a distance from the release site (Ulrich and Bettler, 2007) and require pooling and spillover of GABA to be activated (Scanziani, 2000) their activation would build over time, serving to stop the UP state in an activity dependent manner (Mann et al., 2009).

The spontaneous transition to the UP state appears to begin in layer 5 of the cortex. Evidence for this comes from observations that, both in vitro (Sanchez-Vives and McCormick, 2000) and in vivo (Luczak et al., 2009; Chauvette et al., 2010), the slow oscillation is found first in layer 5 before more superficial layers, and the slow oscillation is weakened or absent in slices lacking layer 5 but present in slices without superficial layers (Sanchez-Vives and McCormick, 2000). In fact, a small subset of cells in layer 5 retain the slow oscillation in the presence of glutamatergic antagonists that block the overall network activity (Compte et al., 2003; McCormick et al., 2003). The frequency of this activity is similar to the slow oscillation and they have been proposed to act as ‘pacemakers’ at a cortical level for the initiation of the up state (Le Bon-Jego and Yuste, 2007). (See however (MacLean et al., 2005) who find ‘core’ cells linked to the initiation of UP states in layer IV.) Neurons of the cortex are heavily interconnected (Douglas et al., 1995) and thus a small initial excitation such as that generated by an intrinsic pacemaker cell can by triggering cascading network activity among the heavily interconnected cells of the cortex (Douglas et al., 1995; Douglas and Martin, 2007).
Layer V neurons, with large projection fields and many synaptic inputs (DeFelipe and Fariñas, 1992), are well-suited to provide this initial excitation (Chauvette et al., 2010). Mechanisms involved in the oscillations between UP and DOWN states are illustrated in Figure 1.4.

**Thalamic involvement in the slow oscillation**

The mechanisms described above are cortical, and, in general, the slow oscillation is described as a cortical rhythm despite being present in thalamocortical and reticular thalamic cells (Steriade et al., 1993a) (for example, see reviews (Steriade, 1999; 2006; Haider and McCormick, 2009; Riedner et al., 2011)). This is a reflection of the fact that the cortical rhythm survives thalamic lesions (Steriade et al., 1993c) and can be generated in isolated cortical slabs (Timofeev et al., 2000) and slices (Sanchez-Vives and McCormick, 2000; McCormick et al., 2003). In contrast, the slow oscillation is not observed in the thalamus following the removal of the cortex in vivo (Timofeev and Steriade, 1996). However, a number of lines of evidence point to a role for the thalamus in shaping of the slow oscillation.

First, careful examination of the slow oscillation following thalamic lesions reveals a less regular rhythm (Crunelli and Hughes, 2010) and intra-thalamic injection of muscimol slows the cortical slow oscillation in a dose-dependent fashion (Doi et al., 2007). Second, in systems with intact cortico-thalamic connections, thalamic bursts often precede cortical ones (Contreras and Steriade, 1997; Grenier et al., 1998; Rigas and Castro-Alamancos, 2007) (a recent review urging readers to not neglect the thalamic contribution to the slow oscillation (Crunelli and Hughes, 2010) reminded readers that an early study of the slow oscillation (Contreras and Steriade, 1995) concluded… “...The
spike bursts of [thalamo-cortical] cells that follow the synchronous inhibitory period…
are good candidates to trigger the depolarizing phases at every cycle of the slow oscillation…”). Third, thalamic input, via stimulation of thalamocortical fibres (Shu et al., 2003b; Rigas and Castro-Alamancos, 2007; Mann et al., 2009) or sensory organs (Petersen et al., 2003) can trigger UP states in the cortex. In vitro, these are indistinguishable from those that arise spontaneously (MacLean et al., 2005).

Why, then, is the slow oscillation not found in thalamic cells in the absence of the cortex in vivo? In contrast to the mechanisms described above for cortical cells, the slow oscillation in thalamic cells depends on an interaction between the non-inactivating opening of a small fraction of T-type calcium channels (leading to an inward current termed the window current (Crunelli et al., 2006)) and potassium leak currents (Blethyn et al., 2006). Cortico-thalamic fibres, acting via metabotropic glutamatergic receptors (mGluR), decrease the leak current on thalamic neurons (McCormick and Krosigk, 1992) and bring its conductance into the correct range to express the slow oscillation (Hughes et al., 2002). This was confirmed by application of exogenous mGluR to isolated thalamocortical neurons, which allows them to express the slow oscillation (Hughes et al., 2002).

Slow oscillation reflects integrated thalamo-cortical circuitry

The thalamus, then, both influences and is influenced by the slow oscillation at the level of the cortex. This illustrates the importance of the slow oscillation in the study of NREM sleep – it is a unifying rhythm that ties together the diverse patterns of activity generated in the thalamus and the cortex (as well as subcortical structures such as the hippocampus (Dickson, 2010)). Its full understanding requires consideration of the entire
thalamocortical system and the full spectrum of NREM sleep related activity. This
unifying effect is acknowledged in the term slow-wave activity which is used to describe
activity in the 0.5-4 Hz range, including both delta and the slow oscillation (Mascetti et
al., 2011).

This is not to say that NREM is a unitary phenomenon; its three primary patterns
(spindles, delta oscillations, and the slow oscillation) are differently regulated and, as we
have seen, have their primary point of genesis in different parts of the brain. Steriade,
who did more to characterize the rhythm than anyone else, summarized the issue well:
“…although the separate description of the three different (spindles, delta, and slow)
sleep oscillations had an heuristic value… the intact brain reveals concerted activities of
neocortical and thalamic neurons leading to grouping of oscillations, which are otherwise
defined as distinct frequency bands…” (Steriade, 2001).

1.2.1.4 Purpose of NREM sleep

What, then, is the purpose of this unified pattern of rhythms that dominates much
of sleep? Some have argued that sleep in general does not have a particular purpose
beyond providing a time of physical rest (Rial et al., 2007) and enforced inactivity during
vulnerable periods (Siegel, 2009). However, this is at odds with the wide range of
detrimental effects of sleep loss, including on the immune (Besedovsky et al., 2012),
cardiovascular (Mullington et al., 2009), and metabolic (Knutson et al., 2007) systems.
Cirelli and Tononi have reviewed a number of reasons why sleep is likely to be important
for the nervous system in particular, including that there is no evidence of species that do
not sleep; no evidence that sleep deprivation does not result in some form of
compensatory mechanism (such as increased sleep following the deprivation); and no
evidence that sleep can be avoided without dire consequences including death (Cirelli and
Tononi, 2008).

NREM sleep reflects prior waking history

With regards to NREM sleep, it is the second factor, that of mechanisms that compensate for a loss of sleep, which is most relevant. (Although not discussed here, important roles for REM sleep have been described, reviewed by (Stickgold et al., 2001; Nir and Tononi, 2010).) It has long been known that sleep deprivation results in a subsequent increase in sleep (an effect termed sleep homeostasis, reviewed in (Borbély and Achermann, 1999; Vyazovskiy et al., 2011)). It was recognized in the 1960’s that this increase was concentrated in the deeper stages of NREM sleep (equivalent stage N3 of the current system) and that even in the well-rested case, stage N3 episodes of sleep were longest at the beginning of sleep and declined over the night (Berger and Oswald, 1962; Williams et al., 1964; Webb and Agnew, 1971).

It was later shown that not only the duration of N3 episodes, but also the EEG power of their characteristic activity (slow-wave activity (SWA), 0.5-4 Hz, including delta as well as some of the slow oscillation (Mascetti et al., 2011)), increased following sleep deprivation in a graded fashion depending on the duration of deprivation (Borbély and Neuhaus, 1979; Tobler and Borbély, 1986; Franken et al., 1991). In contrast, a nap before regular sleep hours decreases the EEG power of SWA (Werth et al., 1996). More detailed analysis has revealed that increases in the average size as well as the slope of each cycle of N3 sleep underlie these changes (Riedner et al., 2007; Vyazovskiy et al., 2007). Thus, it is now accepted that the one of the most accurate reflections of sleep
homeostasis is the prominence of brain activity associated with NREM, particularly stage N3, sleep (Vyazovskiy et al., 2011).

*Behavioural studies show NREM sleep is involved in memory consolidation*

What is the purpose of this increased NREM sleep activity and the associated SWA power? A clear suggestion comes from the many behavioural studies that show a dependence of memory consolidation on sleep (reviewed in (Stickgold and Walker, 2007; Walker, 2008; Diekelmann et al., 2009)). The first such association was suggested by (Ebbinghaus, 1913), who noted a greater retention during a verbal learning task when the testing interval included a period of sleep. In fact, the increased retention was so large that he did not accept it as correct and attributed the difference to experimental error! The first to accept that sleep was associated with memory formation or protection was (Jenkins and Dallenbach, 1924). These and other early studies of sleep and memory were reviewed by Van Ormer (Van Ormer, 1933). In most cases, these early studies did not include a comparison period of equal time spent awake, though, making it difficult to distinguish whether memories were being actively consolidated, or simply protected from new experiences, during sleep. Since this time studies have clearly shown that NREM sleep results in enhanced memory retention the following day by comparing to a comparable time awake (Barrett and Ekstrand, 1972; Fowler et al., 1973; Plihal and Born, 1997; Gais et al., 2000; Walker et al., 2002; Tucker et al., 2006), by showing that performance on memory tasks after sleep is proportional to the amount of NREM sleep (Stickgold et al., 2000a; 2000b); by impeding normal NREM sleep via infusion of cholinesterase inhibitors (Gais and Born, 2004) or acoustic sleep disturbances (Aeschbach et al., 2008); or by enhancing the slow oscillation of deep sleep via external
electrical stimulation (Marshall et al., 2006). In addition, SWA is increased during sleep following social challenges (Meerlo et al., 2001), verbal learning tasks (Mölle et al., 2004), procedural motor learning tasks (Huber et al., 2004; Hanlon et al., 2009), or exploration (Vyazovskiy et al., 2006) but decreased following sensory deprivation (Iwasaki et al., 2004; Huber et al., 2006). EEG grids in humans (Huber et al., 2004; 2006) and rodents (Iwasaki et al., 2004) as well as LFP electrodes in rodents (Hanlon et al., 2009) show the increase is restricted to the area of the brain involved in the task.

**Theories of synaptic change that accompany SWA in NREM sleep**

There are two main models of what this learning- and homeostatic-related SWA represents: replay of neural patterns associated with learning to promote synaptic plasticity (Sutherland and McNaughton, 2000; Schwindel and McNaughton, 2011); and down-scaling of new synapses created during normal waking or learning, to maintain a consistent overall excitatory balance in the cortex (Tononi and Cirelli, 2006; Diekelmann and Born, 2010). These models are illustrated in Figure 1.5.

The first model, known as active system reconsolidation, rests on an idea advanced by (Marr, 1970), namely that memories are encoded in short-term representations during waking, before being permanently encoded during sleep, when the brain is free of ongoing processing needs. In particular, it posits that memories are encoded temporarily in the hippocampus during exploratory activity, and then being ‘replayed’ and transmitted to the cortex during NREM sleep via fast hippocampal sharp waves ripples (SPW-R), all under the synchronizing action of the slow oscillation (Mölle and Born, 2011). Evidence for this theory includes:
- NREM sleep predominantly (but not exclusively, for example (Huber et al., 2004)) benefits hippocampal dependent declarative memory consolidation (Stickgold and Walker, 2007)
- recordings showing that cortical and hippocampal sequences of neuronal firing during waking are replayed together during NREM sleep (Qin et al., 1997; Ji and Wilson, 2007) but not during REM sleep (Siapas and Wilson, 1998; Kudrimoti et al., 1999; Wierzynski et al., 2009);
- hippocampal SPW-R predominate at the transition between cortical DOWN and UP states of the slow oscillation (Battaglia et al., 2004; Mölle et al., 2006) and are temporally linked with both spindles and delta waves (Sirota et al., 2003);
- selectively suppressing hippocampal SPW-R reduces sleep-related improvements in spatial memory (Girardeau et al., 2009).

In addition, a clever experiment directly demonstrated the effects of memory reactivation during SWA by delivering an odor, previously associated with a learning task, during stage N3 sleep in humans (Rasch et al., 2007). This enhanced recall on the learning task following sleep, presumably by activating the learning-related neural patterns associated with the odor.

The second model, that of synaptic homeostasis, comes from the observation that while synaptic potentiation and formation may be an effective mechanism for learning-related plasticity in the brain, it must be balanced by equivalent reductions in synaptic strength to avoid excess excitation (Miller and MacKay, 1994; Turrigiano, 2008). Unfettered increases in synaptic strength and number also place energy and size burdens
on the brain (Howarth et al., 2012). Rather than balancing increasing and decreasing of synaptic strengths in parallel, the synaptic downscaling theory of sleep suggest that synaptic potential proceeds mostly unhindered during waking before being downscaled during sleep (Tononi and Cirelli, 2001). Evidence is accumulating rapidly in support of this theory, and includes the following:

- genes associated with synaptic potentiation (e.g. Arc and BDNF) are decreased during sleep (Cirelli and Tononi, 2000), while those associated with synaptic depression (e.g. CAMK4, calcineurin) are increased (Cirelli et al., 2004);
- cortical excitability as measured via frequency of spontaneous mini-excitatory post-synaptic potentials (Liu et al., 2010), spiking rates (Vyazovskiy et al., 2009), size of cortical evoked potentials (Hulse et al., 2011), or responsiveness to transcranial magnetic stimulation (Huber et al., 2012), increases with time awake and decreases following sleep;
- direct visualization of synapses in both flies (Bushey et al., 2011) and mice (Maret et al., 2011) show that waking is associated with increasing numbers of synaptic spines, while sleeping is associated with a decrease;
- artificially enhancing synaptic potentiation by infusion of brain-derived neurotrophic factor (BDNF) enhances subsequent SWA, but not REM, sleep activity in a region restricted to the infusion (Faraguna et al., 2008).

As mentioned in section 1.1, activity-dependent synaptic potentiation is a major factor in our understanding of learning and memory. Active-system consolidation, with its focus on coordinated replay of salient sequences of neural activity, might be expected
to promote synaptic potentiation via Hebbian plasticity by repeated activation of pairs of neurons. This would be in keeping with the observed benefits of sleep on memory, discussed earlier in this section. On the other hand, synaptic homeostasis emphasizes synaptic weakening and an elimination of non-relevant potential accrued during waking. Can these opposite effects be reconciled?

In fact, neither model of the role of SWS suggests that it contributes directly to synaptic potentiation. It has been known for some time that LTP is poorly induced during SWS (Leonard et al., 1987; Bramham and Srebro, 1989) and genes associated with LTP are down-regulated during SWS (Thiel et al., 1994; Jones et al., 2001). Thus, instead of triggering LTP directly, replayed activity during SWS may ‘tag’ particularly salient synaptic changes, preserving them on a background of overall synaptic down-regulation (Ribeiro et al., 2004). Although the molecular underpinnings of this ‘tagging’ are not clear, they may include persistently elevated calcium levels in selected cells (Rosanova and Ulrich, 2005; Diekelmann and Born, 2010). Alternatively, induction of some plasticity related genes, which respond strongly to pulsatile intracellular oscillation of calcium levels at SWS frequencies (Frey et al., 1993; Abel et al., 1997) could contribute (Sejnowski and Destexhe, 2000).

In contrast to SWS, REM sleep is associated with potent induction of LTP (Leonard et al., 1987; Bramham and Srebro, 1989) as well as expression of LTP associated genes (Ribeiro et al., 2007) and is it suggested episodes of REM sleep serve to consolidate the synaptic changes preserved during SWS (Diekelmann and Born, 2010; Walker and Stickgold, 2010). In keeping with such a two-stage process, the behavioural benefits of sleep depend on both the amount of early SWS and late REM (Stickgold et al.,
and short stretches of sleep benefit memory formation most when they include both SWS and REM (Mednick et al., 2003).

Although a great deal remains to be learned about how sleep can both enhance learning and memory while decreasing overall cortical synaptic strengths, it seems clear that the localized functions related to SWS discussed here are incompatible with a global event, synchronizing the entire cortex. This has implications for the current understanding of SWA, and is addressed directly in section 1.4.

1.3 Spontaneous activity in the developing brain

In the preceding section, I have discussed patterns of activity in the brain when it is not required to be purposefully interacting with the environment. During early life, the brain is unable to purposefully interact with the environment, as many sensory (Rose and Ellingson, 1970; Jewett and Romano, 1972; Krug et al., 2001; Petersson et al., 2003; Colonnese et al., 2010) and motor (Grillner, 2004; Yang et al., 2004; Ladle et al., 2007) systems do not mature until well after birth – yet these systems display a variety of patterns of neural activity (Feller, 1999; Blankenship and Feller, 2010). There is a great deal of interest in this activity because it has been recognized to play essential roles in the normal development of the nervous system, acting in concert with genetically driven, molecular determinants of development (Goodman and Shatz, 1993; Tessier-Lavigne and Goodman, 1996; Chilton, 2006) to ensure formation of functional neural circuits (Pallas, 2001; Hanganu-Opatz, 2010). Understanding activity in the developing cortex is of particular interest, because abnormal neural connections in the cortex are implicated in illnesses such as autism and schizophrenia (Belmonte et al., 2004; Uhlhaas et al., 2008;
Minshew and Keller, 2010), and because mechanisms of plasticity in the developing
cortex overlap with those of the mature cortex (Frank, 2011).

1.3.1 EEG activity in the developing brain

As with the adult, the first studies of activity in the developing cortex were
performed by Berger, using EEG (Berger, 1932). He was unable to obtain any signals of
activity prior to 3 months of age. Subsequent studies of early EEG activity in human
(Lindsley, 1936; Smith, 1938; 1939; Hughes et al., 1949; Ellingson, 1958) as well as
animals (Jasper et al., 1937; Grain, 1952; Charles and Fuller, 1956; Deza and Eidelberg,
1967; Huttenlocher, 1967; Jouvet-Mounier et al., 1970; Gramsbergen, 1976) were more
successful. There is a remarkably similar pattern of development and sequence of
maturation between species (Ellingson and Rose, 1970), with the exception that
adjustments are made for the differing level of maturity at the time of birth. In particular,
the cortical development at mid-gestation in the human is approximately equivalent to
that of the rat at birth (Clancy et al., 2001). EEG recordings from premature human
infants are used generally as a proxy for in utero brain activity, and
magnetoencephalography recorded in utero suggests this is valid (Rose and Eswaran,
2004).

1.3.1.1 Patterns of EEG activity in the infant

These studies established perhaps the most striking feature of the earliest
recordable EEGs (at approximately 24 weeks post-conception in humans, around the time
of birth in rats); their lack of state-dependent differentiation. They do not display the
defined patterns of continuous cortical activity that represent a particular behavioral state such as sleep or waking in the adult. Instead, the EEG consists mainly of bursts of high-amplitude undifferentiated waves, separated by periods of silence (Engel, 1965; Deza and Eidelberg, 1967; Anderson et al., 1985; Selton et al., 2000). At 28 weeks post-conception, the human EEG remains discontinuous but a prominent pattern of short 10-25 Hz bursts super-imposed on a slower 0.5-2 Hz background emerges. The dominance of this pattern is illustrated by its many descriptions in the early literature (Khazipov and Luhmann, 2006), including ‘spindle-shaped bursts of fast waves’ (Ellingson, 1958) ‘spindle-like fast rhythms’ (Watanabe and Iwase, 1972), and ‘fast activity’ (Goldie et al., 1971). These are now referred to delta brushes in the clinical setting (André et al., 2010) and usually spindle bursts in the research setting. They may be grouped by an underlying slow (0.1-0.5 Hz) activity human (Vanhatalo et al., 2002) and rat (Seelke and Blumberg, 2008). Homologous patterns of activity in the rodent cortex during the first week of life will be discussed in much more depth shortly.

State dependent activity in the developing brain

State-dependent patterns of EEG activity emerge in the human EEG around 32 weeks post gestation, with the emergence of continuous fast activity during active periods of sleep, and alternating slow activity and silence during quiet sleep, as well as the disappearance of delta brushes (Dreyfus-Brisac and Monod, 1965; Parmelee et al., 1968; Dreyfus-Brisac, 1975). At birth, sleep and waking are clearly differentiated by EEG (Bartoshuk and Tennant, 1964; Torres and Anderson, 1985) but the adult REM and NREM patterns of activity, as well as the adult patterns of alpha activity, take up to a decade to mature (Jenni et al., 2004; Niedermeyer, 2011). This trajectory is generally
mirrored in the rodent; in particular, SWA during sleep emerges around P12 (Deza and Eidelberg, 1967; Jouvet-Mounier et al., 1970; Gramsbergen, 1976; Seelke and Blumberg, 2008) and also continues to mature for some time (Frank and Heller, 1997).

It is not difficult to understand the absence of state-dependent activity in the early EEG when we consider the mechanisms that generate it in adult. As discussed in section 1.2.1, some such mechanisms are intrinsic membrane properties, particularly $I_T$, intrathalamic connections, and high synaptic connectivity within the cortex. In early life, $I_T$ is small, only about one-third of the adult value (Pirchio et al., 1990) and corresponding calcium depolarizations cannot sustain $\text{Na}^+/\text{K}^+$ spikes (Warren and Jones, 1997). Intrathalamic connections between reticular neurons are not mature, so that inhibitory potentials are not synchronized at the destination relay cells (Warren and Jones, 1997). Synaptic connectivity between cells is low (Ben-Ari, 2001; Khazipov and Buzsaki, 2010), and cortical interneuron circuitry is not functional (Sipila et al., 2010). Therefore, the cortico-thalamic circuitry is not able to sustain ongoing self-generated activity (Colonnese and Khazipov, 2012); interestingly, at least one factor, inter-thalamic circuitry, approaches maturity at the same time as ongoing cortical activity emerges (Warren and Jones, 1997).

1.3.2 Sleep and waking in the infant

The absence of robust state-dependent patterns of EEG activity in early life does not mean that such states do not exist (Blumberg and Seelke, 2010). The human pre-term infant (Prechtl et al., 1979), the human fetus (Nijhuis et al., 1982; de Vries et al., 1985), and the newborn rat (Gramsbergen et al., 1970; Jouvet-Mounier et al., 1970; Corner,
1977) all show distinct states of behavior related to respiration (in the ex utero cases), movements, and heartbeat (although at the youngest ages, changes of these variables may occur independently (Prechtl et al., 1979)).

### 1.3.2.1 Sleep states in the infant

Some of the clearest transitions between states occur with regards to movements. As early as 10 weeks gestation in the human (Van Dongen and Goudie, 1980), and P2 in the rat (Gramsbergen et al., 1970), alternating periods of quiescence and atonia, quiescence with general atonia but small multi-limb twitches, and large movements involving the whole body are seen ((Corner, 1977; Robinson et al., 2000; Blumberg et al., 2005)). These states are termed quiet sleep, active sleep, and waking, respectfully, and a consistent cycle of wake, quiet sleep, then active sleep can be seen in rats as young as P2 (Karlsson et al., 2004), with very short durations and rapid transitions in early life lengthening markedly with increased age (Seelke et al., 2005). One example cycle is shown in Figure 1.6.

*Active sleep is a precursor to REM sleep in the adult*

A major theme of research has been the whether active and quiet sleep represent developmental forms of REM and NREM sleep, or an immature ‘pre-sleep’ that is replaced with adult sleep stages (Corner, 1977; Blumberg and Lucas, 1996; Frank and Heller, 1997; 2003; Blumberg et al., 2005; Blumberg and Seelke, 2010). Over the past decade, evidence has accumulated to support the former hypothesis:

- Active sleep is accompanied by atonia (Karlsson and Blumberg, 2002) resulting from active inhibition of motoneurons via inhibitory projections from the
medulla (Karlsson and Blumberg, 2005; Karlsson et al., 2005) in infants. Analogous effects are present during REM in adults (Chase and Morales, 1983) under similar brainstem medullary influence (Hajnik et al., 2000);

- Twitching of active sleep is accompanied by twitching of extra-ocular muscles in infants (Seelke et al., 2005), as in adult REM sleep (Chase and Morales, 1990);

- Quiet sleep occupies the first component of sleep infants, as NREM does in the adult, and when SWA emerges at around P12, it occupies the ‘slot’ between waking and active sleep (Seelke et al., 2005; Blumberg and Seelke, 2010).

**1.3.2.2 Mechanisms of active sleep**

Thus quiet sleep is the developmental precursor to NREM sleep, and active sleep to REM. However, although there is a wealth of research regarding the structure and function of NREM sleep in adults (see section 1.2.1.4), there is little such research on quiet sleep in infants. One reason for this is that there is little to study; quiet sleep is characterized by an absence of structured behavior or cortical activity. In contrast, active sleep, with its characteristic repetitive twitches, has proven to be a much richer topic of study. Such movements have been noted since the earliest studies of behavior, in a range of animal species (Windle et al., 1933; Raney and Carmichael, 1934; Windle et al., 1935; Barcroft et al., 1936) as well as humans (Hooker, 1952). These early studies, conducted through the lens of reflex-driven activity dominant at the time, mentioned these movements only in passing while focusing more on sensory-driven patterns of movement. Based on these cursory observations, an early review concluded that “…the
most probable explanation of the “spontaneous” movements is that they are due to the activation of the central nervous and the muscular systems in the adult manner, i.e. by the arrival in the central nervous system of impulses via sensory nerves…” (Barron, 1941).

_Twitches of active sleep arise from spinal networks and descending signals_

Decades later, Hamburger showed they were not triggered by sensory impulses by observing their persistence following isolation (Hamburger and Balaban, 1963) and de-afferentation (Hamburger and Wenger, 1966) of the spinal cord. It is now recognized that three mechanisms contribute to the generation of twitches:

- Intrinsic motoneurons properties, particularly the transient expression of L-type calcium channel in early life. This produces the previously described (section 1.2.1.2) low threshold slow calcium current $I_T$ which can give rise to intrinsic bursts of activity (Berger and Takahashi, 1990; Russier et al., 2003);

- Synaptic mechanisms within the developing spinal cord network (O'Donovan and Chub, 1997; Chub and O'Donovan, 1998);

- Descending influences from the brainstem. This was demonstrated via spinal transection between the fore- and hind-limbs of P5-8 rats pups (Blumberg and Lucas, 1994). The frequency of twitching in the hindlimbs decreased by about 50%, and was no longer synchronized with forelimbs. Clues about the nature of this influence were obtained by transecting the brainstem (Kreider and Blumberg, 2000). Separation of the forebrain from the brainstem left twitching of active sleep unaffected, but transection of the pons decreased it markedly. Occasional, random twitching, characteristic of younger prenatal rats (Narayanan et al., 1971), persisted. Such twitching is presumably generated by
the two mechanisms above. The specific area of the brainstem responsible was later localized to the laterodorsal tegmental nucleus by lesion and multi-unit recordings (Karlsson et al., 2005).

1.3.2.3 Twitches of active sleep generate spindle bursts and delta brushes

Thus, the brainstem, but not the cortex, is involved in the generation of twitches of active sleep. We have also seen that the cortex does not exhibit rhythmic activity related to the current behavior state (section 1.3.1.1). Is the cortex completely removed from the events of infant sleep?

Khazipov and colleagues showed in 2004 that it is not (Khazipov et al., 2004). Recording from the hind- and forelimb somatosensory cortex of rats during the first week of life, they found that spontaneous limb twitches, as well as limb stimulation, were followed 100-200 ms later by rhythmic discharges from units of the somatosensory cortex. At the population level, these rhythmic discharges gave rise to bursts of spindle shaped oscillations at ~10 Hz in field potential recordings, often overlying a slower depolarization in the delta frequency range (1-5 Hz). Analogous results were also shown in preterm human infants; the well-known delta brush pattern in the somatosensory cortex follows spontaneous hand and foot movements (Milh et al., 2007); example from both rat and human infants are shown in Figure 1.7. The delta and fast components differ in their source, the former being mostly representative of cutaneous inputs and the latter, representative of proprioceptive inputs (Marcano-Reik and Blumberg, 2008).

This pattern of activity has come to be known as a *spindle burst*, although it is worth noting that they have little other than their name and shape in common with the
spindles of adult NREM sleep (section 1.2.1.1). We have already discussed how the mechanisms that generate adult spindles are not in place in early life; furthermore, adult spindles emerge at earliest 4 weeks after birth in humans (Ellingson, 1982), when delta brushes in humans would be pathological (André et al., 2010).

**Spindle bursts are a common pattern of activity within sensory systems**

An additional difference is that, unlike the spindles of adult sleep, they are generated by ascending sensory inputs, and remain localized to the corresponding sensory system. Twitches in the fore- and hindlimb give rise to spindle bursts in the fore- or hindlimb sensory cortex, respectively (Khazipov et al., 2004). Importantly, this characteristic generalizes across the cortex; spontaneous retinal activity (a prominent feature of the visual system prior to eye opening (Feller and Scanziani, 2005; Firth et al., 2005; Torborg and Feller, 2005)) or optic nerve stimulation gives rise to spindle bursts in the visual cortex (Hanganu et al., 2006), and whisker twitches or whisker stimulation give rise to spindle bursts in the whisker somatosensory cortex (Minlebaev et al., 2007; Yang et al., 2009). In all cases, disrupting ascending sensory inputs (via spinal transection, whisker pad anesthesia, or retinal inactivation) decreases the incidence of spindle bursts significantly in the corresponding cortex.

**Network mechanism of spindle burst generation**

Some spindle bursts do continue following the elimination of sensory inputs, which reflects a network effect of some sort that contributes to their generation. In the cortex, synaptic dependency is demonstrated by the near complete loss of spindle bursts in the absence of glutamatergic activity (Minlebaev et al., 2007; 2009). Interestingly, the
two components of spindle bursts rely on different glutamate receptors: the slow delta-band component relies on n-methyl-D-aspartate (NMDA) and alpha-aminopropionate (AMPA) receptors, while the fast oscillation reflects primarily AMPA receptor activity (Minlebaev et al., 2009). With increasing age, as synaptic connections develop, spindle burst occurrence increases despite decreasing twitch frequency (Marcano-Reik et al., 2010). The subplate, a transient cortical layer during development that forms wide internal connections as well as reciprocal connections with the cortex and thalamus (McConnell et al., 1994; Piñon et al., 2009; Viswanathan et al., 2012), is also likely involved. Indeed, removal of the subplate results in an almost complete loss of spindle bursts (Tolner et al., 2012). In sum, spindle bursts reflect a synaptic network of cortical and subplate neurons that have a small degree of autonomous activity but are normally triggered by ascending sensory inputs from the periphery.

1.3.2.4 Other patterns of activity in the developing cortex

Many other patterns of activity in the developing cortex have been described (reviewed in Allene and Cossart, 2010). Because of differing preparations, ages, and recording techniques, it is difficult to relate these to each other activity (Khazipov and Buzsaki, 2010), but some links can be made. One example is the giant depolarizing potential, which has been extensively studied in the hippocampus but is also present in the cortex (Allene et al., 2008; Allene and Cossart, 2010). Seen as calcium waves in cortical slices, it occurs ever few minutes, rising and falling over a few seconds, and synchronizes a relatively small region of cortex. It is suggested to be the in vitro equivalent of the spindle burst (Allene et al., 2008)
A second example are the so-called *early network oscillations*, slow (tens of seconds) calcium waves in cortical neurons (Garaschuk et al., 2000; Corlew et al., 2004; Allene et al., 2008; Golshani et al., 2009). It has been suggested that these are cellular correlates of the slow activity transients (SAT) described in EEG (Vanhatalo and Kaila, 2006; Seelke and Blumberg, 2008) which are large and slow-waves (~5 s) and can be associated with the twitches and spindle bursts of active sleep. SATs also exist in the rat visual cortex, where they are again related to spindle bursts and are linked to spontaneous retinal activity (Colonnese and Khazipov, 2010).

Recently, sensory-induced gamma oscillations that the developing thalamocortical circuits have been described (Minlebaev et al., 2011; Yang et al., 2012). They have been suggested as a mechanism to synchronize related thalamic and cortical regions, as well as vertical cortical columns, to enhance their connectivity, a hypothesis that will doubtless be explored in the future. These, too, were closely associated with spindle bursts (Yang et al., 2012).

### 1.3.3 Purpose of spindle bursts

Spindle bursts, then, are the primary network activity of the *in vivo* brain, unifying many other patterns of activity (Khazipov and Buzsaki, 2010). It is natural to wonder how they contribute to the primary job of the developing nervous systems – that is, preparing to be functional in maturity. Although our understanding of the function of specific patterns of cortical activity during infancy is, well, in its infancy, there are interesting clues.
First of all, infant sleep is not only actively generated by the brainstem but it is homeostatically regulated, as in adults (Blumberg et al., 2004). The longer infants rats are awake, the greater the stimulus required to keep them awake; furthermore, when sleep restriction ends, they quickly enter a period of vigorous active sleep with many limb movements. Applying a similar logic to that used in adults, it is likely that active sleep has an important function. In addition, spontaneous movements in early human infants has a strong predictive value for long-term health outcomes, suggesting they are a component of an important developmental process (Prechtl, 1997).

1.3.3.1 The role of NMDA receptor mediated activity dependence in brain development

In general, the importance of an active brain during development is clear when one considers the number and complexity of synaptic connections within it. Using activity to refine development of the brain permits an efficient process that is instructed by the existing structure, adapting as necessary to imperfections or outside influences that might alter the normal path of development. Indeed, while initial neural pathfinding and gross patterning of the brain is determined by genetic and molecular features (Tessier-Lavigne and Goodman, 1996; O'Leary et al., 2007), silencing neural activity prevents the normal formation of more precise features (Stryker and Harris, 1986; Löwel and Singer, 1992; McCasland et al., 1992; Catalano and Shatz, 1998).

Much of our understanding of the role of this activity comes from studying the developing visual system. During development, retinal cells shift from projecting widely and imprecisely to projecting retinotopically and in an eye-segregated fashion. This process depends on waves of spontaneous activity that sweep across the retina (retinal
waves) and sequentially activate retinal ganglion cells as well as their targets (Wong, 1999; Firth et al., 2005).

Because retinal waves are localized to small regions of the retinal, they can carry information about the retinotopic location of retinal cells to downstream projection regions – retinal cells with correlated activity are likely to be physically close to each, which those with uncorrelated activity are not. Disrupting the spatial organization of the retinal activity, without changing its overall intensity, confirms the importance of this signal as downstream subcortical regions fail to mature with normally precise visual maps (Xu et al., 2011).

A key point in considering this contribution is the involvement of glutamatergic NMDA receptors. In visual (Daw et al., 1999; Aton et al., 2009; Frank, 2011) as well as somatosensory (Iwasato et al., 2000; Datwani et al., 2002) cortex, for example, normal development depends on functional NMDA receptors. It is generally accepted that this represents an important role for Hebbian synaptic potentiation in the maturation of neural circuits (Cramer and Sur, 1995; Katz and Shatz, 1996; Penn, 2001; Yamamoto and López-Bendito, 2012). Mechanistically, it is known that axonal arbors are highly mobile during synaptogenesis, forming transient synapses with many possible post-synaptic target cells (Wu et al., 1999a; Jontes et al., 2000; Niell et al., 2004). Detection of correlated activity in pre- and post-synaptic cells via NMDA receptors prompts synaptic maturation (Wu et al., 1996; Rajan et al., 1999) which in turn stabilizes the parent branch via Ca\(^{2+}\)-dependent mechanisms similar to those seen in the adult (Wu and Cline, 1998; Rajan et al., 1999; Haas et al., 2006; Ruthazer et al., 2006). Connections between neurons active together are strengthened, and those between unrelated neurons are lost (Cline,
2001; Cline and Haas, 2008). Returning to the example of retinal waves, physically close retinal cells would activate in a correlated fashion, and post-synaptic neurons with multiple nascent synapses from these retinal cells would also activate, strengthening these synapses. In contrast, post-synaptic cells with fewer synapses from cells of this region of retinal would not be strongly depolarized, and theses synapses would progressively weaken.

We will now consider more specifically possible roles for activity in the limb, barrel and visual cortices.

1.3.3.2 Activity dependent development of the limb sensory cortex

A developmental role for spontaneous movements in early life has been recognized for some time with regards to peripheral aspects of the sensory and motor system – for example, without early muscle activity, joint and bone formation is abnormal (Murray and Selby, 1930; Hamburger and Waugh, 1940), and motoneurons (Gould and Enomoto, 2009) and neuromuscular junctions (Sanes and Lichtman, 1999) do not mature. More pertinent to the current discussion, it is clear that limb twitches during development have an instructive role to the nervous system. This was shown directly by manipulating the sensory feedback that arrives following a spontaneous twitch in two-week old rat pups (Petersson et al., 2003). When an air puff was used to artificially stimulate the side of the tail opposite to the movement direction (a reversal of what would occur normally), the rat later made more errors in withdrawing from stimuli applied to the tail. The authors concluded that the sensory feedback provided by the twitch is used to aid in the maturation of pain-withdrawal reflexes.
Could the spindle bursts that accompany these twitches have a similar instructive role in the more complex organization of the cortex? It is well established that, although the gross patterning of the somatosensory cortex may occur independently of activity, refinement relies on activity in the sensorimotor system (Pallas, 2001; Price et al., 2006; Inan and Crair, 2007; Hanganu-Opatz, 2010). In particular, injuring or deafferenting a limb during the first few days of life in the rat results in a failure of the development of the corresponding sensory cortex as well as changes in sensory regions of the remaining limbs (Wall and Cusick, 1986; Dawson and Killackey, 1987; Waters et al., 1990; Pearson et al., 1999).

Transecting the sensory regions of the spinal cord confirms that this effect reflects loss of ascending sensory, particularly proprioceptive, inputs (Jain et al., 2003). While it is difficult to link this process to a particular pattern of brain activity, the altered development of the sensory cortex is only seen if the sensory feedback is disrupted during the first week of life, which overlaps with the peak period of expression of muscle twitches and spindle bursts.

1.3.3.3 Activity dependent development of the barrel cortex

More detailed research has been conducted in the whisker system, where there is a well ordered topographical representation from the whiskers to the cortex (Erzurumlu et al., 2010). As shown in Figure 1.8, the rodent whiskers are organized into five rows (labeled A-E) of four to seven whiskers each. There is a one-to-one representation of the whiskers in the sensory cortex, with the cortical representation of the individual whiskers being called barrels, in reference to their cytoarchitctural appearance (Woolsey and van...
der Loos, 1970). This is also shown in Figure 1.8. The one-to-one correspondence between whiskers and barrels provides a powerful model for examining the relationship between sensory inputs and cortical development (Erzurumlu and Kind, 2001; Inan and Crair, 2007; Fox, 2008; Li and Crair, 2011). As with the limbs, lesioning or trimming the whiskers in early life results in an abnormal development of the corresponding barrel (van der Loos and Woolsey, 1973; Killackey et al., 1976; Killackey and Belford, 1980; Simons and Land, 1987); conversely, mutant mice with additional whiskers develop corresponding barrels, at the expense of the normal barrel representation (Welker and van der Loos, 1986). Removing sensory inputs directly by cutting the infra-orbital nerve (leaving motor innervation intact) results in failure of the thalamo-cortical afferents to become restricted to their individual barrels, confirming the important role of ascending sensory inputs (Jensen and Killackey, 1987). The role of ascending sensory information depends on functional NMDA receptors in the cortex (Iwasato et al., 2000), suggesting it acts via Hebbian learning mechanisms as in the visual system. Again, there is a critical period during the first week of life in which alterations in sensory feedback become reflected in the sensory representation at the cortex (Jeanmonod et al., 1981; Simons et al., 1984; Fox, 1992; McCasland et al., 1992).

Do these adaptive changes reflect a role of spindle bursts in the barrel cortex? There is indirect evidence that they do. The normal development (Fox et al., 1996; Iwasato et al., 2000; Dagnew et al., 2003; Lee et al., 2005) of the barrel cortex, as well as the plastic responses following whisker removal (Schlaggar et al., 1993), depends on glutamatergic transmission via NMDA receptors. A second requirement for the normal
barrel pattern is the intact subplate (Tolner et al., 2012). These same factors are necessary for the expression of spindle bursts (Minlebaev et al., 2009; Tolner et al., 2012).

1.3.3.4 Activity dependent development of the visual cortex

There is also indirect evidence from the visual system that spindle bursts may guide development. We have seen that spindle bursts in the visual cortex result from ascending sensory activity from spontaneous waves of retinal activity (Hanganu et al., 2006). A previously mentioned, it is well recognized that retinal waves are essential to the normal development of the visual system (Wong, 1999; Firth et al., 2005; Torborg and Feller, 2005). Much of this research has focused on the impact of retinal activity on retino-thalamic maturation, but it is clear that it impacts primary and even secondary cortical regions as well (Ackman, 2012). In particular, blocking retinal waves and presumably the subsequent cortical spindle bursts during the first week of life prevents normal ocular dominance column and receptive field formation in ferrets (Huberman et al., 2006) as well as normal precise connections from thalamus to the visual cortex in mice (Cang et al., 2005). Ablation of the subplate, also necessary for spindle bursts, also prevents the normal development of the visual cortex (Ghosh and Shatz, 1994; Kanold et al., 2003). These changes are not reversed by subsequent normal vision (Hooks and Chen, 2007), again implying a critical function for spindle-burst associated patterns of activity in early life.
1.3.3.5 NMDA receptors, spindle bursts, and developmental plasticity

In summary, then, cortical development depends on activity, which refines neural connections via NMDA-receptor mediated Hebbian mechanisms. One source of this activity is via ascending sensory inputs, and the spindle bursts generated by these inputs also depend on NMDA receptors, as described in section 1.3.2.3. An intriguing possibility that follows is that spindle bursts are an essential pattern of activity that underlies the prolific activity dependent plasticity of early life, perhaps by synchronizing related groups of pre- and post-synaptic neurons. Falling spindle burst activity with increasing age could contribute to the closure of critical periods for cortical plasticity (Feldman, 2001; Hensch, 2004; Erzurumlu and Gaspar, 2012).

Processes other than the decrease in spindle bursts are also likely to influence the decreasing plasticity of the system in question. For example, NMDA receptor subunit expression undergoes large changes during the first week of life (Monyer et al., 1994; Flint et al., 1997), to configurations which are less favourable to synaptic potentiation (Erisir and Harris, 2003). Nevertheless, it is reasonable to suggest that spindle bursts represent the network correlate of NMDA receptor mediated activity-dependent plasticity in the developing cortex.

1.4 Large-scale spatial organization of spontaneous activity

We have now seen the characteristics and possible functions of two key forms of spontaneous activity in the adult and infant brain; SWA and spindle bursts, respectively. One stark difference between them is their large-scale organization across the cortex. SWA acts simultaneously across large and unrelated regions of the brain, synchronizing
them with synchronized shifts of membrane polarity; spindle bursts act within a single sensory domain, reflecting activity in the periphery corresponding to that domain.

Does this reflect the supposed functions of these forms of activity? In the case of SWA, these functions include (see section 1.2.3.4) replay of neural sequences between the cortex and the hippocampus, or scaling of synapses modified by prior learning – in either case, functions that could be localized to particular neural systems. In the case of the spindle bursts of the infant brain, they are believed to aid in the development of cortical sensory systems – but such systems depend on tight integration with each other, as well as higher cortical regions, in the adult brain. Is there a more detailed spatial structure of these forms of spontaneous activity that reflects these functions?

1.4.1 The example of resting state connectivity in fMRI

One of the most prominent developments in our understanding of spontaneous activity has come from the discovery that slow patterns of activity within the brain are continually synchronizing functionally related structures. This discovery has brought the large-scale patterns of spontaneous activity into sharp focus and is worth considering briefly.

1.4.1.1 Spontaneous BOLD oscillations are not noise

In 1993, fMRI was first used to study responses in the brain to visual inputs and motor tasks (Kwong et al., 1992; Ogawa et al., 1992). Its appeal was immediately clear, in that the BOLD signal it generates gives an indication of synaptic activity from the entire brain. Since then has been used extensively to study the functional brain systems
that underlie sensory processing, emotion, cognition, and motor outputs (Belliveau et al., 1991; Norman et al., 2006; Logothetis, 2008; Op de Beeck et al., 2008).

Because the BOLD signal increase in response to tasks is small relative to the overall variance of the signal, images of the BOLD signal across the brain during the task at hand are typically compared to images in the absence of the task to extract a clear signal (Raichle, 2009). However, a seminal study in 1995 suggested the background signal being removed was not entirely, or even primarily, noise. It was noted that the BOLD signal was highly correlated between related regions of the brain (in this case, contralateral motor cortices) even in the absence of any task (Biswal et al., 1995). BOLD signals from few other regions of the brain were correlated with that of the motor cortex. Recognizing that is was unlikely that a source of non-physiological noise could contaminate the data in this pattern, the authors, “… concluded that low frequency fluctuation of blood flow and oxygenation is indeed a neurophysiological index.”

1.4.1.2 Spontaneous BOLD oscillations reflect spatial structure of the brain

A second set of key observation came soon after. Using PET, Raichle and colleagues showed that a set of midline cortical areas, primarily the medial prefrontal cortex, the precuneus, and the posterior cingulate cortex, were deactivated when a cognitive task was performed (Shulman et al., 1997), and shortly after, they showed these regions were activated during rest in the absence of any task, leading them to term this activity the default mode of the brain (Raichle et al., 2001). Finally, Greicius and colleagues showed the activity in these regions at rest is highly correlated (Greicius et al., 2003). The high level cognitive functions ascribed to these regions by lesion studies and
other task-basked studies (Gusnard et al., 2001) suggests they form a network of brain regions (since termed the default mode network) that communicate at rest to process memories, plan for the future, or other perform other self-referential tasks (Fox and Raichle, 2007).

Activity, represented by BOLD signals, fluctuates in a coherent way among other related systems of the brain, such as visual, auditory, and motor regions (Fox et al., 2005; Damoiseaux et al., 2006); such regions, examples of which are shown in Figure 1.9, are described as being functional connected or related. In part, this functional connectivity reflects the anatomical connectivity of the brain; comparisons of anatomical connectivity and BOLD signal correlations has shown overlap of these measures (Koch et al., 2002; Vincent et al., 2007; Hagmann et al., 2008; Greicius et al., 2009; Honey et al., 2009), and models of activity within regions of the brain using structural connectivity alone can predict much of the functional correlation in the macaque brain as recorded using fMRI (Honey et al., 2007).

That correlated activity between related regions might also represent a functional structure of the brain is suggested by the observation that activity within these networks can alter behavior, for example by impacting motor outputs (Fox et al., 2007) or sensory perceptions (Monto et al., 2008). In turn, they are altered by learning in a way that reflects the nature of the learning task (Lewis et al., 2009; Albert et al., 2009a; 2009b). They are also reflected in high level behavior, as their coherence is progressively degraded during dementia (Brier et al., 2012), improves along with cognitive function following treatment of epilepsy (Pizoli et al., 2011), and predicts behavior in children (Shannon et al., 2011).
1.4.1.3 Spontaneous BOLD oscillations organize the brain into small world networks

Strong evidence of the physiological significance of correlated fMRI activity also comes from using it to define networks of connected brain regions. Examining this network structure is essential to understanding how the brain balances concurrent needs for segregation and integration of information (Bassett and Bullmore, 2006; Bullmore and Sporns, 2009). An important realization in the study of networks within the brain was that functional regions of the brain are organized into “small-world” networks, characterized by a small number of long-distance connections placed within a network dominated by short, local connections (Watts and Strogatz, 1998).

Small worlds were initially seen by examining networks defined via anatomical connections detected by traditional anatomical techniques (Hilgetag et al., 2000; Sporns et al., 2000; Latora and Marchiori, 2001) and later also by diffusion tensor imaging (Iturria-Medina et al., 2008). Networks defined via correlated fMRI signals, similarly, have small-world properties (Eguíluz et al., 2005; Salvador et al., 2005; Achard et al., 2006; Wang et al., 2009). Such a network strikes a balance between fast local spread of information in a regular network of short connections, and fast long-distance spread within networks of random long connections (Bassett and Bullmore, 2006; Bullmore and Sporns, 2009). The resultant connections allow fast, coherent communication between specialized nodes (Lago-Fernández et al., 2000) even when not directly connected (hence a “small-world”) while balancing the metabolic cost of creating and maintain long distance connections (Bassett and Bullmore, 2006; Kaiser and Hilgetag, 2006).

In addition to giving insight into the network operation of the brain, the reflection of small worlds within networks defined by correlated resting fMRI activity is a good
indicator that fMRI signals are significant. Noise intrinsic to the fMRI signal would be expected to effect signals from all regions of the brain, producing spurious networks with many random long-distance connections; noise from local vasomotor sources would be expected to effect localized regions of cortex, producing networks lacking long-distance connections. The small-world properties of resting state networks, in contrast, are less to reflect sources of noise and it particularly reassuring that they reflect physiological needs of the brain.

1.4.2 Large-scale organization of slow-wave activity in the adult

There is no clear consensus on what resting state networks in the brain do (He and Raichle, 2009b; Koch, 2009; He and Raichle, 2009a; Koepsell et al., 2010), or even what the underlying neural correlate is (Viswanathan and Freeman, 2007; Drew et al., 2008; Nir et al., 2008; Jerbi et al., 2010; Schölvinck et al., 2010). However, they have brought a great deal of attention to the impact that connectivity has on the brain and how examining the large scale structure of activity can reveal underlying features that might otherwise go unnoticed (Friston, 2011). Do similar effects of connectivity hold true for SWA?

1.4.2.1 Evidence for spatial structure in slow-wave activity

First, it is necessary to consider whether a regional structure exists within SWA. Many studies of SWA have emphasized its ability to synchronize large populations of cells across large distances (Steriade et al., 1993c; Contreras and Steriade, 1995; Amzica and Steriade, 1995a; Destexhe et al., 1999; Timofeev et al., 2000; Volgushev et al., 2006; Chauvette et al., 2010; Volgushev et al., 2011) as well as its relationship to underlying
global EEG signals (Dossi et al., 1992; Steriade et al., 1993d; Achermann and Borbély, 1997; Volgushev et al., 2011). It is clear, however, that there exists a finer structure superimposed on this global effect. As early as 1949, it was shown that the slow oscillations of sleep did not have a homogenous distribution on the brain, which was noted to be “…contrary to the opinions of early workers that the cortex acts as a whole.” (Brazier, 1949). A well studied example is the difference in the power of NREM sleep EEG activity between frontal and posterior regions of the cortex following sleep deprivation (Huber et al., 2000; Finelli et al., 2001; Massimini et al., 2004; Münch et al., 2004; Zavada et al., 2009; Leemburg et al., 2010). More precisely, high-resolution recordings of slow-waves using high-density surface (Massimini et al., 2004) EEG showed the SWA traveled in a posterior direction across the cortex and modeling of the origin of EEGs revealed preferred points of origin in frontal midline areas (Murphy et al., 2009). And finally, intracranial depth EEG from regions across human brain showed many slow-waves were local or traveled from anterior to posterior regions of the brain (Nir et al., 2011; Botella-Soler et al., 2012).

1.4.2.2 Slow-wave activity and the spatial structure of the brain

How do these regional patterns of activation relate to the large-scale structure of the brain? There is relatively little research on the topic of SWA and the large-scale anatomical structure of the brain. One indication that there is a role for intracortical connections in SWA comes from the observation that disrupting such fibers reduced the correlation between cortical regions on opposite sides of the disruption (Amzica and Steriade, 1995b). Another indication comes from a particular strain of acallosal mice, in
which the normal callosal fibres are re-routed during development resulting in enhanced intrahemispheric connectivity and reduced interhemispheric connectivity. In these mice, correlations of SWA between hemispheres are reduced (Vyazovskiy et al., 2004) while the power of SWA within each hemisphere was increased (Vyazovskiy and Tobler, 2005). And as seen earlier when discussing NREM sleep and learning (section 1.2), SWA can reflect the functional architecture of the brain; following learning, SWA can increase or decrease within localized functional systems (Huber et al., 2004; Iwasaki et al., 2004; Huber et al., 2006; Hanlon et al., 2009). Finally, a good indication that there is a regional structure to SWA comes from rats and cats raised from birth in darkness. These animals have decreased SWA in the visual cortex but normal SWA elsewhere (Miyamoto et al., 2003).

Additional indirect evidence for a spatial structure in SWA comes from fMRI studies of BOLD oscillations during NREM sleep. Slow-waves are associated with BOLD signals in areas very similar to the default mode network (Dang-Vu et al., 2008; Murphy et al., 2009), including within the cingulate (Murphy et al., 2009). These structures described as are highly interconnected and have been described as a ‘structural core’ of the cortex and the BOLD signals in the regions during slow-waves suggest a role for generating or directing their activity (Hagmann et al., 2008; de Pasquale et al., 2012).

1.4.2.3 Small world networks in slow-wave activity

As described above, SWA is primarily understood as a global (Volgushev et al., 2011) or travelling (Massimini et al., 2004) phenomenon, and thus relatively little research has addressed its network structure. The first study to do so was in 2004, using
magnetoencephalopathy to obtain widespread signals of cortical activity during waking. Examining the coupling between these signals showed that slow activity, under 8 Hz, organized the cortex into a small-world network, with high clustering among local signals but also relatively low path lengths from one cluster to another (Stam, 2004). Slow-wave activity during sleep was examined specifically using EEG, and similar results were found (Ferri et al., 2007; 2008). Recording from a small number (19) of electrodes, these studies provided only overall measures of network clustering and path length – they did not attempt to link coupling of EEG signals to anatomical connections. Nevertheless, the finding of small-world characteristics is consistent with a role for anatomical connections in shaping SWA activity as described in section 1.4.2.2.

1.4.3 Large-scale organization of activity in the developing brain

There are good *a priori* reasons to think that spontaneous activity in the developing brain could have a large-scale spatial structure as opposed to being isolated within localized regions. During development, the cortex progresses from being weakly connected to being richly connected into sets of hierarchical networks (Sporns et al., 2004; Power et al., 2010). As discussed in section 1.3.3, it is clear that activity-dependent processes are involved in this formation of localized connections between neurons, and it is likely that that are involved in the formation of long-distance connections also. An early example was the absence of the corpus callosum in animals in which the eye was removed in infancy (Rhoades and Fish, 1983; Rhoades et al., 1984). Elimination of sensory inputs to the whisker system in early life similarly impairs formation of the corpus callosum from the corresponding homotopic barrel (Koralek and Killackey, 1990).
More specifically, blocking synaptic activity in developing callosal neurons during the first weeks of life via expression of potassium inward rectifiers (Mizuno et al., 2007) or tetanus toxin (Wang et al., 2007) results in an impaired or absent callosum in the adult. Intra-hemispheric connections are also impaired by elimination of sensory inputs; the abnormalities in the barrel and visual cortex that are seen in the absence of whisker or retinal inputs in early life involve reduced levels of intracortical projections within related sensory regions (McCasland et al., 1992; Ruthazer and Stryker, 1996; Rhoades et al., 1997; Dagnew et al., 2003).

1.4.3.1 Resting state networks in infancy

If activity were necessary for the development of long-distance projections between cortical areas, we would expect that cortical areas connected in adulthood are active together in infancy. One strategy to test this is to examine correlations of activity in fMRI. As in adults, BOLD signals are correlated within related systems of the brain as young as 30 weeks gestation (Kiviniemi et al., 2000; Lin et al., 2008; Smyser et al., 2010). These are organized in consistent networks across infants, (Fransson et al., 2007; 2009; 2011), and an incomplete version of the default-mode network can be observed soon after birth (Gao et al., 2009). These networks have small world properties shortly after birth (Yap et al., 2011) which become more pronounced with age (Fair et al., 2009). These findings must be interpreted cautiously, though, not only because coupling between brain activity and blood-flow is not robust in early life as in adulthood (Colonnese et al., 2008) but also because the discontinuous, externally driven brain
activity of early life very different from the continuous, internally generated activity of adulthood (Colonnese and Khazipov, 2012)

1.4.3.2 Reports of activity outside of primary sensory cortices

An additional reason for caution is that we know very little about developmental activity outside of the primary sensory regions during development. In fact, there are only a few reports in the literature about such activity. One recent study collected patterns of activity from midline areas of the rat brain during the first two weeks of life, reporting bursts of fast activity in the gamma-range, sharp-biphasic bursts, and SATs (Seelke and Blumberg, 2010). It is not known how these patterns relate to the spindle-bursts of the sensory cortex, or to sensory events in the periphery. A second recent study collected activity from the medial prefrontal cortex of the rat (Brockmann et al., 2011). They found spindle bursts developed in this region shortly after birth, although later than in primary sensory regions. Later, at about two weeks of age, spindle bursts were replaced with continuous ongoing activity. Again, it is not known how this activity related to other patterns of activity in the brain or the periphery.

1.4.3.3 Small world networks in the developing brain

As in the adult brain, diffusion tensor imaging (Hagmann et al., 2010; Yap et al., 2011; Tymofiyeva et al., 2012), measures of cortical thickness (Fan et al., 2011; Khundrakpam et al., 2012), and correlations of resting fMRI signals (Fair et al., 2009; Supekar et al., 2009; Gao et al., 2011) have been used to study the network organization of the infant and child brain. By both anatomical and functional measures, the brain
networks have small-world properties from birth onwards. These findings are seen as significant because they suggest that efficient, effective network organization is an intrinsic property of the cortex, as well as providing the possibility of early prediction of developmental outcomes following neonatal hypoxia (Tymofiyeva et al., 2012).

1.5 Studying the spatial detail of spontaneous activity in infancy and maturity

1.5.1 Use of voltage-sensitive dyes to study spontaneous activity in the rodent cortex

Questions surrounding the spatial structure of SWA in the adult brain and spindle-burst activity in the developing brain have, in a sense, opposite motivations; in the case of SWA, it is likely there is spatial detail superimposed on the its wide-spread synchronizing effect on the brain, while in the case of spindle bursts, there could be synchronous effects beyond the limited role within sensory systems that is currently recognized. Put another way, SWA across the cortex is likely to be less uniform than currently known, and spindle bursts more uniform.

Nevertheless, both questions converge on a similar solution, namely collecting signals of neural activity from large areas of the cortex at high spatial and temporal resolution. To do this, I have used voltage-sensitive dye (VSD) imaging in the developing rat and the adult mouse.

1.5.1.1 Voltage-sensitive dye imaging

VSD imaging employs dyes that bind to cell membranes and change their fluorescence behavior depending on the surrounding voltage environment. Thus by
exciting these dyes and measuring the emitted fluorescence, they can be used to transduce membrane potential to optical signals (Grinvald and Hildesheim, 2004). They were first used to record signals or activity from isolated nerves (Salzberg et al., 1973; Cohen et al., 1974; Tasaki and Warashina, 1976), followed by imaging from populations of invertebrate neurons (Grinvald et al., 1977; 1981) and mammalian cortex (Orbach et al., 1985). A major advance on this front was the development of dyes who were excited by wavelengths of light that did not overlap with the wavelengths absorbed by hemoglobin, dramatically reducing the contamination of the signal by heartbeat noise (Shoham et al., 1999).

The spatial and temporal resolution of VSD is limited only by the sensitivity of the imaging apparatus, which in current practice is in the millisecond range temporally and the micron range spatially. Noteworthy weaknesses are that VSD stains all membranes, including glia, providing a high background signal, a need for to expose the cortex to apply the dye, and a lack of ability to resolve vertical contributions of the signal from different cortical layers. Nevertheless, it provides fast and high resolution signals of neural activity and has been used to study cortical dynamics in a large range of ways (Grinvald and Hildesheim, 2004; Petersen, 2006; Homma et al., 2009; Peterka et al., 2011).

1.5.1.2 The rodent cortex

One example of using VSD imaging to study cortical activity is shown in Figure 1.10. Here, the activity within sensory and motor cortices is recorded after stimulation of the rodent whisker. The size of the rodent cortex is well suited to VSD imaging, as it is
small enough that signals can be collected from many regions. A noteworthy example is the signal recorded from the motor cortex, which begins shortly after the signal in the sensory cortex. In the rodent, strong excitatory connections exist between the barrel and the motor cortex (White and DeAmicis, 1977; Farkas et al., 1999; Ferezou et al., 2007) which have important functions for object detection (Ferezou et al., 2007; Petersen, 2007; Matyas et al., 2010). These signals are precisely projected so that each whisker excites a defined region of the motor cortex. Because of the precise nature of this connection, the rodent whisker system has emerged as an important model for the study of cortical dynamics and integration of sensory and motor activity (Kleinfeld et al., 2006; Petersen, 2006; 2009).

1.5.2 Structure of slow-wave activity in the adult mouse cortex

Although they were first described in the cat, oscillations between UP and DOWN states are prominent in the rodent cortex, both under anesthesia (Petersen et al., 2003; Ruiz-Mejias et al., 2011; Crunelli et al., 2012) and when awake (Poulet and Petersen, 2008; Haider and McCormick, 2009; Luczak and MacLean, 2012; Poulet et al., 2012). In Chapter 2 of this thesis, I use VSD imaging to study the spatial layout of this activity at high resolution in awake and anesthetized animals. I show that activity within related functional systems, including the sensorimotor circuits of the whisker system and midline associative areas, is highly correlated, revealing a functional structure overtop of the cortex-wide synchronized effect of SWA.
1.5.3 Structure of spindle-related activity in the developing rat cortex

Spindle bursts were first recorded from the developing rat, and much of the subsequent work has been performed in rat pups during the first weeks of life in both naturally sleeping and anesthetized states (Blumberg, 2010; Khazipov and Buzsaki, 2010).

1.5.3.1 Spindle-related activity in the limb and tail sensory cortex

In chapter 3 of this thesis, I examine spontaneous activity in the limb and tail sensory cortices of the anesthetized rat pup. Here, the aim was to determine whether activity in the somatosensory cortex was associated with activity in other parts of the cortex. A prominent observation was that spindle-related activity on the cortex spreads medially, suggesting a role in developing connections between sensory and midline regions of the brain.

1.5.3.2 Spindle-related activity in the barrel and motor cortices

In chapter 4 of this thesis, I examine more closely the possibility that spindle-related activity in sensory cortex synchronized with non-sensory areas of the brain. Given the well-known excitatory connections that exist between the motor and sensory components of the adult whisker system, I recorded spontaneous activity from these regions at a time when these connections between them are being formed, during the first two weeks of life. I also recorded from these areas following stimulation of the whisker. The goal was to determine whether correlations of activity between these regions was limited by the maturation of their excitatory connections, or whether they could be
activity together spontaneously prior to this time, suggesting a possible role in the development of the connection.
1.6 Figures

Figure 1.1 Sleep stages and their characteristics. a) Surface EEG recordings show transition from waking to NREM and subsequently REM sleep. An individual spindle is highlighted. b) Sleep stages over an entire sleep cycle. Section a) is shown in more detail above, section c) below. c) REM sleep is characterized by rapid eye movements not present in NREM sleep. From Pace-Schott and Hobson, 2002. Used with permission.
Figure 1.2. Slow oscillation as recorded from the somatosensory cortex. Note the periodic shifts in membrane potential seen intracellularly and reflected in the depth and surface EEG. From Amzica and Steriade, 2002. Used with permission.
Figure 1.3. The cortical slow oscillation can group thalamic rhythms. Left column, traces show field potential from cortical area 5 (panels A and B) and intracellular recordings from reticular thalamic neurons (RE, panel A), thalamocortical neuron (TC, panel B), or corticothalamic neurons (Cx, both panels). Neurons and their connections are illustrated in right column. Lowercase letters represent synaptic projections (right column) or the effects of these projections (left column) and correspond between right and left columns. A) UP state in cortex (downward deflection) prompts depolarization in RE neuron and subsequent bursting, which is projected to TC neurons and ultimately reflected in cortex as spindles. B) UP state in cortex depolarizes TC cell, initiating delta oscillation via interactions of sodium and calcium currents. This is projected to the cortex as delta oscillations. From Steriade and Timofeev, 2003. Used with permission.
Figure 1.4. Generation of UP and DOWN states in cortical cells. An initial excitation (7 o’clock position; from afferent input or intrinsically active cell, see text for details) triggers recurrent synaptic excitation in the cortical network (11 o’clock position). Over time, activity dependent adaptation currents prompt DOWN state and after-hyperpolization (3 o’clock position). As these recover, the cycle repeats. From Compte et al., 2003. Used with permission.
Figure 1.5. Possible learning related functions to slow-wave sleep (SWS). a) Synaptic downscaling during sleep. Synapse strength increases with new experiences during waking, and new synapses are formed (indicated by yellow synapses at the end of waking showing increased synaptic weight (W)). They are downscaled during subsequent sleep. b) Slow oscillations synchronized hippocampal sharp wave-ripples and thalamic spindles, which drive coordinated replay in the cortex. Subsequent REM episodes may enhance plasticity. See text for details. From Diekelman et al., 2010. Used with permission.
Figure 1.6. Waking (W), quiet sleep (QS), and active sleep (AS) in an eight-day old rat. Upper trace shows muscle tone. Note the onset of atonia with quiet sleep, and the progression to active sleep characterized by limb twitches. From Blumberg, 2010. Used with permission.
Figure 1.7. Movements in human or rodent infants generate bursts of cortical activity. A) Simultaneous hand movement (via piezo-electric device) and EEG from premature human infant (30 week post-conception). Note the bursts of activity following hand movements. B) Similar relationship between movement and cortical activity shown in a two-day old rat pup. Hindlimb movements recorded via acoustic device. Activity in somatosensory cortex (S1) recorded with field potential. C) Bursts consist of fast activity nested within slower delta-range wave. Field potential recording from four-day old rat pup, filtered at different frequencies. Panel A from Milh et al., 2007, panel B from Khazipov and Luhmann, 2006, panel C from Minlebaev et al., 2009. Used with permission.
Figure 1.8. Organization of the rodent whisker system. A) Stimulation of a single whisker causes activation of an individual barrel, shown in yellow. The motor cortex is subsequently excited, as discussed in section 15.1.2. B) Whiskers are organized into five rows of four to seven. C) At the cortex, individual barrels receive afferents from individual whiskers and are labeled accordingly. From Aronoff et al. 2010. Used with permission.
Figure 1.9. Spontaneous BOLD oscillations within functionally related regions are correlated. A) Analysis of PET signals in the resting and engaged brain reveals a set of regions that decrease their activity during tasks, called the default mode network. Upper panel shows these regions from a lateral (left) and medial (right) aspect. Lower panel shows spontaneous BOLD oscillations from two of these regions (shown in yellow and orange arrows in A. Note their synchronous fluctuation. B) Similar analysis reveals that BOLD oscillations are correlated within other functional networks of the brain. Six of these networks are illustrated. Each slice shows the regions of the brain whose BOLD signal is correlated with the region shown on the brain in the center. Panel A from Raichle and Snyder, 2007, panel B from Zhang and Raichle, 2010. Used with permission.
Figure 1.10. VSD images provide high spatial and temporal resolution of activity. A) Whisker stimulation in the adult mouse activates both sensory cortex (S1, red and orange) and motor cortex (M1, blue and purple). Note the distinct regions activated by C2 and E2 whisker. S2 represents secondary somatosensory cortex. B) VSD images showing activity across hemisphere. Times following whisker stimulation shown. Scale bar represents percent fluorescence change. From Aronoff et al. 2010, used with permission.
CHAPTER 2. SLOW-WAVE ACTIVITY IN THE ADULT MOUSE REFLECTS FUNCTIONAL CORTICAL SYSTEMS

2.1 Introduction

As I have shown in the preceding introduction, the brain is never at rest, even when the body may be. In fact, during periods of inactivity the brain remains remarkably active (Raichle, 2006; Duyn, 2011). One prominent form of such activity is delta-waves, oscillations of 1-4 Hz found during the deeper stages of non-REM sleep (termed slow-wave sleep) (Davis et al., 1937; Amzica and Steriade, 1998; Riedner et al., 2011). In quietly-resting awake animals, similar oscillations have been observed to synchronize sensory regions of the cortex (Petersen et al., 2003; Crochet and Petersen, 2006; Poulet and Petersen, 2008; Haider and McCormick, 2009).

Underlying slow-waves in both awake and sleeping animals is the slow oscillation, oscillations of intercellular membrane potentials during which population of neurons oscillate between bursts of activity and silence (see section 1.2.1.3). These two states, called the UP and DOWN states respectively, last hundreds of milliseconds to a few seconds each (Steriade et al., 1993c; Destexhe et al., 1999; Steriade et al., 2001; Petersen et al., 2003; Crunelli et al., 2012) and result from connections intrinsic to the cortex (Sanchez-Vives and McCormick, 2000; Timofeev et al., 2000) with modulation from the thalamus (Hughes et al., 2002; Rigas and Castro-Alamancos, 2007; Crunelli and Hughes, 2010). Although the UP and DOWN oscillations synchronize nearly the entire cortex (Vyazovskiy et al., 2011), there is a distinct spatio-temporal pattern to their
initiation (Luczak and MacLean, 2012) such that a networks of cells activates in a particular order during the transition between states (Mao et al., 2001; Cossart et al., 2003; Luczak et al., 2007; Peyrache et al., 2010). These distinct patterns are very similar to the patterns of activation following sensory stimulation (Watson et al., 2008; Jermakowicz et al., 2009; Luczak et al., 2009).

There is spatial and temporal structure to the slow oscillation at the cortical level also. Slow-waves do not occur synchronously across the cortex, but usually begin in the anterior frontal regions (Huber et al., 2000; Finelli et al., 2001; Münch et al., 2004; Zavada et al., 2009; Leemburg et al., 2010) and travel to the posterior of the cortex (Massimini et al., 2004). Furthermore, sensory stimulation (Kattler et al., 1994; Vyazovskiy et al., 2000) and motor learning (Huber et al., 2004; Vyazovskiy and Tobler, 2008; Hanlon et al., 2009) increase subsequent slow-wave activity locally in related cortical regions, while forced immobility (Huber et al., 2006) decreases it.

There is some evidence that this regionalization extends to large-scale circuits at the cortical level. The spatial layout of spontaneous activity in the visual cortex reflects functional connections, such that spontaneous patterns of activity are similar to evoked patterns in the visual cortex (Kenet et al., 2003) and can reflect previous visual experiences (Han et al., 2008). A similar overlap between spontaneous and evoked patterns has been seen in the barrel cortex of the mouse (Ferezou et al., 2006).

One influential hypothesis that explains these spatial and temporal details of slow-wave activity is that it is involved in synaptic reorganization and downscaling following synaptic potentiation that occurs during waking (Tononi and Cirelli, 2003; 2006). A second idea is that slow-waves group the replay of previous neural activity within
systems of the brain, to facilitate synaptic strengthening (Sutherland and McNaughton, 2000; Schwindel and McNaughton, 2011). In either case, slow-wave activity would be involved in the maintenance or adjustment of long-range cortical connections, and demonstration of slow-wave activity resonating within such long-range circuits would support this idea. On the other hand, homogeneity of slow-wave activity across and within hemispheres would not support the idea that they are involved in adjusting synaptic strengths within cortical connections. In this study, we sought to explore this topic by recording slow-wave activity across very wide regions of the cortex with high spatial and temporal resolution. We accomplished this by using VSD (Shoham et al., 1999; Grinvald and Hildesheim, 2004) applied to a large (7 x 7 mm) bilateral region of cortex exposed in the mouse. In contrast to the studies discussed above which examined activity within one or two functional domains of the cortex, we recorded activity within multiple domains in both hemispheres simultaneously. We find that, while slow-wave activity from disparate regions of the cortex is synchronized, the fine-scale spatial resolution provided by VSD imaging reveals a specific spatial structure than reflects cortical circuits.

2.2 Methods

2.2.1 Animal model.

We used adult male C57BL/6J mice (>2 months of age) for the experiments we describe, with the approval of the University of British Columbia Animal Care Committee. We induced anesthesia with urethane (0.12% w/w) or isoflurane (0.5–2% mixed in O₂) for anesthetized and awake recording, respectively. Body temperature was
maintained at 37 °C. A single cranial window was made over both mouse cortical hemispheres (relative to bregma, 2-2.5 mm anterior to 3.5-4 mm posterior; and 3.5-4 mm lateral on either side). Lateral craniotomies (Figure 2.12) were performed unilaterally (relative to bregma 2.5 mm anterior to 4.5 mm posterior; and from bregma to 5 mm laterally) on the right hemisphere.

2.2.2 VSD imaging.

For in vivo VSD experiments, we carefully removed the dura within the craniotomy window. We applied the dye RH1692 (Optical Imaging) (Shoham et al., 1999), dissolved in HEPES-buffered saline at an optical density to the cortex for 90 min. To minimize movement artifacts, we sealed the brain with 1.5% agarose made in HEPES-buffered saline and a glass coverslip. We collected images at 150 Hz using a digital camera (1M60 Pantera, Dalsa) and EPIX E1DB frame grabber along with XCAP 3.2 software (EPIX). VSD was excited with a red light-emitting diode (LED) (Luxeon K2, 627 nm) and images were taken through a macroscope composed of front-to-front video lenses (8.4 X 8.4 mm field of view, 65 µm per pixel). The entire imaging apparatus was located in a darkened enclosure isolated from external light and sound.

When collecting activity evoked by stimulation of the limb, we illuminated the LEDs and began collecting images 100 ms later. Two hundred ms after the initiation of the camera, a single pulse of stimulation (~200 µm displacement over ~10 ms) was given to the limb using a custom-built piezoelectric device (Harrison et al., 2009) and image collection was stopped 720 ms later (108 frames total). The stimulator was attached to the dorsal surface of the paw between the second and third digits. We also collected identical
sets of images with no stimulation, to correct for time-dependent decreases in the VSD signal. An overall sequence of images in response to stimulation was generated for each mouse by taking the mean of 5-10 individual responses and filtering this mean with a Gaussian filter of 1 pixel standard deviation, using custom-script in ImageJ (Harrison et al., 2009). To prevent changes in responses related to repetitive stimulation, we stimulated at most once every 9 s.

When collecting spontaneous cortical activity, we illuminated the LEDs and began collecting images 100 ms later. We collected images in 33.3 s (5000 frame) or 26.7 s (4000 frame) epochs. We filtered the images using a Butterworth zero phase-shift band-pass filter (0.5-6 Hz) and spatially filtered using a Gaussian filter of 1 pixel standard deviation. Change in fluorescence intensity at time F was isolated from background signal by calculating \((F - F_0)/F_0\) where \(F_0\) is the average fluorescence signal. Finally, we reduced sources of noise by processing using principle-component analysis of each 5000 frame epoch and reconstructing the epoch using the first 40 principle components (Reidl et al., 2007). We combined these epochs into sequences representing 6-10 min of activity for each pup and conducted our analysis on these aggregated image sets. For simplicity, we use the terminology the ‘VSD signal’ to refer to the percent change from background of the VSD signal.

2.2.3 Recording behavior in awake animals during VSD imaging.

Separate groups of mice were used for awake and anesthetized recordings. We surgically attached a microconnector (CLP-106-02-FDH; Samtec USA) on the skull of young (6 – 8 weeks old) male C57BL/6 mice (n = 5) for immobilization by connecting to
a fixed connector. After 4 d of recovery, we began training that consisted of gradually increasing time periods restrained over a period of 2-3 weeks. Once familiar with the recording apparatus, we performed a craniotomy under isoflurane anesthesia (0.5–2% maintenance mixed with oxygen) as described in section 2.2.2 and then transferred the mouse to an awake imaging setup in which the head was restrained under the camera and the body was in a relaxed posture. An analgesic, bupremorphine, was injected (0.075 mg/kg, i.p.) 8 h before awake VSD recording to minimize pain upon waking.

To wake the animals, the isoflurane and oxygen were stopped, the anesthesia mask was removed, and VSD imaging data was obtained over the next 2–3 h. We eliminated visual, olfactory, or auditory stimulation during the time of imaging. The 627 nm LED light used for VSD excitation or the infrared light used for behavioral observation did not result in a visual response, and data from the first second of awake imaging was discarded as a precaution against visual stimulation. We used two behavioral monitoring cameras to confirm that the animals were indeed awake and relatively unstressed since whisking, feeding, and drinking were observed. We used the second Dalsa 1M60 camera (75 or 150 Hz) to capture whisker movements under infrared illumination. Because we were interested in resting activity in the absence of sensory inputs, we removed any periods of behaviour. We examined synchronized videos from two infrared cameras to eliminate any periods of gross movements. We also examined light level changes detected from 325 µm X 325 µm regions over whiskers to detect any small behavioural movements and eliminated these periods.
2.2.4 Data analysis

To measure the correlations between cortical regions, both cortical hemispheres were divided into 10 regions, shown in Table 2.1. All cortical regions were identified based on stereotaxic coordinates and their stereotyped position relative to the hindlimb sensory cortex located by stimulation. In most experiments, the functional locations of the left and right forelimb was also collected and used to confirm these placements. VSD signals for reach region were extracted from a 5 x 5 pixel (0.325 µm) region centered on the region’s coordinates.

To create correlation maps of activity as recorded by VSD, we calculated the pairwise correlation between the ROI VSD signal and the VSD signal from each other pixel. The value of the correlation was placed back into the position of the non-ROI pixel, as shown in Figure 2.6. When comparing correlation maps calculated from different seed

<table>
<thead>
<tr>
<th>Region</th>
<th>A/P position relative to hindlimb S1</th>
<th>M/L position relative to hindlimb S1</th>
</tr>
</thead>
<tbody>
<tr>
<td>anterior cingulate (dorsal part) and anterior segment of sondary motor cortex (AC/M2)</td>
<td>1.43 mm A</td>
<td>1.04 mm M</td>
</tr>
<tr>
<td>forelimb primary somatosensory cortex (FL)</td>
<td>0.91 mm A</td>
<td>0.91 mm L</td>
</tr>
<tr>
<td>anterior cingulate (dorsal and ventral part) and posterior segment of sondary motor cortex (pM2)</td>
<td>0 mm</td>
<td>1.04 mm M</td>
</tr>
<tr>
<td>hindlimb somatosensory cortex (HL)</td>
<td>0 mm</td>
<td>0 mm</td>
</tr>
<tr>
<td>primary barrel cortex (BC)</td>
<td>1.43 mm P</td>
<td>1.56 mm L</td>
</tr>
<tr>
<td>parietal association cortex (PT)</td>
<td>1.170 mm P</td>
<td>0.455 M</td>
</tr>
<tr>
<td>retrosplenial cortex (RS)</td>
<td>1.495 mm P</td>
<td>1.04 mm M</td>
</tr>
<tr>
<td>primary visual cortex (V1)</td>
<td>2.34 mm P</td>
<td>0.845 mm L</td>
</tr>
<tr>
<td>sordary visual cortex (V2)</td>
<td>2.34 mm P</td>
<td>0.195 mm M</td>
</tr>
<tr>
<td>primary motor cortex (M1)</td>
<td>1.625 mm A</td>
<td>0.325 mm M</td>
</tr>
</tbody>
</table>

Table 2.1. Cortical regions and positions. A, anterior, P, posterior, M, medial, L, lateral.
regions, or calculated from spontaneous and evoked activity, we linearized the image, removed the non-signal (mask) portion, and calculated the correlation.

When calculating the frequency of bursts of VSD activity, we used a threshold of 0.1 % ΔF/F. To determine the number of independent peaks of activity as shown in Figure 2.12, we thresholded each image at the 60th percentile. Regions larger than 0.2 mm² were classified as bursts.

2.2.5 EEG recording.

A Teflon-coated chlorided silver wire (0.125 mm) was placed on the cortical surface. A reference electrode was placed on the nasal bone. The cortical signal was amplified and filtered (0.1–1000 Hz) using a differential alternating current amplifier.

2.2.6 Statistical analysis.

Correlation values were calculated using Pearson’s method. Correlation values were corrected using Fischer’s z-transformation before statistical analysis. ANOVA, one- or two-way as appropriate, was used to compare the frequency of spontaneous activity across different cortical regions and between waking and anesthetized states. In this and subsequent chapters, p values associated with ANOVA refer to main effects. Whenever possible, exact p values are reported as suggested in statistical guides (Bailar & Mosteller, 1988). F- and t-statistics were examined but are not generally reported. We adjusted for multiple comparisons using Tukey’s method when comparing means directly. As suggested in statistical guides, exact p values are reported (Bailar &
Mosteller, 1988) and values are reported as mean +/- standard error unless otherwise stated (Cumming et al., 2007).

2.3 Results

In this study, we recorded VSD signals from large regions of the bilateral mouse cortex in the anesthetized and awake states. Figures 2.1 (anesthetized) and 2.2 (awake) show examples of the images we collected, and the signals that we generated from functional regions as described in sections 2.2.4. In the awake animals, we also collected video of whisker movements, as well as video of the entire animal, so that we could eliminate times when the animal was active.

2.3.1 Bursts of depolarization correlated between and within hemispheres

In both states, we found prominent bursts of depolarization across the exposed cortex. The frequency of these bursts was lower in the awake animals, as shown in Figure 2.3. In the anesthetized state, the frequency of bursts ranged from 1.02 +/- 0.04 Hz in the retrosplenial cortex to 0.711 +/- 0.03 Hz in the visual cortex; in the awake animal, bursts were again most frequent in the retrosplenial cortex (0.850 +/- 0.06 Hz) while least frequent in the barrel cortex (0.675 +/- 0.03 Hz). Two-way ANOVA showed a significant effect of both region (p = 0.0082) and state (p = 3.08 x 10^-8) on frequency. In this analysis as well as all subsequent interhemispheric analyses, we took the mean of the right and left values after first confirming hemisphere selection had no significant effect of the value in question using a two-way ANOVA.
A prominent feature of the activity we recorded was interhemispheric synchrony. Figure 2.4A shows the homotopic correlation values for each of the ten regions we examined, in the awake and anesthetized states. In both states, the regions most correlated with their homotopic region were along the midline. In the awake animals, this was pM2 (0.900 +/- 0.0046), and in the anesthetized animals the most correlated was RS (0.956 +/- 0.0019). Also in both states, the least correlated with homotopic regions were posterior lateral regions. In the case of the awake animals, this was BC (0.69 +/-0.0021) while it was V1 in the anesthetized animals (0.65 +/-0.013). The homotopic correlation was lower in the awake state for all regions except FL and V1. Overall, the effects of state and region were both significant (two-way ANOVA, p = 0.0012 and p = 2.9 x 10^-13), respectively.

Signals were also strongly correlated within hemispheres. Figure 2.4B shows the correlation value for each cortical region with cortex along a circle of radius 1, 2, and 3 mm (Figure 2.4B, top, middle and bottom respectively). In both awake and anesthetized states, the correlation decreased for all cortical regions at higher distances (two-way ANOVA, effect of radius, p < 10^-10 in both states), but this decrease was most marked in primary sensory regions (FL and HL) and least in anterior medial regions (M1 and aM2/AC). In contrast to homotopic correlations (Figure 2.4A), interhemispheric correlations were higher in the awake animals and this difference was significant (three-way ANOVA, effect of state, p = 1.92 x 10^{-9}).
2.3.2 Cortical regions are not uniformly correlated with each other

We next sought to determine if there was a spatial structure to the correlation between brain regions. In Figure 2.5, we show the pair-wise correlation between VSD signals from functional regions (this is in contrast to Figure 2.4, in which correlations are presented for all points at a given distance). In the anesthetized animals, there is a clear band of high correlations between homotopic regions (demarcated by black circles); consistent with Figure 2.4A, this is less marked in the awake animals. In panels B and C, we show the pairwise correlations for VSD signals from two regions (HL and PT) with signals from each other region. The values on these bar graphs are equivalent to the values shown in the grid of Figure 2.5A along the rows labeled B (for HL) and C (for PT). For both regions, the highest correlation is with the homotopic region, and there was no significant effect of right vs left hemisphere in the correlation value with each other region but a strong effect of cortical region (two-way ANOVA, effect of hemisphere, p > 0.05; effect of cortical region, p < 10^{-8}). In the case of correlation with PT, other regions were nearly as highly correlated as the homotopic region (RS and pM2, for example). Panels D-F are identical to A, except representing data from the awake animals. Similar to the anesthetized animals, there was no effect of right vs left hemisphere but there was an effect of cortical region (two-way ANOVA, effect of hemisphere, p > 0.05; effect of cortical region, p < 10^{-12}).

2.3.3 Correlation maps provide precise representation of correlated activity across the brain

Examining the results presented in Figure 2.5, it is clear there are differences in the spatial pattern of correlation relative to each cortical region (for example, the
relatively focused homotopic correlation seen with HL compared to the broader
correlation seen with PT). To examine these differences more closely, we created
correlation maps for each cortical region, in which each pixel represented the correlation
between the VSD signal collected at that pixel and the VSD signal collected from the
seed pixel (Figure 2.6A). Examples of these maps from an anesthetized and awake
animal are shown in Figure 6B and D. In the top row are maps based on seed pixels in
primary sensory regions – in the lower row, maps are based on seed pixels in medial
association areas. There is a noteworthy similarity between the maps of the lower row. In
panels C and F, contour outlines of the correlation maps at 0.85 (anesthetized) and 0.9
(awake) are shown. Outlines of correlation maps from barrel cortex are included – these
maps shown in Figure 2.10. Note that the outlines of the medial regions overlap along a
bilateral anterior-posterior region, while the outlines of the sensory regions occupy more
distinct territories.

To quantify the differences between these correlation maps, we calculated the
pairwise correlation between the correlation map generated from each cortical region.
These correlation values are shown in Figure 2.7. The layout of this Figure is similar to
that of Figure 2.5, except correlations between correlation maps are presented, rather than
between VSD signals. As in Figure 2.5A, Figure 2.7A shows a clear band corresponding
to homotopic correlations in the anesthetized state, which is present but weaker in awake
animals also (panel D). Panels B shows the pair-wise correlations between the HL
correlation map and other maps, and Panel C shows the correlation between the PT
correlation map and other maps, respectively. Note the high correlation between the PT
correlation maps and maps generated from other midline regions (aM2/AC, RS, M2); and
low correlation between PT maps and primary sensory regions (FL, HL, BC, and V1). In contrast, the HL correlation maps are strongly correlated with the map from their homotopic region, and less correlated with all other maps. Panels D-F are identical to panels A-C except representing data from awake animals. Note that the correlations with the HL correlation map are generally increased, corresponding to the less focused regions of high correlation as seen in Figure 2.6 D and E. Note also that the correlations between the PT correlation map and maps from other midline regions are higher than with sensory regions, as in the anesthetized animals.

Figure 2.8 directly compares these the overlap of correlation maps in the awake vs. the anesthetized states. Panel A shows the correlation between the correlation maps created from each pair of homotopic regions. Correlation maps created from homotopic medial association regions (RS, M2, PT) were very highly correlated in the anesthetized state (for example, correlation of 0.987 +/- 0.0006 in the case of RS) and less correlated in lateral sensory regions (for example, 0.542 +/- 0.03 in BC). This pattern held true for awake animals also, although the values themselves were lower (0.791 +/- 0.03 in the case of RS; 0.25 +/- 0.069 in the case of BC). To test the effects of cortical region and state, we performed a two-way ANOVA analysis. Both cortical region and state had significant effects on the value of the homotopic correlation values (two-way ANOVA, effect of state, p = 0.0037; effect of cortical region, p = 3.2 x 10^-7). It is noteworthy that the correlation of homotopic VSD signals (Figure 2.4A) had a similar pattern, with signals from medial association areas more correlated than those in lateral regions, and signals in the anesthetized state more correlated than those in awake animals.
We next examined the similarity among the correlation maps created for the different cortical regions. Our goal here was to test the similarities and differences among correlation maps as shown in the examples in Figure 2.6; in particular that maps from sensory regions overlapped less than those from medial association areas. We combined the homotopic correlation maps created from the right and left hemispheres by taking the mean of values created from right and left regions. For example, the map created from the PT on the right hemisphere was correlated with each other map created from regions on the right hemisphere, and the map created from PT on the left hemisphere was correlated with each other map created from the left hemisphere. Then, these two sets of values were averaged giving a single set of values representing the correlation of the PT map with each other map. After removing the autocorrelation values, we performed a two-way ANOVA to assess the contribution of cortical region and state to the variability of these correlations. Two examples of this analysis are shown in Figure 2.8B. In the upper panel, the HL correlation map has been correlated with each other map in the awake and anesthetized state; in the lower panel, correlations with the PT correlation map are shown. These values are similar to those of Figure 2.7 B,C and E,F; as described above, the two hemispheres have been combined, and the subsequent values for anesthetized and awake states placed together. Note the high correlation between PT and other medial regions (for example, the correlation between the PT correlation map and the RS correlation map was 0.96 +/- 0.01 in the anesthetized state) which reflects the similarities visible in Figure 2.6. In contrast, the HL map is much less correlated with these maps (correlation between the HL correlation map and the RS correlation map was 0.25 +/- 0.10 in the anesthetized state).
We repeated this analysis for the eight other cortical regions (not shown). In all cases, the correlation between the region’s correlation map and other correlation maps was significantly affected by the cortical region used to generate the correlation map (two-way ANOVA, effect of cortical region; p < 0.001). In other words, there was heterogeneity among the correlation maps that was reflected in variable correlation values among them. The effect of state (awake vs. anesthetized) on the correlation values among the maps was variable. It had a significant effect in 6 regions (BC, RS, HL, FL, V2, and M1).

To ensure the spatial layout of correlation maps was essential to the results described here, we randomly reshuffled the pixels in the correlation maps and repeated this analysis. Pair-wise correlation values between the scrambled maps ranged between 0.014 and -0.012 (awake) and 0.013 and -0.0169 (anesthetized). In other words, once the spatial layout of the pixels was removed, all meaningful similarities between correlation maps were eliminated.

2.3.4 Similar cortical regions are correlated with hindlimb sensory cortex during both spontaneous and stimuli evoked activity

One feature visible on Figure 2.8B is that the correlation map generated from the HL region is relatively unique, in that its correlation with other maps is low. Also notable is that this effect is less pronounced in the awake state, which reflects the less focused region of high correlation visible in the correlation maps of Figure 2.6. To examine this effect more closely, we compared the correlation maps created from each cortical region to a template map of cortical areas activated by stimulating the hindlimb. This template map was created by calculating the correlation between each pixel and the primary
hindlimb region following stimulation. In doing so, we created a map that represented the hindlimb sensory circuits activated by stimulation. This process is shown in Figure 2.9A. Here, montages show activity following hindlimb stimulation. The sensory template created via correlation of each pixel with the primary sensory region is shown at right. Comparing this template map to the correlation maps created from spontaneous activity gave us a measure of how spontaneous activity reflects the underlying sensory circuits. An example of such activity in an anesthetized animal is shown in Figure 2.9B; here, a sequence of images shows activity beginning in the HL sensory region, then flowing away, before returning to this sensory region. Figure 2.2C iii shows a similar sequence in an awake animal.

We found that the sensory template was uniquely correlated with the HL correlation map as shown in Figure 2.9C, although this effect was much more pronounced in the anesthetized animals (awake 0.43 +/- 0.067; anesthetized, 0.804 +/- 0.031). In both cases, there was a significant effect of cortical region on the correlation between the sensory template and the spontaneous correlation map; this effect was stronger in the anesthetized animals (one-way ANOVA, effect of cortical region; anesthetized animals, p = 1.18 x 10^{-13}; wake animals, p = 4.30 x 10^{-3}). Reflecting this difference, a two-way ANOVA examining the effect of state on these values found it to have a significant effect (p = 2.56 x 10^{-3}). Regions whose correlation maps were significantly less correlated with the sensory template than the HL correlation map (Tukey’s HSD test) are indicated by ticks in Figure 2.9C; in the anesthetized animals, all other correlation maps were significantly different, while in the awake animals, only V1 and V2 were. Following randomization of the pixel locations of the sensory template,
pairwise correlation values between the scrambled sensory map and the spontaneous correlation maps ranged between 0.0089 to -0.0084 in awake animals and -0.0093 to 0.0070.

2.3.5 Correlation between barrel and motor cortices during spontaneous activity

We interpret the preceding results as evidence that spontaneous cortical activity reflects underlying connectivity within the cortex. To address this issue further, we next examined the correlation of VSD signals from across the cortex with the VSD signal from the barrel cortex. It is well-known that the BC is closely connected with anterior, medial motor regions of the cortex via monosynaptic connections (Ferezou et al., 2007; Lim et al., 2012). Figure 2.10A shows example correlations maps from the right and left BC of one anesthetized animal. Note the island of higher correlation in the anterior medial cortex. In the anesthetized state, we found that the more highly correlated cortical region with the BC was the adjacent V1 (0.76 +/- 0.085), followed by anterior motor regions (ACC/M2, p = 0.73 +/- 0.47; M1, p = 0.712 +/- 0.046) (Figure 2.10B). One-way ANOVA of these correlation values (with auto-correlation removed) was significant (p = 0.0157), although correlation with motor areas was not significantly higher (Tukey’s HSD test). In the awake animal, this pattern was not seen, and there was no significant difference among the correlation values (one-way ANOVA, p = 0.45). This difference between anesthetized and awake was reflected in a two-way ANOVA examining the effects of cortical location and state on correlation values with BC, which showed a significant effect of state (p = 1.13 x 10^{-5}).
2.3.6 Activity in anterior regions of brain precedes activity in posterior regions

We next examined the temporal relationships among VSD signals from different regions of the brain, to determine how activity spread across the cortex. Previous studies had found an anterior-to-posterior spread of slow-wave activity (Massimini et al., 2004; Nir et al., 2011), so we examined the timing of signals relative to one posterior region of the brain (V1) and one anterior region of the brain (ACC/M2). As a measure of temporal relationships, we computed the delay of one signal relative to the other needed to achieve maximal correlation, as shown in Figure 2.11. We found a symmetrical relationship between the timing of activity in other regions relative to V1 and ACC/M2. The greatest delay of any signal relative to ACC/M2 was V1 in both the awake (12 +/- 3 ms) and the anesthetized states (27 +/- 8 ms), while the signal that preceded V1 by the most was ACC/M2 in both awake and anesthetized states (same values as these relationships are mirror-images). Therefore, activity in anterior regions of the brain preceded activity in posterior regions. To examine whether the spread of activity was significantly faster in the awake state we performed a two-way ANOVA on the delays needed to obtain maximal correlation relative to activity in both V1 and AC/M2. In both cases, the effect of state was significant (V1, $p = 2.4 \times 10^{-3}$; AC/M2, $p = 6.7 \times 10^{-8}$).

2.3.7 Patterns of cortical activation are dynamic

Although, in general, activity in anterior regions of the brain preceded that in posterior regions, we noted that activity did not necessarily follow a uniform path across the cortex – see for example Figure 2.9B. We recorded from a single, unilateral
hemisphere in four animals to examine this more closely. This portion was not done in awake animals.

Isolated peaks of activity did not necessarily spread in a uniform path across the brain, but could be dynamic. Figure 2.12A shows two examples of such bursts, where activity moves in spiral, nonlinear patterns. To emphasize the value of using high-resolution signals, we have included EEG signals showing a single unitary wave of depolarization. Spread of activity could also be discontinuous, as shown in Figure 2.12B. We calculated the maximum number of individual peaks during each bursts by thresholding the VSD image and counting the isolated regions, as a measure of discontinuous activity. The distribution of maximum peak numbers is shown in Figure 2.12C.

2.4 Discussion

2.4.1 Similarities to descriptions of slow-wave activity as a global cortical phenomenon

The first reports of the slow-wave oscillations between cortical UP and DOWN states emphasized their widespread and synchronous nature, and many studies using EEG or multiple electrode recordings have continued to emphasize such features (Buzsaki et al., 1988; Steriade et al., 1993c; 1993d; Contreras and Steriade, 1995; Amzica and Steriade, 1995a; 1995b; Achermann and Borbély, 1997; Destexhe et al., 1999; Sanchez-Vives and McCormick, 2000; Timofeev et al., 2000; Volgushev et al., 2006; Chauvette et al., 2010; Volgushev et al., 2011). Our results are not at all inconsistent with these findings. We can consider VSD signals recorded from a small region of the brain as similar to a LFP within that region (Chemla and Chavane, 2009); such signals are highly
synchronized (Figures 2.1, 2.2), even at large distances relative to the size of the mouse cortex (Figure 2.4). Slow changes in cortical activity – as described in section 1.4 – rhythmically influence cortical excitability (Vanhatalo et al., 2004) and can ‘nest’ faster rhythms (Canolty and Knight, 2010), and likely underlie these widespread patterns of activation.

Studies with higher spatial resolution have noted regional differences in the onset of slow-waves, with anterior regions preceding posterior ones (Huber et al., 2000; Massimini et al., 2004; Münch et al., 2004; Zavada et al., 2009; Leemburg et al., 2010; Riedner et al., 2011), resulting in a wave of activity traveling in an anterior-posterior direction (Massimini et al., 2004; Nir et al., 2011). Comparing VSD signals recorded in anterior regions of the brain to those recorded from posterior regions, we found a similar result (Figure 2.11).

We found that correlations decreased with distance, as shown previously (Destexhe et al., 1999; Volgushev et al., 2011). However, we also found this effect to be heterogeneous, as the decrease is less pronounced for anterior motor regions of the brain (Figure 2.4, 2.5). As discussed later, this is due to long-range connection that exist between these motor areas and sensory regions of the brain (Ferezou et al., 2006; Lim et al., 2012)

2.4.2 VSD signals reveal underlying heterogeneous spatial structure to slow-wave activity

Typically, studies of the spatial structure of SWA compare the signals recorded from a reference electrode (generally within Area 5 or 7) to other signals recorded from varying distances (Destexhe et al., 1999; Timofeev et al., 2000; Volgushev et al., 2011). Because VSD signals are simultaneously captured from multiple functional regions

93
(Figures 2.1,2.2), we were able to compare the degree to which *multiple* regions were correlated with cortex at different distances, including on the opposite hemisphere. One pertinent finding related to interhemispheric relationships was that the most lateral regions of the brain (V1, BC, FL) were the least well correlated with their homotopic regions on the opposite hemisphere. This likely reflects the importance of passive synchronizing via transcallosal fibres, rather than active synchronizing via subcortical structures, of activity between the two hemispheres (Mohajerani et al., 2010). Those regions furthest from the midline would have the greatest distance-related conduction delay.

While many studies emphasize the global nature of slow-waves, some (Finelli et al., 2001; Huber et al., 2004; 2006) have shown local differences in the power of slow-wave activity, and a recent study using electrodes implanted in the brains of human patients showed many slow-waves remain restricted to a single functional region of the brain (Nir et al., 2011). The high-spatial resolution and large imaging region of VSD imaging is well suited to explore this local nature further. Our strategy was to create correlation maps for each functional region (Figure 2.6). These maps revealed differences between the spatial patterns of activity associated with each cortical region that were not evident when looking at isolated VSD signals. For example, we found that correlation maps for sensory regions were relatively distinct; there was little overlap between the regions of cortex that were highly correlated with HL, FL, or VL (Figure 2.7).

We believe this fits within a growing recognition that oscillations between UP and DOWN states tap into functional networks of cortical cells (Harris, 2005; Castro-Alamancos, 2009; Haider and McCormick, 2009; Luczak et al., 2009; Luczak and
During the transition to UP states, the spatio-temporal pattern of spikes within a local network of cells is highly stereotyped (Luczak et al., 2007), for example, and this pattern of cell activation during UP states generated via thalamic simulation in slices (MacLean et al., 2005) or sensory stimulation in vivo (Luczak et al., 2009) is very similar to that of spontaneous UP states. On a larger scale of network also, slow-wave oscillations reflect functional connections with the visual (Kenet et al., 2003; Han et al., 2008) and somatosensory (Ferezou et al., 2006) cortices. In this study we expand on these findings by showing that the pattern of slow-wave activity is shaped by sensory circuits at a bilateral, cortex-wide scale (Figure 2.9,2.10). Particularly interesting is the high correlation between the barrel and motor regions of the cortex (Figure 2.10), given the well-recognized strong reciprocal connections between these regions of the cortex (Kleinfeld et al., 2002; Veinante and Deschênes, 2003; Ferezou et al., 2006; 2007; Aronoff et al., 2010; Matyas et al., 2010; Lim et al., 2012). Although a previous study demonstrated a loss of correlation between two regions following the transection of the intervening cortex (Amzica and Steriade, 1995b), the strong correlation between the barrel cortex and anterior motor regions is evidence that the importance of cortical connectivity is not homogenous but rather reflects precise, functional connections.

While the correlation maps of sensory regions were relatively distinct, those of other area of the cortex overlapped (Figures 2.6,2.7). In particular, the maps of PT, RS, ACC/M2, and V2 were particularly well correlated with each other (Figure 2.7). These regions are all considered association areas of the mouse brain (Torrealba and Valdés, 2008; Lu et al., 2012), and share many reciprocal connections (Reep et al., 1994; Condé et al., 1995; Shibata et al., 2004; 2009) giving further support to contention that the fine
spatial structure of slow-wave activity represents underlying cortical circuitry. It is noteworthy that these regions all lie with the default mode network, a set of structures that are synchronized by slow oscillations of the BOLD signal in primates (Raichle et al., 2001; Damoiseaux et al., 2006; Rilling et al., 2007; Vincent et al., 2007) as well as rats (Lu et al., 2012), and that similar overlap of slow-wave activity with default-mode network exists in humans (Murphy et al., 2009). The functions of the default mode network remains a subject of active debate and investigation but are presumed to include processing of ongoing and prior multi-modal sensory inputs to generate predictions about the future (Gusnard and Raichle, 2001; Raichle and Snyder, 2007; Zhang and Raichle, 2010). Given that spontaneous oscillations of the BOLD signal are associated with delta-band (1-4 Hz) activity in the rat (Lu et al., 2007), our results suggest that slow-wave oscillations may have important functions within the default mode network also.

**2.4.3 Comparison between awake and anesthetized states**

In cats and primates, a general feature of waking states as compared to sleeping or anesthetized states is a decrease in amplitude and an increase in frequency of population cortical activity as recorded on EEG or LFP (Steriade et al., 1993b; Destexhe et al., 1999; Steriade et al., 2001; Haider and McCormick, 2009; Vyazovskiy et al., 2009). However, in the quietly resting rodent, neurons show highly synchronized oscillations of membrane potential at the slow-wave frequencies (0.5-4 Hz) (Poulet and Petersen, 2008) and these oscillations are reflected at the population level in VSD and LFP signals (Petersen et al., 2003; Crochet and Petersen, 2006; Poulet and Petersen, 2008; Poulet et al., 2012). Here we show that these oscillations can synchronize large regions of the cortex (Figure 2.6).
We found that correlation maps of slow-wave activity in the awake animals were broader than those of the anesthetized animals (Figure 2.7). In other words, they were more correlated with, and less distinct from, each other. Similarly, there was higher correlation between the association correlation maps and the HL sensory template (Figure 2.9). This may be due in part to the increased propagation speed of activity as represented by the smaller delay needed to achieve maximal correlation between different regions of the brain (Figure 2.11). Different areas of the brain became activated more closely in time, and thus the uniqueness of activity within any one area is decreased.

It may also be due to different cortical dynamics in the awake state. The proposed functions of SWA in the sleeping state (see section 1.2.3.4), which suggested a detailed spatial structure for SWA, are not proposed to be active in the waking brain. Even in the absence of ongoing sensory or motor tasks, the awake brain is tasked with processing demands that are not present during sleep or under anesthesia (Haider and McCormick, 2009). Circuits involved may be intermingled among simpler circuits involved in sensory processing, and may obscure the structured activity observed in the sleeping or anesthetized brain. This effect has been proposed to explain similar loss of structured activity at the level of cortical micro-circuits (Luczak and MacLean, 2012). It is also recognized that in the awake state, cortical regions are more functionally connected, such that stimulation via TMS spreads substantially longer-distances during waking than during deep sleep (Massimini et al., 2005).

When examining a single sensory domain, it is reasonable to remove any possibility of sensory activity via deafferentation (Poulet and Petersen, 2008). However, it is not feasible to accomplish this when examining activity across multiple regions of
the brain. Could, then, the depolarizations we observe in the awake animal be sensory-driven rather than spontaneous? We believe this to be unlikely, for three reasons. First, we minimized the effects of any possible inputs by a) discarding the first second of recordings after the LEDs illuminated b) maintaining an otherwise darkening and silent environment and c) removing any sequences that included whisker or limb movements. Second, the pattern of delays between anterior and posterior regions of the cortex was consistent (Figure 2.11). Although the delays were shorter in the awake animal, activity in posterior regions followed activity in anterior regions, as in the anesthetized animal. This suggests a common mode of initiation in the two states, and it is particularly relevant that activity in the barrel cortex was delayed relative to activity in the anterior medial regions of the cortex (Figure 2.11B). When activated via sensory stimulation, the initial activity is in the barrel cortex and anterior motor regions follow at a delay of ~8 ms (Ferezou et al., 2007)). Third, we note strong activity in posterior midline association regions, as in the anesthetized state, which is not characteristic of limb or whisker responses (see Figure 2.9 for limb response, Figure 1.10 for whisker responses). In fact, in both states, bursts were most frequent in the retrosplenial area and least frequent in sensory regions (Figure 2.2).

In summary, slow-wave activity in the awake and anesthetized states had similar spatial patterns of activity and temporal relationships, although the absolute values differed, with activity being less frequent and spreading more quickly in the awake state.
2.4.4 What do the current results suggest about the function of slow-wave activity?

One of the driving forces behind interest in slow-wave activity is that it is one of the best characterized physiological correlates of sleep homeostasis – that is, the increasing urge to sleep as the time awake increases (Daan et al., 1984; Borbély and Achermann, 1999; Leemburg et al., 2010; Vyazovskiy et al., 2011). Slow-wave activity is most prominent immediately after sleep begins, and increases or decreases as a function of the interval since the previous sleep episode (Tobler and Borbély, 1986; Franken et al., 1991; Werth et al., 1996; Akerstedt et al., 2009; Leemburg et al., 2010; Vyazovskiy et al., 2011). A prominent hypothesis that explains this observation is that this activity represents a period of synaptic scaling, during which synapses that were strengthened during waking are down-scaled to preserve the overall excitatory balance of the cortex (Tononi and Cirelli, 2003; Massimini et al., 2009; Tononi and Cirelli, 2012). The most functional and relevant synaptic increases would be preserved or even further strengthened (Walker, 2010; Mölle and Born, 2011). Our results are consistent with this hypothesis. Important learning related changes can occur within very local cortical circuits (Komiyama et al., 2010; Fu et al., 2012) and local slow-waves within these circuits could facilitate this process, perhaps by permitting synchronized faster oscillation within components of the circuit (Stickgold and Walker, 2007). It bears noting that spontaneous activity within functional circuits may not have any particularly important function, but rather may simply reflect synaptic connectivity (Luczak and MacLean, 2012). However, localized changes in slow-wave power that follow a specific learning task (Huber et al., 2004; 2006) suggest otherwise.
In the awake animal, the function of slow-wave-activity is less clear. Spontaneous activity has clear effects on responses to sensory inputs (Lakatos et al., 2005; Monto et al., 2008; Busch et al., 2009; Nierhaus et al., 2009); at the cellular level this is observed as changes in the input-output characteristics of neurons depending on ongoing synaptic background activity (Shu et al., 2003a). In particular, sensory inputs during a spontaneous UP state in the awake rodent result in a weaker and more localized response (Petersen et al., 2003). This results from activity-dependent depression of thalamocortical synapses resulting from the enhanced activity of the UP state (Castro-Alamancos and Oldford, 2002), and can be seen in enhanced cortical responses to thalamic inputs following periods of silence in thalamocortical fibres (Swadlow and Gusev, 2001). The alternation between stronger, global responses and weaker, local responses may provide a means for the brain to finely resolve sensory inputs in time and space, while also being able to integrate and associate multi-modal inputs across cortical regions (Petersen et al., 2003). Alternatively, waxing and waning sensory excitability may allow the brain to allocate attention to different sensory modalities (Lakatos et al., 2008; Palva and Palva, 2011) or to self-regulate excitability (Hasenstaub et al., 2007) as needed. Finally, the slow oscillation may contribute to awareness and sensory-integration by linking disparate brain regions (He and Raichle, 2009a) (but see (Koch, 2009; He and Raichle, 2009b)). In general, the role of spontaneous activity in the waking brain remains unclear and more research is needed (Fox and Raichle, 2007; Palva and Palva, 2011).
2.4.5 Conclusion

In the introduction of this study we established our goal of determining whether the spatial structure of slow-wave activity reflects connections within and between hemispheres of the cortex. Using VSD imaging, we have shown that slow-wave activity is shaped by these connections, in both the quiet awake and anesthetized state.
2.5 Figures

Figure 2.1 Voltage-sensitive dye (VSD) imaging in the adult anesthetized mouse. A) Simultaneous VSD signals from 10 cortical regions (5 functions regions on right and left hemispheres) of an anesthetized mouse. Schematic at left shows brain regions. Abbreviations described in text. B) Montages showing VSD images at time indicated in gray in panel A. A, anterior; R, right; L, left; P, posterior.
Figure 2.2: Voltage-sensitive dye imaging in the awake adult mouse. A) Cartoon showing set-up used to record from awake mouse. VSD imaging camera and illumination lights not shown. Image at left shows frame of whisker movement video used to differentiate periods of quite wakefulness from periods of activity. Whisker has been highlighted in red. B) Simultaneous VSD signals from 10 cortical regions (5 functions regions on right and left hemispheres) of an awake mouse. Schematic at left shows brain regions. B) Montages showing VSD images at time indicated in gray in panel A.
Figure 2.3: Frequency of VSD bursts in the awake (black) and anesthetized (blue) state, for each cortical region examined. * denotes significant effect (p < 0.05) of cortical region on burst frequency, tested using one-way ANOVA.
Figure 2.4: Homotopic and intrahemispheric correlations of VSD signals. Correlations for each brain region in the adult mouse for awake (black) and anesthetized (blue) states. Intrahemispheric correlations show the correlation of each brain region with brain regions at a circular distance of 1, 2, and 3 mm. * denotes significant effect (p < 0.05) of cortical region on correlation, tested using one-way ANOVA.
Figure 2.5: Pair-wise correlations between VSD signals from brain regions in the awake and anesthetized mouse. A) Grid representing pair-wise correlations between cortical regions. R suffix, right hemisphere; L suffix, left hemisphere. Black circles denote homotopic correlations. Labels B and C show values presented in bar graph format in B and C below. B) Correlation of VSD signals from each brain region with VSD signal from HL left (black) or HL right (white). C) Correlation of VSD signals from each brain region with VSD signal from PT left (black) or PT right (white). D,E,F) As described for B,C,D except for awake animal. Correlations between homotopic, as well as autocorrelation, are noted here and in subsequent figures. * denotes significant effect (p < 0.05) of cortical region on correlation, tested using one-way ANOVA.
Figure 2.6: Correlation maps show precise spatial layout of correlation of VSD signals from all pixels with VSD signal from each brain region. A) Cartoon showing creation of correlation map. Reference/seed pixel is extracted, correlation with each other pixel is calculated and placed into position of comparison pixel. B) Correlation maps for 6 different seed pixels (top row, primary sensory-area seed pixels; bottom row, association-area seed pixels). C) Outlines of correlation maps at 0.85 for 7 cortical regions. Note the relative overlap of medial association areas compared to the sensory areas. Note also the second region of cortex correlated with BC seed pixel in anterior motor section of cortex. D,E) As described in B,C, except for awake animal.
Figure 2.7: Pair-wise correlations between correlation maps generated from each cortical region. A) Grid representing pair-wise correlations between correlation maps. R suffix, right hemisphere; L suffix, left hemisphere. Black circles denote homotopic correlations. Labels B and C show values presented in bar graph format in B and C below. B) Correlation of correlation map from each brain region with that of HL left (black) or HL right (white). C) Correlation of correlation map from each brain region with that of HL left (black) or HL right (white). D,E,F) As described for B,C,D except for awake animal. * denotes significant effect (p < 0.05) of cortical region on correlation, tested using one-way ANOVA.
Figure 2.8: Correlation maps from medial cortical areas are highly correlated with maps from homotopic regions as well as maps from other medial areas. A) Correlation between correlation-maps from homotopic regions for awake (black) and anesthetized (blue) states. B) Upper: correlation between correlation-maps from HL regions for awake (black) and anesthetized (blue) states. Lower: correlation between correlation-maps from PT regions for awake (black) and anesthetized (blue) states. * denotes significant effect (p < 0.05) of cortical region on correlation, tested using one-way ANOVA.
Figure 2.9: Slow-wave activity can reflect underlying sensory architecture. A) Montages of images showing brain activity following hindlimb stimulation. At right, correlation map for stimulation sequence, HL, R seed pixel. B) Montage of spontaneous activity showing isolate activation of HL region of brain bilaterally at beginning and end of sequence. Note also isolated deactivation of HL region at time 53 ms. C) Correlation between sensory stimulation correlation map (HL seed pixel) and spontaneous correlation map generated from each cortical region. Horizontal lines show mean correlation of sensory activity with correlation map of spontaneous activity with HL seed pixel in awake (black) and anesthetized (blue) states. Vertical ticks show significant difference from correlation with HL correlation map (arrow). * denotes significant effect (p < 0.05) of seed region on correlation between correlation map and HL evoked correlation map, tested using one-way ANOVA.
Figure 2.10: Role of long-range cortical connections in slow-wave activity revealed in whisker sensorimotor circuits. A) Correlation maps with BC seed pixel in anesthetized (upper) and awake (lower) states. B) Correlation of VSD signal from each cortical region with VSD signal from BC in awake (black) and anesthetized (blue) states. Blue and black horizontal lines show mean correlation between VSD signal from BC and M1. * denotes significant effect (p < 0.05) of cortical region on correlation between region and BC VSD signal, tested using one-way ANOVA.
Cross-correlations reveal consistent anterior-posterior spread of slow-wave activity. A) Mean cross correlations between VSD signals from 6 cortical regions and VSD from anterior region (M2/ACC; left column) and from posterior region (V1; right column) in the anesthetized (upper) and awake (lower) state. Black dots denote time of maximal correlation. Note the increasing delay to maximal correlation for more posterior regions relative to M2/ACC; and the increasing advance for maximal correlation for more anterior regions relative to V1. B) Delay needed for maximal correlation of VSD signal from V1 (upper) and ACC/M2 (lower) in awake (black) and anesthetized (blue) states. * denotes significant effect (p < 0.05) of cortical region on delay, tested using one-way ANOVA.

Unilateral imaging region (of right hemisphere) reveals that slow-wave activity is composed of complex local events. A, Two examples of complex patterns depolarizations. Surface EEG is displayed on the left, and images corresponding to the gray region are shown. Top at right shows spiral path and instantaneous velocity of a single peak (in black circle, first panel). B, Single example showing that the number of discrete peaks of depolarization evolves rapidly over time. Vertical lines connect VSD images to the appropriate point on the record above that provides an index of the number of peaks detected. Scale bar, 1 mm. C, Histogram showing the number of depolarizations with differing number of maximum number of discrete peaks of depolarization during 90 s of activity in four animals. Example in C) would be counted as maximum of three.
CHAPTER 3. VOLTAGE-SENSITIVE DYE IMAGING REVEALS DYNAMIC SPATIOTEMPORAL PROPERTIES OF CORTICAL ACTIVITY FOLLOWING SPONTANEOUS MUSCLE TWITCHES IN THE NEWBORN RAT

3.1 Introduction

In the previous chapter, I described how the spatial structure of spontaneous active in the adult might reflect functions related to synaptic scaling or consolidation. In the developing nervous system, the function of activity is likely to be different. Early neural activity, combined with genetically determined molecular factors, shapes the connections of the developing mammalian nervous system. (Katz and Shatz, 1996; Tessier-Lavigne and Goodman, 1996; Ben-Ari and Spitzer, 2010; Hanganu-Opatz, 2010). Distinct patterns of neural activity are found in different neural systems (Yvert et al., 2004; Crépel et al., 2007; Tritsch et al., 2007; Watt et al., 2009), and in some cases the function of these patterns has been determined (Torborg and Feller, 2005; Huberman, 2007; Wu et al., 2010). Distinct patterns of neural activity are present within the developing mammalian cortex as well. Understanding these patterns is crucial as they may provide a template for future task-related activity by controlling axon growth and synaptogenesis (Katz and Shatz, 1996; Tessier-Lavigne and Goodman, 1996; Allene and Cossart, 2010; Ben-Ari and Spitzer, 2010; Hanganu-Opatz, 2010). The best studied pattern in vivo, the spindle burst, is a 300-600 ms event comprised of a fast 5-20 Hz burst often associated with a slower ~ 3 Hz oscillation (Khazipov et al., 2004; Yvert et al., 2004; Crépel et al., 2007; Tritsch et al., 2007; Watt et al., 2009). It generally follows a peripheral sensory event such as a retinal wave or a spontaneous muscle twitch, suggesting a role in the development of functional sensory cortices (Torborg and Feller,
Whether such activity could contribute to the maturation of long-range connections present in the adult brain (Wallace et al., 2004; Ferezou et al., 2007; Frostig et al., 2008; Desai et al., 2011) is not known. The majority of research has examined its properties within a single sensory domain, either somatosensory (Milh et al., 2007; Marcano-Reik and Blumberg, 2008; Yang et al., 2009; Colonnese and Khazipov, 2010; Minlebaev et al., 2011) or visual (Hanganu et al., 2006; Colonnese et al., 2010) and it is generally described as local and immobile (Yang et al., 2009). Nevertheless, dynamic spindle bursts have been reported (Khazipov et al., 2004) and they are synchronized between hemispheres (Yang et al., 2009). In this study, we directly study the dynamic properties of cortical activity in early life using VSD imaging to provide high spatiotemporal resolution signals of cortical activity (Grinvald and Hildesheim, 2004; Chemla and Chavane, 2009) from large regions of the cortex of 4-6 day old rats in vivo. While high frequency patterns were not well represented in the VSD signal, we found prominent bursts of lower frequency activity in sensory cortices. Following spontaneous twitches in the tail and limbs, these bursts may represent the slow depolarizations that accompany some spindle bursts (Marcano-Reik and Blumberg, 2008). Sensory regions were synchronized by these twitch-induced bursts and we found they were dynamic, spreading towards the midline of the brain. This asymmetric spread may be involved in the maturation of connections with medial motor regions. Furthermore, this spread of activity within the sensory regions and into midline areas closely mirrored the activity
produced by directly stimulating the periphery, suggesting that these spontaneous bursts provide a recurring template of functional sensory circuits to the cortex.

3.2 Methods

3.2.1 Animals and surgical preparation

The University of British Columbia Animal Care Committee approved all procedures involved in this study. Working with male Wistar rat pups (P4-P6), we induced anesthesia using 1-1.5% isoflurane in oxygen and maintained body temperature at 37 C using a heating pad. After locally anesthetizing the scalp with lidocaine, we made a single 7X7 mm craniotomy over both hemispheres. The location of this craniotomy varied but always included hindlimb and tail primary somatosensory cortex. We then attached a custom made headplate to the skull using dental cement. This headplate included with internal channels for circulating hot water that kept temperatures near the surface of the cortex near 37 °C.

3.2.2 Cortical EEG recording

In order to monitor EEG activity throughout the experiment, a Teflon coated chlorided silver wire (0.125 mm) was placed on the edge of the craniotomy window. A reference electrode was placed on the nasal bone. The cortical signal was amplified and filtered (0 –1000 Hz) using a differential alternating current amplifier (DAM 50 model, World Precision Instruments).
3.2.3 VSD imaging

Prior to moving the animal to our imaging set-up, we decreased the isoflurane to 0.25%. We maintained anesthesia between 0.25-0.5% throughout imaging, adjusting the level as needed to minimize whole-body movements. We performed imaging of VSD signals as described in Chapter 2. A second identical camera was fixed behind the animal to capture images of limb and tail movements, illuminated by infrared LEDs. We collected 12 bit images at 6.67 ms time resolution (150 Hz) using an EPIX E1DB frame grabber with XCAP 3.1 imaging software (EPIX) from this camera. When collecting spontaneous cortical activity associated with limb or tail twitches, we collected synchronized images from both cameras for 33.3 seconds at a time.

3.2.4 VSD signal analysis

We processed the VSD signals as described in Chapter 2. To determine the position of bursts on the cortex, we visually detected the onset and offset of bursts. Within this visually detected range, the exact timing of burst onset was determined using custom-written Matlab scripts that thresholded each image at its 60th percentile. While the area above this threshold was over 0.2 mm², we classified it as a burst. To calculate the instantaneous spatial correlation between cortical activity and somatosensory circuits, we first created a template of sensory activity by taking the mean image of cortical activity over the 40 ms following cortical activation via stimulating the limbs or tail. We then removed the non-signal mask region for this image and each image of spontaneous activity. Lastly, we calculated the correlation between the template and each individual frame of activity.
3.2.5 Video analysis

To determine the timing of movements of the limbs and tails, we detected changes in the light level from 325 µm X 325 µm regions over the hindlimbs and tail. Using custom written Matlab scripts, we filtered this signal between 1 and 20 Hz and took the absolute value, then detected instances in which this signal crossed a threshold of four times the standard deviation of the background activity. This process was partially automated but relied on supervision including reference to the original videos to exclude large whole body movements and to determine the exact timing of small movements.

3.2.6 Statistical analysis

Results are presented as mean +/- standard error, unless otherwise specified. We used t-tests and ANOVA tests to test for differences between and among means, as described. Correlation values were calculated using Pearson’s method. We transformed correlation values using Fisher’s z-transformation prior to statistical tests. All analyses are performed on data collected from 10 pups.

3.3 Results

In this study, we used VSD imaging to record membrane potential changes over large stions of the developing rat cortex in vivo. Our experimental set-up, along with an image of the cortical region we recorded VSD signals from, is shown in Figure 3.1A. Changes in these signals reflect changes in the membrane potential within each pixel (65 µm X 65 µm). All signals presented were collected under light anesthetic (0.25-0.5% isoflurane).
3.3.1 Spatial and temporal differences cortical responses following limb and tail stimulation

To determine the precise location of sensory regions corresponding to the limbs and tail in the developing cortex, we stimulated the hindlimbs and tail and recorded the subsequent cortical activity. This also allowed us to examine the spatial and temporal properties of cortical sensory processing at young ages. In adult rodents, imaging tools have shown subthreshold activity spreads over large regions of the cortex after such stimulation (Wallace et al., 2004; Ferezou et al., 2007; Frostig et al., 2008; Desai et al., 2011; Mohajerani et al., 2011), but the extent to which this exists during development is not known. Images showing patterns of activity following limb and tail stimulation are shown in 3.1B, and an example of the association between the VSD signal and EEG signal is shown in Figure 3.1C. We found pronounced differences between the cortical activity that followed limb stimulation and tail stimulation. The most prominent difference was that responses to limb stimulation were dominated by responses in the contralateral cortex, while responses to tail stimulation were bilateral, as is visible in Figures 1B and E. This is consistent with recent studies showing limb (Marcano-Reik and Blumberg, 2008) and whisker (Quairiaux et al., 2011) responses are primarily unilateral in early development. Throughout this study, we refer to the area of cortex activated by stimulating the left and right hindlimbs as HLS1,R and HLS1,L respectively. Similarly, we refer to the regions activated by stimulating the tail as TLS1,R and TLS1,L. These cortical regions are shown in Figure 3.1D. We used the center of these regions (calculated in each animal), to collect a temporal signal of S1 cortical activation following stimulation, as show in Figure 3.1E. Following limb stimulation, we found a robust contralateral, but weak ipsilateral activation; the mean ratio of the peak VSD signal in the
contralateral cortex to the peak VSD in the ipsilateral cortex was 3.6 +/- 0.31, and a paired two-tailed t-test of the peak signal intensity confirmed this difference (p = 1.8 \times 10^{-9}). In contrast, stimulation of the tail yielded approximately equal responses in both hemispheres; the ratio of peak VSD signal on the left hemisphere to that on the right was 0.92 +/- 0.12, and a two-tailed paired t-test of the responses from the two hemispheres did not meet the threshold for significance (p = 0.42).

In addition to differences in the spatial properties of cortical activation, temporal properties differed between tail and limb stimulation as well. The mean time for the VSD signal to increase beyond 4 standard deviations above background activity following stimulation of the right hindlimb was 88 +/- 1.1 ms, and 92 +/- 0.61 ms following left hindlimb stimulation (not shown). Following tail stimulation, the VSD signal increased more slowly, taking 123 +/- 2 ms to increase beyond 4 standard deviations above background on the right hemisphere, and 121 +/- 1.2 ms on the left. A two-way ANOVA test performed on these values showed a significant difference between the time to activate between limbs and tail (p = 5.0 \times 10^{-9}), but no effect of hemisphere (p = 0.87).

To more precisely examine the spread of activity on the cortex following stimulation, we collected VSD signals from a 2 mm square around the earliest point of activation and examined the dynamics of activity within this restricted area (Figure 3.2A). Before being analyzed, images following right hindlimb stimulation were flipped on a medial-lateral axis so that medial and lateral directions became consistent between the two hemispheres. A prominent feature of the pattern of activity was an asymmetrical spread of VSD signal increases over time following stimulation. This can be noted in the line graphs in Figure 3.2A, which show a cross-section of mean activity spread on a
medial-lateral axis (black line) and anterior-posterior axis (gray line). To quantify this difference, we calculated the mean of the activity at eight locations relative to the point of maximal activation, at three different times following stimulation (locations are shown in rightmost image of upper panel, Figure 3.2A, as colored squares – all 0.975 mm from the point of maximum activation). As shown in Figure 3.2B, 100 ms after stimulation of the limbs, there was no significant difference among the VSD signal at the 8 points (one-way ANOVA, p = 0.22). By 180 ms after stimulation, a pronounced effect of location on the size of the VSD signal was apparent (one-way ANOVA, p = 1.9 x 10^{-6}). The largest difference along a single axis was along the lateral-medial axis, with mean VSD signal at the lateral point equal to 0.05 +/- 0.01 %ΔF/F compared to 0.13 +/- 0.01 %ΔF/F at the medial point. This difference was found to be significant using Tukey’s HSD test. The difference among the VSD signals at the examined points persisted 300 ms after stimulation (one-way ANOVA, p = 1.6 x 10^{-5}), with the difference between the lateral point (0.05 +/- 0.01 %ΔF/F) and the medial point (0.17 +/- 0.01 %ΔF/F) again found to be significant. We performed an identical analysis of VSD signals following tail stimulation, except using slightly later time-points to account for the slower activation of the tail cortex following stimulation (see above). These results are shown in Figure 3.2C. The effect of cortical location on the VSD signals did not meet the threshold for significance for any of the time points examined (one-way ANOVA, 133 ms after simulation, p = 0.12; 213 ms after simulation, p = 0.37; 333 ms after stimulation, p = 0.078).
3.3.2 Spontaneous limb and tail twitches stimulate dynamic bursts of cortical activity in the absence of external stimulation

Given its high temporal and spatial resolution, VSD imaging holds promise for gaining insight into the structure of spontaneous activity in the developing cortex. The value of these high resolution images can be seen in Figure 3.3A. Here, VSD signals from individual subregions of the somatosensory cortex, identified via sensory-evoked responses as described in Figure 3.1, are shown, allowing a detailed examination of the interactions between movements in the periphery and the patterns of activity on the cortex. The upper panel shows VSD signals recorded from three regions of S1, while signals of limb movements extracted from video are shown in the middle panel. To quantify the extent to which activation of developing sensorimotor circuits contributed to the overall cortical activity, we calculated the instantaneous spatial correlation between ongoing cortical activity and a template of sensorimotor activation, created from the mean VSD activity pattern evoked by stimulating the limbs and tail. This process is described in more detail in Methods (section 3.2). The instantaneous spatial correlation is shown in the lower panel of Figure 3.3A. Figure 3.3B shows montages of images of brain activity at 100 ms intervals from regions highlighted in gray in Figure 3.3A. Note that bursts in a particular region are accompanied by an increase in the correlation with that sensory template.

While there was a clear relationship between limb twitches and cortical bursts, it was not a one-to-one relationship. Figure 3.4A shows a sequence of VSD signals, limb twitches, and ongoing sensory template correlation in which there is mix of cortical bursts that follow twitches in the associated limb and bursts that do not follow twitches. To quantify the contribution of twitches to bursts of cortical activity, we examined the
relationship between spontaneous twitches and bursts of VSD signal in corresponding subregions of S1 more closely. For a particular burst of VSD signal in a subregion of S1, we examined whether it was preceded by a twitch in its corresponding limb (e.g. was a burst in HLS1,R preceded by a twitch in the left hindlimb) and, similarly, whether a twitch was followed by a burst in the corresponding subregion of S1. Figures 4B combines results across animals and across subregions of S1 by showing the probability of a twitch being followed by a burst in the corresponding region of S1 (left panel) or a burst being preceded by a corresponding twitch (right panel) as a function of time.

As the example VSD signals in Figure 3.3 and 3.4 show, bursts of cortical activity can be isolated in a single cortical region, driving a high correlation with a single sensory template, or can occur in multiple regions, resulting in moderate correlations with multiple templates. To explore whether high correlation bursts were more likely to arise from twitches in the associated limb, we binned bursts by their correlation with the appropriate sensory template. For example, the image of brain activity at the time of a burst of VSD signal in HLS1,R was correlated with the template of sensory activation generated by stimulating the left hindlimb. This burst was binned with others of similar correlation values and the presence or absence of a twitch in the left hindlimb preceding the burst was used in the calculation of the probability that bursts of this correlation bin were preceded by a twitch, as shown in Figure 3.4C. A one-way ANOVA revealed a strong effect of correlation with sensory template on the likelihood of being preceded by a twitch (p = 1.2 *10^-8). The likelihood ranged from 28 +/- 6% for bursts that had a correlation coefficient with their corresponding sensory template in the range of 0 to 0.1,
to 79% +/- 5% for those that had a correlation coefficient with their corresponding sensory template above 0.6.

3.3.3 Synchronized spontaneous limb twitches contribute to correlated activity

Spindle bursts can synchronize activity between hemispheres, potentially assisting the development of homotopic connections in the cortex (Marcano-Reik and Blumberg, 2008; Yang et al., 2009). We took advantage of the large imaging area of our preparation to examine such interactions between hemispheres, as well as within hemispheres, for the delta component captured by VSD. Figure 3.5A shows mean images of VSD signals from one rat pup at the time of all bursts in a given region of S1. An obvious feature is the presence of activity in both hemispheres, even when the mean image is calculated based on bursts in the hindlimb region, which is unilateral when evoked via stimulation (Figure 3.1B and 1E). To quantify this feature across all animals, we calculated the correlation between the VSD signals from subregions of S1 as shown in 5C. A one way ANOVA revealed significant differences among these correlations (p = 0.002) with the highest correlation being between tail S1,L and tail S1, R. This is not surprising given that these two regions respond bilaterally to sensory input to the tail. As a control, we calculated the correlation between the VSD signal in the tail S1,L and the tail S1,R offset by 3 s. The mean of this correlation across pups was -0.13 and a paired t-test comparing it to the correlations between the non-lagged signals showed a highly significant result (p = 3.6 x 10^-8), making it unlikely that any rhythmic movement artifacts (heartbeat, respiration) contributed to the described correlations.
Interactions between the peripheral limbs are one source of these correlations. Figure 3.5B shows two examples of twitches interacting to produce synchronized activity on the brain. In the upper panel, the right and left hindlimbs twitch sequentially, and generate sequential bursts in HLS1,L and HLS1,R. In the lower panel, a tail twitch causes the tail to strike the right hind paw, causing bursts bilaterally TLS1 and HLS1,L. The potential for interactions among twitching limbs is high, as we found that 71 +/- 1.9% of twitches were accompanied by a twitch in at least one additional limb or tail within 800 milliseconds, and 40 +/- 3.6% were accompanied by twitches in both additional limb/tail in the same time period. Figure 3.5D, shows the cumulative probability distribution for these concurrent twitches over time. To quantify the contribution that synchronization of twitches made to the coincidence of cortical bursts of activity, we calculated the likelihood that a twitch would be followed by a burst in its associated region of cortex (e.g., left twitch followed by burst in HL, S1R, termed matched), as well as in a non-associated region of sensory cortex (unmatched) (Figure 3.5E). We found that 34 +/- 4.3% of twitches were followed by a matched burst and 24 +/- 3.8% were followed by an unmatched twitch. When we restricted this calculation to twitches that were isolated from any other twitch by 500 ms, the proportion of twitches that were followed by a matched burst was 38 +/- 6.7% which was not significantly different from the non-isolated twitches (paired two-tailed t-test, p = 0.41). The proportion of twitches followed by an unmatched burst, however, fell to 9.9 +/- 4.9% which was significantly lower than the same value for the non-isolated bursts (paired two-tailed t-test, p = 8.2 x 10^{-5}). To allow direct comparisons to earlier studies that examined the incidence of spindle bursts following hindlimb twitches (without examining tail movements) (Marcano-Reik and
Blumberg, 2008) we also examined the incidence of ipsi- and contralateral bursts following limb twitches (Figure 3.5F). After any limb twitch, the likelihood of a burst in the contralateral hindlimb sensory cortex was 40 +/- 4.8 %, and 28 +/- 4.0% in the ipsilateral cortex. After isolated limb twitches, the likelihood of a burst in the contralateral hindlimb cortex was unchanged at 40 +/- 6.7% (paired two-tailed t-test, p = 0.99), while the likelihood of a burst in the ipsilateral cortex fell to 11 +/- 4.8% (paired two-tailed t-test, p = 1.2 x 10^-5). Thus, while signals of cortical activity are correlated among the sensory regions we examined, there is a large decrease in the incidence of bursts in unmatched regions of the cortex when examining isolated twitches. This supports the conclusion that synchronized twitching is a contributor to the correlation of VSD signals in different regions of S1.

3.3.4 Cortical bursts following limb twitches spread towards the midline of the brain

Having shown similarities between the spatial patterns of cortical activity that follow stimulation of the limbs and those that follow limb twitches, we next examined the dynamic nature of spontaneous cortical activity. For example the bursts in Figure 3.3B show a medial, asymmetrical spread. Figure 3.6A shows two additional bursts from two pups that are dynamic, moving in both cases from S1 medially towards the midline. The correlation of ongoing activity with the three sensory templates is shown to the left of the montages in Figure 3.6A, with the time spanned by the montage shown with gray background. To examine the burst spread across all animals, we performed an analysis similar to that described in Figure 3.2, except that rather than using bursts following stimulation of the limbs, we used all bursts during spontaneous activity for which the
correlation with the corresponding sensory template exceeded 0.3. This threshold ensured we were examining bursts in which there was a relatively strong and isolated activation in the sensory cortex. In the upper montage of Figure 3.6, for example, the brain image at the time of a burst in HLS1,R was highly correlated with the sensory template corresponding to such a burst, that of the left hindlimb; in the lower montage, the brain image at the time of a burst in HLS1,L was highly correlated with its corresponding template, that of the right hindlimb. Figure 3.7B shows that one hundred ms prior to the peak of the burst, there was no significant difference among the VSD signal at the 8 cortical points (one-way ANOVA, p = 0.46). At the peak of the burst, there was a significant difference among these points (one-way ANOVA, p = 1.8 x 10^{-7}). The largest difference, as with the bursts following stimulation of the limbs, was between points on the medial-lateral axis. Mean VSD signal at the lateral point was 0.052 +/- 0.0082 %ΔF/F as compared to 0.14 +/- 0.016 %ΔF/F at the medial point. Tukey’s HSD test showed VSD points on all axes except the anterior-posterior axis were significantly different. Two hundred ms after the peak of the bursts, there remained a strong effect of location on the VSD signal (one-way ANOVA, p = 2.5 x 10^{-5}), and points on the medial-lateral axis remained significantly different from each other. The lower panel of Figure 3.6B shows the similar analysis for bursts of the tail cortex. As with the bursts following tail stimulation (Figure 3.2), the effect of cortical location on the VSD signals was not significant at any of the time points examined (one-way ANOVA, 100 ms prior to peak, p = 0.37; at peak, p = 0.54, 200 ms after peak, p = 0.25).

More evidence that limb-movement triggered bursts of activity in S1 spread medially is shown in Figure 3.8. Here, we calculated the correlation between signals of
limb movement and the VSD signal from different regions of the cortex, delayed relative
to each other by varying amounts. Figure 3.8A shows an example of this calculation. In
this example, a sequence of VSD images collected under light anesthesia is shown at left.
The VSD signal collected from a single pixel at the center of the overlaid black box is
shown at right, delayed 200 ms relative to the movement signal of the left hindlimb,
shown directly below it. The value of the correlation between these two signals was put
back into the same pixel. Repeating this process for each pixel, we generated a map
showing the degree to which each part of the cortex was correlated to the movement
trace. Figure 3.8B shows examples from one pup showing the correlation maps described
in Figure 3.8A for the movement traces of the left hindlimb, the right hindlimb, and the
tail. As a control, a correlation map for VSD signals delayed 2 s relative to the right hind
movement trace is also shown in Figure 3.8B. Highly correlated pixels are clustered
within and medial to S1, except in the control image which shows very low correlation.
Figure 3.8C shows these results combined across animals and across sub-regions of
S1. We calculated the cross-correlation of activity in each region of S1 with the
movement trace of the corresponding limb (e.g. VSD signal from HLS1, R with the left
hindlimb movement trace) to represent the extent to which cortical activity and limb
movements were related. To determine how this relation varied in regions surrounding
S1, we also performed the cross-correlation of the movement trace with the VSD signal
taken medially, laterally, anteriorly and posteriorly to each subregion of S1. Figure 3.8C
shows the means of these cross correlations. As shown in the left panel Figure 3.8D, the
delay yielding the maximal correlation was highest at the most medial position relative to
S1 (243 +/- 4.9 ms, compared to 200 +/- 6.9 ms at the center of S1) and the delay was
strongly influenced by the position medial or lateral to the center of the associated sensory cortex (one-way ANOVA, \( p = 1.2 \times 10^{-4} \)) but not by the position anterior or posterior to the sensory cortex (one-way ANOVA, \( p = 0.83 \)). This was consistent with a spread of activity towards the midline. The right panel of Figure 3.8D shows the value of the correlation. It was highest in S1 (0.32 +/- 0.019) and decreased symmetrically in surrounding directions.

Although we focused our analysis on bursts of VSD signal in S1 and their association with peripheral spontaneous twitches, in order to align with previous research in these areas, we also examined the VSD signal from other regions of the cortex. A prominent difference is that bursts of VSD signal outside of S1 are not associated with twitches. Two examples of such bursts are shown in Figure 3.9A. In both cases there was no associated twitches. To compare the spatial layout of bursts associated with a twitch with those that were not associated with a twitch, we calculated the relative frequency of bursts following a twitch (within 300 ms), or not associated with a twitch, and presented these results as a color-mapped image of relative frequency for each point on the cortex, as shown in Figure 3.9B. Right and left hemispheres were aligned independently to the center of HL S1, and HL and TL S1 from one pup are shown for reference. Note that those bursts that followed a twitch are concentrated within S1, while those that did not are located in areas surrounding S1. A notable limitation is that we did not collect twitches of the forelimbs, thus activity in the forelimb S1 cortex (lateral and anterior to the hindlimb S1) should not be assumed to be independent of twitches of the forelimb. To quantify this difference across all animals, we calculated the correlation coefficient between the mean image of each burst not associated with a twitch and all three (Right
hind, Left hind, and Tail) sensory templates. We took the maximum of these three values as the spatial correlation of this burst with a sensory pattern. We compared this value with the correlation between the mean image of brain activity following an isolated limb or tail twitch and the corresponding sensory template as well as their non-corresponding sensory templates (e.g., the correlation between a VSD burst following a tail twitch and the tail sensory template (matched) as well as the left and right hindlimb sensory templates (unmatched)). The means of these three sets of correlations are shown in Figure 3.9C. As is subjectively apparent in Figure 3.9B, the spatial pattern of bursts associated with a twitch is significantly closer to bursts evoked by direct stimulation than the spatial pattern of bursts that do not follow a twitch. Correlation for a burst following a twitch was 0.36 +/- 0.04 with its matched sensory template, and 0.034 +/- 0.05 with its unmatched template. The maximum correlation between a burst that did not follow a twitch and all three sensory templates was 0.13 +/- 0.04. These values differed significantly (one-way ANOVA, p = 1.3 x 10^{-8}). Combined with the results shown in Figures 3-6, we conclude that while slow cortical activity in S1 is closely associated with peripheral twitches, activity in non-sensory areas exists independent of twitches in the peripheral limbs.

### 3.4 Discussion

In this study we recorded cortical activity using VSD imaging during the early post-natal period, P4-P6. We used the high resolution recordings provided by this technique to gain greater insight into the structure of cortical activity at a time of rapid
cortical maturation via synaptic formation and dendritic growth (Ben-Ari, 2001; Tau and Peterson, 2010).

### 3.4.1 Comparison with other studies of developmental cortical activity

The study of correlated neural activity in developing mammalian cortex is currently characterized by a variety of patterns, described in a range of preparations including cortical slices (Garaschuk et al., 2000; Corlew et al., 2004; Sun and Luhmann, 2007; Allene and Cossart, 2010) and the in vivo intact brain (Khazipov et al., 2004; Adelsberger et al., 2005; Marcano-Reik and Blumberg, 2008; Golshani et al., 2009; Seelke and Blumberg, 2010; Minlebaev et al., 2011). Most relevant to our results are patterns of activity described in vivo. Most prominent of these is the spindle burst, an event lasting 200-600 ms and consisting of a burst of 10-25 Hz oscillations found in the somatosensory (Khazipov et al., 2004; Minlebaev et al., 2007) and visual (Hanganu et al., 2006) cortices. Oscillations in the gamma range of frequencies (30-40 Hz) also exist every 10-30 seconds (Yang et al., 2009; Seelke and Blumberg, 2010), while long (40 s) bursts of 20-30 Hz activity repeat every 20-30 minutes (Yang et al., 2009). Bursts of gamma-range activity have also recently been described in the developing barrel cortex, under control of excitatory thalamic synapses (Minlebaev et al., 2011). Finally, two recent studies report slow (> 0.1 Hz) oscillations in the developing sensory cortices (Colonnese and Khazipov, 2010; Seelke and Blumberg, 2010).

The activity we describe using VSD is most closely related to spindle bursts. Like spindle bursts, this activity follows limb twitches (Figure 3.3), can be evoked by direct stimulation of the limbs (Figure 3.1), is primarily located in the sensorimotor cortex.
(Figure 3.9), and occurs approximately every 10 seconds. Furthermore, spindle bursts can exist on top of slower depolarizations in the frequency range that we examine here (0.1-10 Hz) (Marcano-Reik and Blumberg, 2008; Minlebaev et al., 2009). Simultaneous recordings of VSD signals and local field potentials in the sensorimotor cortex would be necessary to confirm a relationship between the signals we observe and previously described spindle bursts.

3.4.2 Functional role of movement-generated cortical activity

The purpose of movement related cortical activity is difficult to test directly, but its properties are consistent with a role in refining homotopic maps within the somatosensory cortex (for review see (Khazipov and Luhmann, 2006; Blumberg, 2010)). The results presented here support and extend this hypothesis. By comparing the pattern of activity evoked across the cortex following twitches and direct stimulation of the limbs, we show that twitches robustly activate the ascending fibers arriving at the cortex. There was a much higher correlation between the activity pattern evoked by the stimulation of the twitched limb than the activity pattern evoked by other limbs (Figure 3.8C), indicating the activity is confined to the appropriate portions of the somatosensory cortex, which is consistent with previous reports (Khazipov et al., 2004). However, when we examined the entire VSD signal, as opposed to only those portions following isolated twitches, we found correlations between sensory regions across the span of our recordings (Figure 3.5). In part, this lack of specificity arises because isolated twitches are not common (Figure 3.5D). Figure 3.5B shows a clear example of such a case. This is in contrast to (Marcano-Reik and Blumberg, 2008), who reported that spindle bursts did
not overlap in opposing hemispheres. However, they showed the fast and slow component of spindle bursts arise from different forms of sensory input; tactile inputs give rise to the fast, while proprioceptive inputs give rise to the slow component. Furthermore, their preparation minimized tactile inputs during spontaneous twitches by keeping the limbs suspending away from any surface. In our preparation the limbs were free to interact with each other and the supporting surface as they twitched. Thus the activity we report may reflect tactile inputs from a complex interaction of the limbs moving under natural conditions.

As described in section 1.4.3 development of callosal projections depends on activity in both pre- and post-synaptic cells (Mizuno et al., 2007; Wang et al., 2007). The results we present here provide an in vivo example of how this rule could promote appropriate callosal development. Correlated activity in related limbs, generated via developing spinal networks (see section 1.3.2.2) generates correlated activity within homotopic regions of the somatosensory cortex, which could promote maturation of callosal fibres via Hebbian plasticity. This process can be seen as long-distance, cortico-cortico analogue to the role of retinal waves as described in section 1.3.3.1.

3.4.3 Medial spread of cortical bursts

One key difference between the activity that we describe and previous descriptions of developmental cortical activity is that the activity we describe has a prominently asymmetrical spread (Figures 2, 7 and 8). Spindle bursts, in contrast are generally described as locally-confined and immobile (Khazipov et al., 2004; Yang et al., 2009). We believe an important difference between the current study and the previous
description of spindle bursts is that we have focused our analysis on the slow component of the spindle burst. This corresponds to the slow envelope component of some spindle-bursts (Marcano-Reik and Blumberg, 2008; Minlebaev et al., 2009). Yang (2009) and Khazipov (2004), in contrast, examined spread of the fast component of spindle bursts, examining signals that had been bandpass filtered with 5 Hz as the low cut-off. Nevertheless, the faster component has been noted to travel (see Supplemental Figure 3 Khazipov (2004) which shows the fast component of a spindle burst moving medially over approximately 2 mm). Similarly, (Marcano-Reik and Blumberg, 2008) found that inactivation of the forelimb somatosensory cortex does not eliminate the occurrence of spindle bursts in that region, which the authors interpreted as evidence for a contribution traveling from neighboring tissue. It is possible that a dynamic, traveling nature may be a minor feature of the fast component, but a major component of the slow component, of spindle bursts.

A traveling component to movement-related activity would be consistent with activity in many developing neural systems (Calderon et al., 2005; Firth et al., 2005; Watt et al., 2009). In sensory areas, they promote homotopic connections by synchronizing cortical regions receiving afferent inputs from adjacent sensory organs (Feller, 1999). In the adult rodent, close anatomical connections exist between the somatosensory and motor cortices (Donoghue and Parham, 1983; Fabri and Burton, 1991) and the motor cortex is rapidly altered by sensory input (Donoghue and Sanes, 1987; Sanes et al., 1990; 1992). Depolarization from the somatosensory cortex that moves medially within the developing cortex may serve to promote the maturation of these anatomical and functional connections. In this way, spontaneous twitches could promote appropriate
connections within the cortex in a manner analogous to how they have been shown to do so at the level of spinal reflexes (Holmberg and Schouenborg, 1996; Petersson et al., 2003; Schouenborg, 2004). Interestingly, the tail representation of the motor cortex is small and indistinct in the rodent (Gioanni and Lamarche, 1985; Tennant et al., 2011), and we found that bursts in the tail sensory cortices, after either stimulation (Figure 3.2) or twitching (Figure 3.7) did not spread medially to the same extent as those in the hindlimb cortices.

Traveling waves of cortical activity have been described in the adult rodent cortex using VSD imaging (Wu et al., 1999b; Berger et al., 2007; Han et al., 2008; Mohajerani et al., 2010). Although these waves are different from the activity we describe in that they reflect continuous, ongoing, internally generated cortical activity that does not emerge until approximately P12 (Seelke and Blumberg, 2008; Colonnese and Khazipov, 2012), they are similar to the waves we describe in that they are highly localized and bilaterally synchronized (Mohajerani et al., 2010) and may reflect a template of sensory experiences (Han et al., 2008).

### 3.4.4 Use of voltage-sensitive dyes to study developmental activity

VSD imaging have proven useful in the study of cortical dynamics across large regions in the mammalian cortex (Tsodyks et al., 1999; Ferezou et al., 2006; 2007; Xu et al., 2007; Huang et al., 2010; Mohajerani et al., 2010), as well as patterns of activity in slices of developing rat hippocampus (Bolea et al., 2006) and localized responses in the developing mouse sensory cortex (Borgdorff et al., 2007) but to our knowledge this is the first time they have been applied to the study of bilateral developmental activity in the
intact *in vivo* brain. Despite being an additional technique with the potential to provide another set of descriptions of cortical activity during development, we believe that VSD imaging can add to our understanding of this topic. It provides high resolution signals that reflect membrane potential changes from the intact brain, an important consideration as cortical connections, which are disrupted by slicing, have been found to be essential to some forms of activity in the developing brain (Sun and Luhmann, 2007). It permits the collection of activity from large regions of the cortex, including multiple sensory regions (Figures 3,4) as well as regions outside of the sensory cortices (Figure 3.8), where our knowledge of activity patterns is limited (Seelke and Blumberg, 2010). Notable weaknesses of VSD imaging include a limited contribution to the signal from deep layers of cortex (Berger et al., 2007; Chemla and Chavane, 2009; Mohajerani et al., 2010), particularly relevant as synaptic inputs to superficial layers 2/3 are weak during early life (Stern et al., 2001; Bureau et al., 2004). This weakness contributes to the more complex EEG signal compared to the VSD signal (Figure 3.1C) (Devonshire et al., 2010). It is also worth noting that VSD signals can reflect subthreshold, dendritic depolarization (Grinvald and Hildesheim, 2004; Chemla and Chavane, 2009) making direct comparisons to patterns of calcium waves attributable to spiking (Schwartz et al., 1998; Garaschuk et al., 2000; Adelsberger et al., 2005) difficult (Berger et al., 2007). Nevertheless, it has recently been suggested that the diverse patterns of developmental activity previously reported are in fact all manifestations of spindle bursts under different experimental conditions (Khazipov and Buzsaki, 2010), and *in vivo* VSD imaging may provide important clues to resolve this issue.
3.5 Figures

Figure 3.1. VSD imaging provides high resolution images of large region of the developing rat cortex. In this and all subsequent figures, 10 animals were used for group data analysis unless otherwise indicated. A) Cartoon showing experimental setup. Panels to right show simultaneously collected video of cortex and body. B) Montages of VSD images following cutaneous stimulation of limbs and tail. White dot is bregma. A, Anterior; L, Left; P, posterior; R, Right. Images at 100 ms intervals. C) EEGs recorded simultaneously with left-hindlimb stimulation shown in panel B. VSD signal from HLS1,R is also shown. D) Map showing region of cortex activated by stimulating tail (green), right hind (red), and left hind (blue), relative to bregma (black circle). Open circles show geometric mean of activated cortex. Position of EEG electrode used for signals in C is shown. E) VSD signals evoked by stimulation of right hindlimb (right) and tail (left). In this and subsequent figures, light shaded region denotes standard error.
Figure 3.2: Activity spreads asymmetrically following hindlimb stimulation. A) Mean images after right and left hindlimb stimulation. Images after left hindlimb stimulation has been flipped on medial-lateral axis. Upper panel: Mean images showing VSD signal. Lower panel: intensity along a medial/lateral (black) and anterior/posterior (gray) axis through the point of initial activation (as shown by the solid line, first box, upper panel). B) Left: VSD signal intensity at 8 spatial points 0.975 mm from the point of initial activation, at three time points, following stimulation. Spatial points are shown and labeled in the right most image of the upper panel of Figure 3.2A. L-M, lateral medial axis; P-A, posterior-anterior axis; PM-AL, posterior-medial to anterior-lateral axis; AM-ML, anterior-medial to posterior-lateral axis. C) As described for 2B, except following tail stimulation. * denotes significant difference (p < 0.05) between VSD signals, tested using Tukey’s HSD test.
Figure 3.3: Bursts of VSD signal in sensory cortex lead to correlation with the overall cortical pattern evoked by stimulating the limbs/tail. A) VSD signal (top), limb movement (middle) and concurrent correlation of instantaneous cortical activation pattern with pattern evoked by limb/tail stimulation (bottom). B) Montages showing cortical activity every 100 ms in shaded gray regions highlighted in A.
Figure 3.4: Cortical bursts and twitches have a variable relationship. A) VSD signal (top), limb movement (middle) and concurrent correlation of instantaneous cortical activation pattern with pattern evoked by limb/tail stimulation (bottom). Note that bursts may or may not be preceded by a twitch in the corresponding limb. B) Left, probability density function of a limb twitch being followed by a burst in the corresponding subregion of S1, across time points relative to the twitch. Right, probability density function of a burst of VSD signal in S1 being preceded by a twitch in the corresponding limb, across time points relative to the burst. C) Probability of a peak of VSD signal in a subregion of S1 being preceded by a corresponding limb twitch (y-axis), across correlation value at the time of the VSD peak (x-axis). Because of the small numbers of correlation values above 0.6 and below 0 we binned these values together. * denotes significant effect (p < 0.05) of correlation value, tested using Tukey’s HSD test for multiple comparisons.
Figure 3.5: Changes in VSD signal are correlated across sensory regions. A) Images from one animal show the mean image of cortical activation for peaks of VSD signal in TLS1, HLS1,L, and HLS1,R, respectively. B) Interactions between limbs contribute to correlated VSD signals in sensory cortices. Montages show cortical activity following limb twitch, shown at right of montages. Dotted lines show time of images within montage. Upper panel, right and left hindlimb twitch in sequence. Lower panel, tail twitches and strikes left hindlimb. B) Matrix showing correlation of VSD signals between subregions of S1. D) Probability density function of one or two additional twitches across time points relative to a given twitch. E) Likelihood of a twitch being followed by a matched or unmatched VSD burst in sequence. Lower panel, tail twitches and strikes left hindlimb. F) Likelihood of a limb twitch being followed by a contralateral or ipsilateral burst, for all bursts (left) vs. isolated bursts (right). * denotes significant difference (p < 0.05) between means, tested using student’s t-test.
Figure 3.6: Cortical activation is dynamic. Montages show examples of cortical activity at time of HLS1,R (top) and a HLS1,L activation (bottom). At left of montages, ongoing correlation with sensory templates is shown. Gray region denotes times represented in montages. Panels at right of montages show instantaneous position of center of burst at 40 ms intervals; green dot denotes position at burst onset and red dot denotes position at burst end.
Figure 3.7. Cortical activation of sensory regions spreads medially A) Upper panel: Mean images showing VSD signal before, during, and after bursts of VSD activity that were correlated with their corresponding sensory templates. Bursts were included if they had a correlation above 0.3 with their corresponding sensory template. Lower panel: VSD signal along a medial/lateral (black) and an anterior/posterior (gray) axis through the center of the sensory cortex. Lines representing these axes are shown in upper panels. C). Top panel: VSD signal intensity at 8 spatial points 0.975 mm from the point of initial activation, at three time points, following stimulation. Spatial points are shown in the right upper most image, of A. L-M, lateral-medial axis; P-A, posterior-anterior axis; PM-AL, posterior-medial to anterior-lateral axis; AM-ML, anterior-medial to posterior-lateral axis. Bottom panel: As described for top panel, expect for bursts correlated with tail template. A, Anterior; L, left; P, posterior; M, medial. * denotes significant difference (p < 0.05) between VSD signals, tested using Tukey’s HSD test.
Figure 3.8. Limb twitches initiate dynamic activity across the cortex. A) Example VSD signals correlated with movement signals from one hindlimb. The correlation between the VSD signal at each pixel and the movement signal was calculated and used to generate a map showing the correlation of VSD signals from each region with the limb movement signal (sequence of VSD images shown at left, VSD and limb movements shown in center, final map of all correlation values shown at right) B) Example images from one pup showing the correlation value of each pixel with limb movement signals. Note the peak of correlation in, and medial to, the associated sensory cortex; note no correlation in the control (2 s delay) image. C) Correlation between VSD signals and limb movements at points relative to sensory cortex across time delays. Time of maximal correlation is shown by vertical line in matching color. Note close cluster of times, except for medial region of cortex (red). Control signal offset by 2 s. D) Upper panels show delay giving maximal correlation across medio-lateral axis (left) and anterior-posterior axis (right). Colors match C). Lower panels show maximal value of correlation across same axes. Med, Medial; Lat, lateral; Pos, posterior; Ant, anterior; Cont, control.
Figure 3.9: Bursts in the absence of twitches may occur in non-somatosensory regions of cortex. A) Montages show two examples of bursts not associated with twitches in any limbs. B) Maps showing relative frequency of activation of cortex for bursts following within 300 ms (right), or not following (left), a twitch. We calculated the mean image of each burst, and aligned all bursts across animals on the center of HL S1. Each pixel is colored to represent the relative number of bursts that overlapped it. Note the different scale bars in the two images. C) Correlation between mean VSD image after a twitch and the corresponding sensory template (Twitch, matched), compared with the same value for bursts that do not follow a twitch (No twitch). Because there is no corresponding sensory template with which to calculate the correlation value, it was calculated with all three templates, and the maximum of the three was considered the correlation with a sensory template. For comparison, correlation between brain activity after a twitch and a two non-corresponding sensory templates are also shown (Twitch, unmatched).
CHAPTER 4. SPONTANEOUS ACTIVITY SYNCHRONIZES WHISKER-RELATED SENSORIMOTOR NETWORKS PRIOR TO THEIR MATURATION IN THE DEVELOPING RAT CORTEX

4.1 Introduction

One new finding from chapter 3 is that cortical activity in early life is mobile and spreads predominantly towards the midline, suggesting a possible role in development of connections between sensory and motor regions of the brain. Integration of signals between these regions is necessary to accurately sense the environment and move within it (Franklin and Wolpert, 2011) and examining activity during early life within sensory and motor systems that are tightly linked in adulthood may provide insight into the development of this integration. A clear example of such tightly linked systems exists in the whisker sensorimotor system of the rodent (Aronoff et al., 2010). The barrel cortex, the primary sensory destination of whisker related sensory inputs, is connected to the motor cortex via mono-synaptic excitatory fibers (White and DeAmicis, 1977; Hoffer et al., 2005; Chakrabarti and Alloway, 2006; Ferezou et al., 2007) and stimulation of the vibrissae or their afferents evokes activity in the motor cortex shortly after activation of the primary barrel sensory cortex (Kleinfeld et al., 2002; Ferezou et al., 2007; Chakrabarti et al., 2008).

How the corticocortical connections that underlie these interactions develop is not well understood. Molecular cues under the control of regional gene expression play important roles in the guidance of thalamocortical axons and the subsequent development
of cortical identity (Miyashita-Lin et al., 1999; Rubenstein et al., 1999; Fukuchi-Shimogori and Grove, 2001; Hamasaki et al., 2004; Shimogori and Grove, 2005) and are likely to have similar roles in the guidance of corticocortical neurons (Barbe and Levitt, 1995; Huffman et al., 2004). The contribution of extrinsic, activity dependent factors in the development of cortical sensorimotor circuits is not known, but activity-independent factors alone are insufficient to allow normal formation of such circuits. Evidence for this comes from studies in which whisker clipping (Keller and Carlson, 1999), transection of the whisker afferent nerves (McCasland et al., 1992; Rhoades et al., 1996), and manipulation of extrinsic serotonin levels (Lane et al., 2002) result in altered or absent intracortical projections from the barrel cortex.

One mechanism via which extrinsic inputs could promote the formation of appropriate cortico-cortical connections is patterned spontaneous activity that correlates depolarizations among disparate brain areas, allowing synaptic stabilization via Hebbian processes. Such mechanisms have been well described in visual systems (Constantine-Paton et al., 1990; Torborg and Feller, 2005) but evidence for them is weak in other systems. The large majority of studies of spontaneous activity patterns in early life have examined activity within a single sensory cortex (Khazipov et al., 2004; Minlebaev et al., 2007; Marcano-Reik and Blumberg, 2008; Yang et al., 2009; Colonnese and Khazipov, 2010). Studies using functional magnetic resonance imaging (fMRI) in very young human infants have shown networks of cortical areas synchronized by very slow oscillations of the BOLD signal (Fransson et al., 2007; 2011); as in adults, these networks have small-world properties even in very young infants (Fair et al., 2009; Yap et al., 2011). Others have examined the integration of sensory cortices into wider networks of
cortical areas (Colonnese et al., 2008; Quairiaux et al., 2011), but to date it is not known if these maturating networks are reflected in spontaneous cortical activity. If they are, it would provide a worthwhile target for further examination of the roles of extrinsic factors in the development of cortico-cortico networks.

In this study we address this issue by using VSD imaging to provide signals of cortical activity with high spatial and temporal resolution (Shoham et al., 1999; Grinvald and Hildesheim, 2004) over a large region of the cortex spanning both medial cortical areas and the barrel cortex from the *in vivo* rat brain of young (P5-P12) pups. Our imaging was conducted under light anesthetic (0.25-0.75% isoflurane). We found that stimulation of the whisker in P5-6 pups evoked activity in a focused and local region of the barrel cortex. Stimulating the whisker in P12 pups resulted in activation of medial motor areas in addition to the barrel cortex. In the intervening ages (P8-P10) the medial activation was variable. Nevertheless, we found that spontaneous activity in the barrel cortex was correlated with cortical activity in the putative motor cortex, regardless of whether whisker stimulation evoked activity in these regions. These results suggest that spontaneous cortical activity may be a factor in the formation of mature cortico-cortical connections.

4.2 Methods

All procedures used in this study were conducted with the supervision and approval of the University of British Columbia Animal Care Committee.
4.2.1 Animals model and surgical procedures

We performed the experiments in this study using Sprague-Dawley rats of either sex, from ages of P5 to P12 (P5, n = 4; P6, n = 5; P8, n = 5; P10, n = 4; P12, n = 7). We induced anesthesia using isoflurane (1.5-2%) mixed in oxygen. Following induction and throughout our imaging procedures, we maintained body temperature at 37 °C. Following subdural injection of local anesthetic, we removed the scalp and attached the skull to a custom built head-plate using dental cement. This headplate has embedded channels through which we passed warm water to maintain cortical temperature at 37 °C, following the completion of preparatory surgery. We fixed the plate to a custom-built platform, and removed the skull and dura of the right hemisphere in a region extending approximately 2 mm posterior of bregma to 5 mm anterior of bregma, and immediately left of bregma to 7 mm lateral of bregma. We clipped all whiskers on both sides except for the E2 whisker to allow for precise stimulation.

4.2.2 VSD preparation, application and imaging

We stained the cortex with VSD as described in Chapter 3. Images of cortical activity were collected under light isofluorance anesthesia (0.25-0.5%, P5-P8; 0.5-0.75% P10-P12). We collected images as described in stion 2.2. A sond synchronized camera focused on the animal allowed us to monitor the pup for any large movements or startles and adjust anesthesia as needed. When stimulating the whisker, the stimulator was attached ~ 1mm from the snout, resulting in a deflection of ~10 degrees. When stimulating the forelimb, the stimulator was attached to the dorsal surface of the paw between the sond and third digits.
4.2.3 Data analysis

We processed raw VSD images as described in chapter 2. To determine the onset of cortical activity following stimulation of the whisker and limbs, we thresholded each image collected following stimulation at 60\textsuperscript{th} percentile. The first image on which a single area greater than 20 pixels\(^2\) (~0.078 mm\(^2\)) crossed this threshold was classified as the onset of activation. The center of this region was classified as the center of the sensory region being examined. We use the terms barrel cortex (BC) and forelimb cortex (FL) to refer to the center of the regions activated by whisker and forelimb stimulation, respectively. To determine the area of activation, we calculated the mean image from the time of onset to the time of maximal VSD signal, and thresholded this mean image at the 60\textsuperscript{th} percentile.

To determine if there was an independent activation in medial/motor regions of cortex following whisker stimulation, we examined the region of cortex anterior and medial to the detected center of forelimb S1. In this isolated region, we repeated the detection algorithm described above, searching for an isolated region of activated cortex larger than 20 pixels\(^2\) (~0.078 mm\(^2\)). If such an area was not found, we used a point 45 degrees anterior-medial to the detected forelimb region, at a distance of 75\% of the distance between the detected forelimb and barrel regions. As this point represents the presumptive motor cortex, we termed this region \textit{M1}. As a comparison region of cortex, we used a point with the same medio-lateral position as the detected or calculated motor cortex, and the same anterior-posterior position as the detected barrel cortex. We refer to this point as simply \textit{parietal} to differentiate it from the motor cortex point in the frontal cortex. These locations are summarized in Figures 4.1C and D. We used these same
points to analyze spontaneous cortical activity. For each region of interest (ROI) described above, we took the mean of the VSD signal from a 5x5 pixel region centered on the ROI. We used the synchronized video of the animal’s hindquarters to manually determine any periods of time when the animal was making any movements other than small one- or –two limb twitches, and removed these time periods form analysis.

Correlation maps were calculated as described in Chapter 2.

We were interested to determine if there was a region of cortex in the anterior-medial region that was highly correlated with activity in the barrel cortex during spontaneous brain activity. In searching for such a region, we had two classes of pups – those in which the M1 point had been detected during whisker stimulation (see P12 pup in Figure 1 examples) and those the M1 point had been estimated, as no signal had been detected (see P8 and P5 pup in Figure 1 examples). In the case of the former, we collected the correlation value at the detected M1 point, and compared it to the parietal comparison point. In those pups in which M1 was estimated (not detected), we thresholded the correlation map anterior and medial to the FL S1 point at the 90th percentile and took the center of this region. This strategy allowed us to find regions in the area of presumptive motor cortex that were correlated to the BC without any a priori knowledge of where exactly these regions would be.

4.2.4 Detection of bursts of VSD activity

To examine relationships between bursts in different cortical regions (Supplementary Figure 3), we set a threshold VSD signal above which we considered the region to be active. In pups age P5-P8, we determined the value of a threshold set at three
times the standard deviation of the VSD signal. By age P10 and P12, cortical activity was nearly continuous (Supplementary Figure 2) and accordingly the standard deviation was not appropriate for thresholding. The values of three times the standard deviation for the P5-P8 pups was 0.10 +/- 0.006 (BC), 0.11 +/- 0.002 (M1), 0.10 +/- 0.004 (parietal) and 0.10 +/- 0.005 (FL). Thus, we used a threshold of 0.10 for the P10-P12 pups.

4.2.5 Statistical analysis

We compared means between groups using one- or two-way ANOVA as appropriate, with Tukey’s Honestly Significant Difference correction for multiple comparisons when comparing means of groups. We used paired one-way t-tests when comparing pairs of correlations. Correlation values were corrected using Fischer’s z-transformation before statistical analysis.

4.3 Results

In this study, we examined activity in the sensory and motor areas of the developing rat cortex. The key finding was that activity could be correlated between the barrel and motor cortices even when activating the sensory region directly via whisker stimulation did not activate the motor cortex. Our experimental approach is shown in Figure 4.1.

4.3.1 Spatial and temporal properties of whisker-evoked activity change with age

Montages in Figure 4.2A show the cortical pattern of the whisker responses to contralateral whisker stimulation at five time points following stimulation in three pups,
The latency of the VSD response decreased with age, from 0.083 +/- 0.015 s at P5 to 0.035 +/- 0.0050 s at P12 (one-way ANOVA, p = 0.018). There was also a significant effect of age on time above 50% of the maximum response, which decreased from 0.263 s +/- 0.031 s at P5 to 0.014 +/- 0.0042 s (one-way ANOVA, p = 4.0 x 10^{-4}). The mean area of cortex activated (see Methods for calculation details) increased with age from 0.40 +/- 0.13 mm² at P5 to 5.5 +/- 1.0 mm² at P12 (one-way ANOVA, p = 0.0012). These results are shown in Figure 4.3. Also shown on Figure 4.3 are the responses evoked by ipsilateral whisker stimulation. There was a significant effect of age on the maximum ipsilateral response, increasing from 0.058 +/- 0.011 % ΔF/F at P5 to 0.15 +/- 0.017 % ΔF/F at P12 (one-way ANOVA, p = 0.0022).

4.3.2 Emergence of whisker-evoked activity in motor cortex

A prominent feature of the P12 example is the presence of an independent medial area of activity following stimulation of the whisker, similar to as described in the adult rodent (Ferezou et al., 2007). We describe this region as motor cortex based on this response and its location; we did not assess whether stimulation elicited movement responses. The outlines of these regions, along with the region activated by stimulating the forelimb, are shown in the left panels of Figure 4.2A.

To allow for a comparison of M1 activation following whisker stimulation across ages, we defined a region from which to collect a signal in the absence of any detected depolarization. This region was anterior and medial to the detected forelimb region, as described in section 4.2. We also defined a point directly medial to the barrel cortex for comparison, as shown in Figure 4.1C. We describe this latter point simply as parietal to
differentiate it from the M1 point, within the frontal cortex. Figure 4.2B shows the mean VSD signals collected from these points following stimulation. Responses in M1 were absent at the earliest ages examined (P5 and P6), variable at P8 and P10 (we detected responses in 5/6 P8 pups and 2/4 P10 pups) and present in all pups at P12.

To test whether these responses were due to a second locus of activity and not simply a uniform spread of activation, we normalized the peak M1 response and the peak parietal response to the peak BC response. These ratios are shown in Figure 4.2C for each age group. The normalized M1 response increased with age from 0.29 +/- 0.025 at P5 to 0.63 +/- 0.024 at P12 (one-way ANOVA, p = 0.0019). In contrast, the normalized parietal response did not change with age, being 0.32 +/- 0.05 at P5 and 0.27 +/- 0.038 at P12 (one-way ANOVA, p = 0.23). Two-way ANOVA of both sets of ratios across ages revealed an effect of age (p = 0.0128) and of cortical location (p = 7.0 x 10^{-4}). Furthermore, the normalized M1 response was significantly correlated with the area of cortex activated following whisker stimulation (r = 0.61, p = 8.3 x 10^{-4}) as well as the latency of activation following whisker stimulation (r = -0.48, p = 0.012). Importantly, the normalized parietal response was not significantly correlated with either the area of activation (p = 0.26) or the latency of activation (p = 0.48) following whisker stimulation.

We interpret these results as evidence that the signal recorded from the medial anterior (presumptive motor) cortex is a distinct feature of maturation of the whisker sensorimotor system and not due to a general increase in the area of activation.
4.3.3 Spontaneous cortical activity preferentially synchronizes motor and barrel cortices

Figure 4.4A shows example sequences of spontaneous cortical activity from two pups of P6, and P12 ages, along with power and frequency properties of these signals across all pups. We used the wide imaging area to examine the correlation of activity between the barrel and motor cortices during spontaneous activity. We observed that these regions could be active together spontaneously, even in those pups in which whisker stimulation activated only the barrel cortex. Figure 4.5 shows four examples of this synchronous activation from four pups ranging from P5-P12. Images on the left show sequential images of spontaneous brain activity, while the image on the right shows the sensory evoked pattern of activity (mean image calculated from onset to peak of activity). In these P5-P8 examples, the barrel cortex and the presumptive motor cortex are active together, while sensory stimulation activated only the barrel cortex; in the P12 example, M1 is active following sensory stimulation of the whisker.

To examine this synchrony more closely, we generated correlation maps that showed the correlation of activity between each imaged point of cortex and a seed pixel, as described in Supplementary Methods. We generated such maps from both spontaneous (6-8 min of activity) and whisker-evoked activity, which allowed us to make direct comparisons between the degrees of correlation between cortical points during spontaneous activity vs. during sensory-evoked activity.

Figure 4.6A shows example correlation maps from three pups. In all cases, the seed pixel is in the barrel cortex. In the three examples, there is a region of high correlation in the motor cortex in the maps generated from spontaneous activity. Note there is also a region of high correlation on the lateral edge of the imaged region, likely
representing the whisker secondary sensory region (Aronoff et al., 2010; Lim et al., 2012). The correlation maps generated from sensory-evoked activity has no region of higher correlation in the motor region in the P6 animal, a small weak region in the P8 animal, and a large robust region in the P12 animal (note the different scaling between the sensory-evoked and spontaneous maps). This is consistent with the emergence of M1 responses following whisker stimulation as shown in Figure 4.2. Figure 4.6B compares the mean correlation across ages. The mean correlation between barrel and motor cortices during whisker evoked activity increased with age (0.010 +/- 0.094 at P5, 0.69 +/- 0.047 at P12; one-way ANOVA, p = 0.0015), while the correlation during spontaneous activity did not (0.62 +/- 0.035 at P5, 0.67 +/- 0.032 at P12; one-way ANOVA, p = 0.54). Results of paired one-tailed t-test comparing the barrel-M1 correlation values following sensory stimulation to those during spontaneous activity at each age were: P5, p = 0.0057; P6, p = 0.0069; P8, p = 0.24, P10, p = 0.017; P12, p = 0.69). Thus in three ages (P5, P6, P10) barrel and motor cortices were significantly more correlated during spontaneous activity than following whisker stimulation. There was a weak (r = 0.38), non-significant (p = 0.06) relationship between correlations during spontaneous activity and correlations during sensory-evoked activity as shown in Figure 4.6C.

To examine the specificity of the relationship between barrel cortex and motor cortex, we compared these correlations to those between barrel cortex and the parietal point, as shown in Figure 4.6D. The correlation between barrel and motor cortices was higher at all ages, and significantly so beginning at P6 (one tailed paired t-test, P5, p = 0.0645; P6, p = 0.0069; P8, p = 0.024; P10, p = 0.013; P12, p = 4.87 x 10^{-4}), providing
evidence that activity in the barrel cortex was not simply correlated indiscriminately with all cortical regions, but preferentially with M1.

4.3.4 Spontaneous bursts of activity in barrel cortex associated with co-incident bursts in motor cortex

There are well described patterns of activity in the forelimb cortex of the rodent at the ages we examined in this study (Khazipov et al., 2004). Since forelimb sensory cortex is located between the barrel and motor cortices (Figure 4.2A), we questioned whether spread of activity related to bursts in the forelimb cortex could be responsible for the correlated activity between the barrel and motor cortex we describe in Figure 4.6. To test this possibility, we examined the activity in the motor and barrel regions of cortex at times when there was no activity in the forelimb area, detecting bursts of activity in the barrel cortex (see Methods for details) that were separated by at least 400 ms from bursts in the forelimb cortex. At all ages, bursts were more likely in the motor cortex than in the parietal comparison point (Supplementary Figure 3B) during the time surrounding bursts in the barrel cortex. The difference was significant at ages P6 and above (one-tail paired t-test of cumulative probability distribution from 200 ms prior to 200 ms post barrel burst; P5, p = 0.20; P6, p = 0.029; P8, p = 0.011; P10, p = 0.035; P12, p = 6.4 x 10⁻⁴).

We also determined the amplitude of the VSD signal in the motor and parietal cortices at the time of bursts in the barrel cortex, shown in Figure 4.6. The intensity of the signal at the parietal point was lower at all ages, and significantly so from P6 and older (one-tailed paired t-test, P5, p = 0.11; P6, p = 0.046; P8, p = 0.034; P10, p = 0.0040; P12, p = 3.1 x 10⁻⁴).
4.4 Discussion

Functional connectivity between sensory and motor cortices is a prominent feature of cortical circuits related to the rodent whisker system (Kleinfeld et al., 2006; Ferezou et al., 2007; Petersen, 2007). In this study, we used VSD imaging to examine the development of this connectivity in rat pups from the ages of P5-P12. Our main findings were that spontaneous activity in the barrel cortex is associated with activity in the motor cortex and that this association precedes the activation of the motor cortex following whisker stimulation.

4.4.1 Earlier studies of the development of the whisker sensorimotor system

Two parallel threads of research interst with the results presented here; studies that examine the development of cortical networks that respond to and process sensory stimuli; and studies that examine the developmental changes of spontaneous cortical activity in these same brain regions. Regarding the former stream of research, our results are in agreement with a series of previous studies showing that functional thalamocortical synapses form shortly after birth (Catalano et al., 1991; McCandlish et al., 1993; Schlaggar and O'Leary, 1994), as well as a recent study examining the maturation of EEG responses across a large expanse of cortex following whisker stimulation (Quairiaux et al., 2011). Using intracortical electrodes, this study found weak activity in M1 emerged between P7 and P10, with much stronger activity detectable by epicranial electrodes emerging at P13. Similarly, we found activity in M1 following whisker stimulation in some animals at P8 and P10, with stronger activation by P12 (Figure 4.2).
With regards to spontaneous patterns of activity in the developing cortex, there is a rich description of activity in the somatosensory cortices (see sections 3.4.1 and 1.3.4.2) with a relative paucity of data regarding activity in other regions. The dominant form of activity in the sensory cortices is the spindle burst, a burst of ~20 Hz oscillations lasting for 200-400 ms that synchronizes activity within the somatosensory or visual cortex (Khazipov et al., 2004; Hanganu et al., 2006; Minlebaev et al., 2007; Yang et al., 2009). These fast oscillations are nested in slower, 1-4 Hz waves (Minlebaev et al., 2009). At about P8, discrete bursts are replaced with continuous rhythmic activity (Seelke and Blumberg, 2008; Kaila, 2011) and similarly, we found significant increasing activity in the 1-4 Hz band of frequency power first apparent at P8 (Figure 4.4).

Relatively little is known about developmental activity in non-sensory regions. Introducing one of the first studies of such activity, the authors commented that, “…very little is currently known about the development of cortical activity outside primary sensory areas...” (Seelke and Blumberg, 2010). They found a variety of patterns including long-lasting (2-5 s) slow transients, shorter discrete biphasic events, and bursts of gamma activity along the midline from occipital, parietal, and frontal (approximately what we describe as motor) cortices. Cortical sharp potentials are in the same range as the signals we study here (~5 Hz); more study is needed to determine whether these events are homologous.

4.4.2 Synchronized activity in barrel and motor cortex during spontaneous activity precedes functional cortico-cortico connections

VSD imaging allows the collection of activity from both sensory and non-sensory areas simultaneously, making it especially useful to examine the correlation of activity
between disparate regions, in addition to the patterns of activity themselves. We found that activity in motor and sensory regions of cortex could be synchronized during spontaneous activity in the absence of any functional connections revealed by stimulation. Could there be activation of the motor cortex at these youngest ages that we failed to detect? Direct stimulation of the barrel cortex would be necessary to conclusively determine this, but our results, combined with those of other studies, make it unlikely. Previous examinations of whisker evoked responses have resulted in similar timelines for the development of responses in the motor cortex (Quairiaux et al., 2011). Furthermore, inputs to the motor cortex arrive mainly from layer V of the barrel cortex (Mao et al., 2011) which in turn receives inputs primarily from layers 2/3 of the barrel cortex (Hooks et al., 2011). Synaptic inputs to layer 2/3 of the barrel cortex are not mature until P12, and whisker stimulation does not reliably generate action potentials in cells of this layer until this time (Stern et al., 2001).

Where then could such synchronizations arise? The subplate, a transient structure present during development (Kanold and Luhmann, 2010), is a likely source. Subplate neurons form bidirectional glutamatergic and GABAergic connections with both the thalamus and the developing cortex (De Carlos and O'Leary, 1992; McConnell et al., 1994; Hanganu et al., 2001; 2002; Viswanathan et al., 2012) in some cases spanning wide expanses of cortex (Luhmann et al., 2009). It is essential for the generation of spindle bursts (Tolner et al., 2012) via an amplification and processing of sensory signals such as muscle twitches or retinal waves generated in the periphery (Luhmann et al., 2009).

The thalamus is another possible source of synchronization during spontaneous activity as projections to both the motor (Galazo et al., 2008) and sensory (Schlaggar and
cortices are present at birth, and simultaneous recordings from the thalamus and the barrel cortex show tight correlation between the two (Minlebaev et al., 2011; Yang et al., 2012). Interestingly, temporal properties of spontaneous thalamic activity were different from those that followed whisker stimulation, as was the delay between the thalamic burst and the cortical burst (Yang et al., 2012). This suggests that spontaneous bursts of activity in the developing barrel cortex do not strictly reflect activation of ascending sensory pathways. Similarly, spindle bursts persist in the absence of peripheral inputs (via pharmacological transection of the spinal cord (Khazipov et al., 2004), inactivation of the whisker pad (Yang et al., 2009) or silencing of the retina (Hanganu et al., 2006)), which indicates activity intrinsic to the subplate or thalamus is an additional mechanism via which spindle bursts can arise. These different mechanisms could account for our observation that barrel and motor cortices could be activated simultaneously during spontaneous activity even when sensory stimulation activated only the barrel cortex.

Finally, long-duration (5-10 sec) depolarizations have recently been described in the infant brain (Vanhatalo et al., 2002; Seelke and Blumberg, 2008; Colonnese and Khazipov, 2010). These events affect the excitability of the cortex (Vanhaatalo et al., 2004), can nest faster events including spindle bursts (Vanhaatalo et al., 2002; Colonnese and Khazipov, 2010; Hartley et al., 2012) and precede limb twitches (Thorgersteinsson et al., 2010). These slow, widespread events suggest a mechanism through which faster events could be coordinated in different parts of the cortex.
4.4.3 Possible functional role for synchronized activity in cortical networks

Widespread connectivity is an essential feature of the mammalian brain (Power et al., 2010; Friston, 2011) and in the rodent, electrical recordings (Wallace et al., 2004), imaging (Frostig et al., 2008; Mohajerani et al., 2010) and optogenetics (Desai et al., 2011; Lim et al., 2012) have revealed functional interactions between discrete regions of cortex. Nevertheless we have only limited understanding of the development of the cortico-cortical connections that underlie this connectivity, especially in comparison to our understanding of the development of thalamocortical afferents and cortical patterning (López-Bendito and Molnár, 2003; O'Leary et al., 2007). It has been clearly demonstrated the development of some long-range connections within the cortex, such as callosal fibres, depend on activity in the presynaptic neurons for maturation (Mizuno et al., 2007; Wang et al., 2007; Mizuno et al., 2010). It has also been shown that correlated activity between pre- and post-synaptic neurons promotes their maturation and connection via Hebbian mechanisms (Löwel and Singer, 1992; Ruthazer et al., 2003) (see also section 1.3.3.1). Our results suggest that such mechanisms could be at play in the developing sensorimotor cortex, with activity correlated by extrinsic factors acting to promote the connectivity observed in the adult.
4.5 Figures

Figure 4.1. Use of VSD imaging to detect activation of barrel and motor cortex in the developing cortex. A) Schematic demonstrating experimental set-up. Tactile stimulator can be attached to whisker (shown) or forelimb. Enlarged image shows imaged region. A, anterior; P, posterior; M, medial; L, lateral. B) False-color image showing isolated brain and region stained by VSD (red). A, Anterior; P, posterior; L, left; R, right. C) Schematic showing locations of cortical regions discussed in this and remaining figures. Dashed lines illustrate relationship of comparison point to M1 and BC. See stion 4.2.3 for details. D) Locations of M1, BC, and FL for all animals relative to bregma. Different shapes denote different ages, as shown. BC, barrel cortex; FL fore-limb sensory cortex; M1, primary motor cortex in this and subsequent figures.
Figure 4.2. VSD imaging reveals patterns of cortical activation following sensory stimulation. A) Images showing patterns of cortical activation following whisker stimulation. First panel of P5 pup shows anterior (A), posterior (P), medial (M) and lateral (L) directions. Note faster time intervals for P12 example. At right, outlines of cortical regions reaching 60% of maximal VSD signal. Total imaged area outlined in gray. B) Mean signals, by age, collected from BC, M1, and parietal points following whisker stimulation. Shaded areas show standard error, dashed line represents time of whisker stimulation.
Figure 4.3 VSD imaging reveals prominent changes of spatial and temporal properties of whisker responses with age. 

A) Time to onset (left) and area of depolarization (right) change significantly over the ages examined. In this and all subsequent bar graphs, bars show standard error. * denotes significant effect (p < 0.05) of age on time to onset or area of depolarization. 

B) Mean responses to ipsilateral whisker stimulation (left) and mean maximum response (left). * denotes significant effect (p < 0.05) of age on VSD signal.

C) Signal from M1 increases with increasing age. Bar graphs show ratio of peak signal recorded from M1 and BC (black), compared to ratio of peak signal recorded from comparison point and BC (green). 

D) Area of depolarization plotted against ratio of peak VSD signal from M1 to BC for each pup. Symbols represent different ages as shown. * denotes significant effect (p < 0.05) of age on VSD signal ratio and significant difference (p < 0.05) between parietal and M1 ratios. Correction for multiple comparisons was performed with Tukey’s HSD.
Figure 4.4. Frequency of spontaneous activity increases with age but can synchronize barrel and motor cortices at early ages. A) Examples showing a sequence of images of brain activity in a P6 and P12 pup. Outlines show cortical areas activated by stimulation of forelimb and whisker. Panels at left show signals recorded from M1 and BC, with gray highlighted region represent time shown in image sequence. B) Mean periodograms across ages for VSD signals collected from barrel (left) and motor (right) cortices. C) Mean power in 1-4 Hz range by age. * denotes significant effect (p < 0.05) of age on power, tested using one-way ANOVA, and significant differences between power in M1 and BC at each age. Tukey’s HSD test was used to correct for multiple comparisons.
Figure 4.5. Synchronous activation can exist during spontaneous activity that is not present during stimulation. Images show example sequences in which barrel cortex (outlined) is activated synchronously with motor cortex. Forelimb region outlined for reference. Filled circle shows estimated M1 region, except in P12 example in which M1 activity was detected. Images at right of dashed line show mean image (onset to peak) of activity following whisker stimulation.
Figure 4.6. Sensory-evoked activity in M1 is not required for synchronized activity in barrel and motor cortex during spontaneous activity. A) Maps showing correlation of activity at each point on imaged cortex with barrel cortex (seed pixel) during spontaneous activity (left) or activity following whisker stimulation (right). Forelimb (circle), M1 (square), and parietal (diamond) points shown for reference. Discrete islands of high correlation during spontaneous activity are outlined for emphasis. B) Mean correlations between barrel and motor cortices during sensory evoked activity (black bars) and spontaneous activity (white bars). * denotes significant effect (p < 0.05) of age on correlation, tested using one-way ANOVA, and significant differences between correlation for evoked and spontaneous at each age. Tukey’s HSD test was used to correct for multiple comparisons. C) Scatter plot illustrating weak relationship between M1-BC correlation during spontaneous (y-axis) and sensory evoked (x-axis) activity. D) Correlations during spontaneous activity between parietal and BC (green) and M1 and BC (black). * denotes significant effect (p < 0.05) of age on correlation, tested using one-way ANOVA, and significant differences between correlation at each age.
Figure 4.7. Bursts of activity in barrel cortex coincide with bursts in motor cortex. A) Mean images at time of burst in barrel cortex from three pups (P8, P10, P12). Putative M1 shown by red circle (P8 and P10), while M1 activated by whisker stimulation outlined in P12. B) Cumulative probability distribution of bursts in M1 (black) and parietal cortex (green) relative to burst in barrel cortex. Dashed line represents barrel cortex burst. * denotes significant difference (p < 0.05) between cumulative probability at 0.2 sec for M1 and parietal, tested using Student’s t-test. C) VSD signal at time of barrel cortex burst (normalized to barrel cortex signal) in parietal (green) or motor (black) points. * denotes significant effect (p < 0.05) of age on VSD signal, tested using one-way ANOVA, and significant differences between VSD signal in M1 and parietal at each age. Tukey’s HSD test was used to correct for multiple comparisons.
CHAPTER 5. CONCLUSIONS

In this final chapter, I will discuss the strengths and weaknesses of the results I have presented, similarities and differences between the chapters, and how the conclusions integrate into the current understanding of spontaneous brain activity. I conclude with unifying themes revealed by these results as well as future experiments that could be motivated by the results presented here.

5.1 Aims and results

In Chapter 1 of this thesis, I introduced two dominant forms of sleep related brain activity in the infant and adult brain, spindle bursts and slow-wave activity (SWA). I discussed theorized roles for these activities, and showed how understanding these roles would be advanced by having a better understanding of the spatial structure of these forms of activities.

In Chapter 2, I presented the results of from the adult brain, showing that there is a spatial structure to SWA, and that this structure reflects functionally related regions of the cortex. In Chapters 3 and 4, I showed that spindle-related activity could be synchronized between regions, such as homotopic sensory cortices, or sensory and motor areas, that are not yet functionally connected. In sum, I believe that I attained the goals set out in the introduction.
5.2 Strengths and weaknesses

5.2.1 Large imaging area

The studies presented here are the first studies to use in vivo VSD imaging in the developing brain. The prime benefit of this technique is to provide direct signals of membrane depolarization, at high spatial resolution, from large regions of the cortex. Thus, not only was I able to collect activity from regions we know little about, I was able to relate it to activity in the sensory cortices, which we know much more about. One of the first studies to examine activity outside of the sensory cortices recently described patterns of activity along the midline of the developing rat brain, recorded using field potentials (Seelke and Blumberg, 2010). By using the signals from the entire cortex provided by VSD, we were able to see how such activity relates to activity in the sensory regions, and make predictions about its purpose (see section 4.4).

This same benefit applies to work in the adult. For example, we are the first to collect signals of SWA at high spatial resolution from sensory, motor, and midline regions simultaneously. This allowed us to demonstrate that the separation between two points on the cortex is less predictive of how their activity will relate than their functional relationship. Thus, activity in the barrel cortex and the motor cortex is more related than activity within the barrel and the forelimb cortex despite a greater separation. Certainly, a role for intracortical connections in the organization of SWA is not entirely surprising given that disrupting intracortical linkages disrupts SWA (Amzica and Steriade, 1995b). However, an unexpected finding was the degree to which this effect reflects targeted functional connections. A second new finding was the high correlation between regions along the midline of the brain, suggesting the central ‘core’ of the brain that links many
resting state networks defined via fMRI (Hagmann et al., 2008; de Pasquale et al., 2012) suggesting these regions may interact within many patterns of brain activity.

5.2.2 \textit{In vivo recordings}

The large imaging area that VSD imaging provides not only permits the study of activity within multiple cortical systems, but it also make imaging the intact brain feasible. There are a multitude of patterns of described in the developing brain, some of which are described in the section 1.3.2.4. It is difficult to make comparisons between these patterns because of differences in preparation (slice, slab, \textit{in vivo}) and recording technique (calcium imaging, field potentials, even fMRI) (Allene and Cossart, 2010; Khazipov and Buzsaki, 2010). Similarly, there are differences in the regularity, speed, and frequency of activity when recording SWA in slices or the reduced brain, as compared to the intact brain. Some of these differences likely arise from differences in input resistance due to decreases synaptic inputs (Paré et al., 1998) and the loss of long-distance cortico-cortical fibres (Ruiz-Mejias et al., 2011). By recording from the intact brain, we obtain the most realistic activity possible.

5.2.3 \textit{Voltage-sensitive dye imaging is invasive}

VSD imaging is an invasive technique, and the same animal cannot be imaged more than once. This weakness is particularly acute for imaging during development. For example, it would have been useful to image the same animals over time, as having different animals for each session introduces unmanaged variables. More importantly, the large exposure of the cortex under the imaging camera means that developing animals
must be anesthetized. Although we were able to train adult mice to accept head restraint, this would be very difficult in young animals; furthermore, the soft skull at this age means even small waking movements generate large artifacts. Although natural sleep and waking cycles would be preferable, anesthesia, including iso-fluorane, is often used to study spindle burst related activity (Golshani et al., 2009; Colonnese and Khazipov, 2010).

5.2.4 Only delta-band signal recorded

A second weakness related to the developmental imaging is that we collected only the slower, delta-wave component of spindle burst activity. This is probably a reflection of the relative strengths of these two components, with the delta component representing a unitary single large burst generated by the massive summation of slower NMDA-R synaptic inputs, while the fast spindle component is generated by faster AMPA-R mediated synaptic events that do not summate (Minlebaev et al., 2009). However this is an issue that deserves more attention, particularly with the recent discovery that even faster gamma-range events are associated with spindle bursts in early life (Minlebaev et al., 2011; Yang et al., 2012).

5.3 Ongoing activity in the adult and infant

As expected, activity patterns in the adult and the infant were very different. Activity in the adult was ongoing and repetitive, while in the infant it was sporadic and linked to peripheral twitches. In chapter 4, where I examined activity over the second week of life, it became more continuous with increasing age. These findings are
consistent with our current understanding of cortical activity in the developing and the adult brain, as discussed in the introduction.

5.3.1 Structural and functional relationships during spontaneous activity

In the adult rat, many connections exist between cortical areas, as can be seen by stimulation (Frostig et al., 2008; Lim et al., 2012) or by anatomical tracing (Reep et al., 1994; Mitchell and Macklis, 2005; Hoover and Vertes, 2011). In both the infant and the adult, spontaneous activity reflected functional relationships. In the developing brain though, particularly through the first week of life in the rat, both cortico-cortical connections (Ivy and Killackey, 1982; Nicolelis et al., 1991; Price et al., 2006) and mature synapses (Marcano-Reik et al., 2010) are sparse. The is very relevant to the adult literature on spontaneous activity, as the influence of anatomy on correlated activity is of great interest (Honey et al., 2007; 2010; Sporns, 2011; Adachi et al., 2012).

Even in the adult, the relationship between structural and functional connectivity is not absolute – structural connections are estimated to explain ~50% of functional relationships in the brain (Honey et al., 2010). A one-to-one concordance of these measure would not be expected, given that functional connectivity can change rapidly, for example by mood (Harrison et al., 2008), learning (Albert et al., 2009b), or simply with time (Chang and Glover, 2010), but structural connectivity changes much more slowly (local synaptic changes, days, (Komiyama et al., 2010; Fu et al., 2012); long-distance white matter changes, weeks (Scholz et al., 2009; Lövdén et al., 2010)).

In some cases in the adult brain, there is a clear difference between functional and structural connectivity. For example, there are strong correlations between spontaneous...
BOLD signals of contralateral visual cortex (Vincent et al., 2007) and amygdala (Roy et al., 2009) of the two hemispheres (i.e. functional connection) even though these structures are not strongly anatomically connected (Leopold and Maier, 2011). Contralateral BOLD responses following electrical micro-stimulation of the somatosensory cortex of the monkey did not overlap with BOLD signals on the contralateral brain during spontaneous activation of the sensory cortex (Matsui et al., 2011), demonstrating a difference between the patterns of cortex related by spontaneous activity and evoked sensory activity.

We can compare the adult and infant case directly by considering how the corpus callosum mediates spontaneous activity. There are recent studies in the adult brain comparing the functional connections between homotopic regions of the cortex in intact brains, in which the hemispheres are anatomically connected via the corpus callosum (Luders et al., 2010), and functional connections in patients in which the callosum is absent and the hemispheres are not directly connected, either due to surgery or agenesis. There are interesting differences between these two cases. When the callosum is acutely removed via surgery, there is a large reduction in functional connectivity between the two hemispheres (Johnston et al., 2008). On the other hand, when absence of the callosum is long-standing, either because of agenesis (Tyszka et al., 2011) or long-ago surgery (Uddin et al., 2008) homotopic functional connectivity is very similar to that of the intact brain (note, however, that a small case-study of three patients with callosal agenesis found reduced homotopic functional connectivity (Quigley et al., 2003)).

I have demonstrated in the infant that activity between homotopic regions of the sensory cortex can be synchronized during spontaneous bursts, even if the signal is not
being carried trans-callosally as indicated by stimulation. A second example that I demonstrated was that activity in the barrel and motor cortices could be synchronized even if stimulating the whisker did not evoke activity in the barrel. Similarly, there are examples of correlated resting states in the infant human brain (Fransson et al., 2007; 2009) when it is not clear that the intracortical connections that could provide a direct anatomical correlate have developed (Kostović and Jovanov-Milosević, 2006).

In sum, these results from the developing rat are not inconsistent with the adult in showing that functional connectivity does not strictly reflect the structure of the underlying brain. I should emphasize that I am not suggesting that similar mechanisms are involved in the synchronization of brain activity in adults and infants, a point that has been made recently in a thorough review of the topic (Colonnese and Khazipov, 2012). In fact, I demonstrated that the homotopic correlation in the infant arises due to activity in the periphery; in the adult, spontaneous activity is ongoing and internally generated. Instead, I am suggesting that my results are consistent with the concept that correlated activity within the brain does not strictly reflect anatomical connections. Rather, it is a means with which to achieve developmental or cognitive goals, and can be achieved via anatomical connections or via other mechanisms (Honey et al., 2010; Sporns, 2011).

5.3.2 What do the current results suggest about the network structure of brain activity?

In sections 1.4.1.3 and 1.4.3.2, I discussed the small-world organization of the adult and developing brains as seen in measures of anatomical connection, as well as by measures of correlations between fMRI signals. As these are prominent frameworks
regarding the large-scale functioning of the brain, it is worth considering how our results may relate.

Relatively little is known about whether SWA organizes the brain into small-world networks, although there are some results using EEG that suggest that it does (Ferri et al., 2007; 2008), as discussed in Section 1.4.2.3. Although we did not measure ‘small-worldness’ (the ratio of local clustering to long-distance connections (Spoormaker et al., 2010)) among the regions we recorded from, our results are consistent these earlier studies, with related clustered regions (such as midline regions) connected via with long-distance connections among clusters (such as between hemispheres and between sensory and motor regions). The high-resolution signals provided by VSD recordings of SWA make it well-suited to more rigorous examination of this issue in the future.

There is also emerging research into the network properties of very slow cortical signals during stage N3 sleep. In these studies, fMRI is used to record signals of brain activity at very slow frequencies (due to the limitations of fMRI) during N3, when the cortex is dominated by SWA (Spoormaker et al., 2010; Larson-Prior et al., 2011). By this measure, the cortical network becomes more local after the transition to deep sleep, with local clustering becoming more prevalent and long-distance correlations weakening (although still meeting the criteria of a small-world network). Our results presented here suggest that this may be true of SWA activity as well as slower bands of activity, as we found more local correlations in the anesthetized state vs awake state (Figure 2.4). In keeping with this possibility, it is worth noting that cross-frequency coupling is a common feature of brain activity (Canolty and Knight, 2010) and that, in particular, the
amplitude of SWA is closely linked to the phase of very slow oscillations during sleep (Vanhatalo et al., 2004).

Studies of brain networks in the developing brain, like their subjects, are relatively immature, but an interesting finding has been a mismatch between the measures of small-worldness in anatomically and structurally defined networks. More specifically, anatomical networks are more clustered, with fewer long-distance connections; while measures of functional long-distance connectivity plateau within the first year of life (Fair et al., 2009; Supekar et al., 2009; Gao et al., 2011), the same measures in anatomical networks continue to increase throughout childhood (Fan et al., 2011; Khundrakpam et al., 2012). These measures become more similar with increasing age through the first decade of life (Hagmann et al., 2010).

It is not surprising that anatomical networks become more small-world with age, as cortico-cortical fibres continue to myelinate and develop mature synapses through the first decade of life (Levitt, 2003; Tau and Peterson, 2010), contributing the long-distance connections that define small-worlds. A number of authors, however, have expressed surprise at the earlier emergence of small-worlds in functional networks of the infant cortex (Hagmann et al., 2010; Fan et al., 2011; Khundrakpam et al., 2012). Our results offer some insights into this issue. In both Chapters 3 and 4, we show that connectivity as measured via spontaneous activity may not directly reflect connectivity as measured via circuit activation. As in the adult, faster patterns of activity in infancy are grouped by very slow oscillations (Vanhatalo et al., 2005; Hartley et al., 2012), providing a link between the long-distance functional connections we recorded in the 0.5-6 Hz bands and the networks characterized by much slower activity recorded via fMRI. Thus, factors
beyond the anatomical connections of the cortex, such as the simultaneous peripheral twitching we describe in Chapter 3, may contribute to the small-worldness of infant brains.

### 5.3.3 Spatial structure of brain activity as compared to the activity itself

A remarkable aspect of the resting states of the brain as seen via fMRI is that we do not know what underlies hem – two possibilities are very slow shifts in the background membrane potential of pyramidal cells (He and Raichle, 2009a), or slow changes in the power of very fast oscillations (Schölvinck et al., 2010) but this is far from established. Even the synaptic nature of the BOLD signal itself is not completely established (Mukamel et al., 2005; Viswanathan and Freeman, 2007; Nir et al., 2008; Logothetis, 2010). Nevertheless, the patterns of synchrony between brain regions have allowed good inferences about how the spontaneous activity of the brain may influence learning, cognition, and mental health (Fox and Greicius, 2010; Zhang and Raichle, 2010; Leopold and Maier, 2011).

We adopted a similar strategy with these projects, concentrating more on the structure of a single well-studied pattern across the brain, and less on the diversity of patterns that might be present. I believe that this strategy works well to generate hypothesis about the purpose of spontaneous activity, as I discuss more in section 5.5.

### 5.4 Activity in the motor cortex

After recording from the adult brain using VSD imaging, we saw the value of using the technique on the developing brain, but, as there were few reports about the nature of
activity in across large regions of the brain, we were not sure what to expect. One prominent feature was the spread of activity towards the midline, where developing motor regions are located. Knowing the tight integration between the barrel and whisker regions, we next recorded from these regions to see if correlated activity was apparent even in early life; as shown in Chapter 4, it is.

5.4.1 Evidence for a functional role of motor cortex activity during development

This fits with a substantial, but indirect, body of evidence suggesting activity in the motor cortex has an important developmental role in early life. A key observation was that blockade of activity during development in the cortex resulted in impaired branching and sprouting of corticospinal neurons within the spinal cord (Martin et al., 1999; Friel and Martin, 2005). These deficits lead to lasting impairments of reaching (Martin et al., 2000), which can be reversed via electrical stimulation of the corticospinal tracts during the time of inactivation. From these observations we can conclude that activity in the motor cortex during early life plays an important role in the development of the motor system.

A clue to the nature of this activity comes from the observation that transecting the dorsal columns of the spinal cord in early life leads to permanent abnormal development of the motor cortex (Qi et al., 2010), showing sensory feedback during development helps drive the maturation of the motor cortex. More elegantly, temporary paralysis of kitten forelimb muscles in early life has negative effects on the development of the cortico-spinal tracts that mirror those seen when the motor cortex is inactivated (Martin et al., 2004). These experiments were done in kittens between 3 and 7 weeks of ages, just past
the time of peak spindle bursts (Huttenlocher, 1967) and twitching during active sleep (Jouvet-Mounier et al., 1970). Most directly relevant to the projects of this thesis, trimming the whisker during the first days of life in the rat leads to shrunken motor cortex representation (Huntley, 1997), as well as lifelong difficulties with sensorimotor integration during active whisker movements (Carvell and Simons, 1996). Taken together, these observations suggest that spindle burst activity could be the original source of the activity in the motor cortex that promotes maturation of the cortico-spinal tract.

5.4.2 Theoretical reasons for a functional role of motor cortex activity during development

In addition to these observations, there are substantial a priori reasons to think that twitches in early life would influence the development of the motor cortex (in addition to the sensory cortex, as discussed in section 1.3.3). Nearly five decades ago, Roffwarg suggested that high levels of active or REM sleep in early life could serve to provide the developing cortex with activity when little was available from the outside world (Roffwarg et al., 1966). With this in mind, it is interesting to note that adaptive plasticity, in which changes in the external environment or the biomechanical system itself drive changes in the motor control system, is a fundamental feature of the neural control of movement (Pearson, 2000; Sanes and Donoghue, 2000; Wolpaw and Tennissen, 2001; Rossignol et al., 2008). Particularly important is detection of errors by comparing expected sensory effects of a movement to the actual effects of the movement and adapting or calibrating motor output to minimize this error (Davidson and Wolpert, 2005; Shadmehr et al., 2010; Franklin and Wolpert, 2011). Adult animals, moving
constantly through their environment, have ample opportunity to receive feedback, learn about their muscles and bodies, and adjust their motor system as necessary. Twitches during active sleep could provide this feedback to allow infants to calibrate the cortical motor system until they begin to make purposeful movements. Indeed, twitches decline rapidly as purposeful crawling begins (at about P8 in the rat, (Altman and Sudarshan, 1975; Marcano-Reik et al., 2010)).

In this scheme, it would be necessary to distinguish activations of the sensory cortex occurring after twitches, which would be useful for calibration of the sensorimotor system, from activations via other sources. Recently, a noteworthy study on this topic showed that the early corpus callosum exerts an inhibitory influence on the opposite somatosensory cortex, so that callosotomy increases the frequency of spindle bursts and decreases the delay between bursts on opposite hemispheres during active sleep (Marcano-Reik et al., 2010). This could be an active strategy to suppress transcallosal spread of activity through developing excitatory projections, ensuring that activation of the somatosensory cortex only occurs after true stimulation of the contralateral limb and not via passive spread.

Two additional sources of possible confounding activations exist. One was demonstrated in Chapter 3 – twitches within one limb or tail that stimulate another. Here, the two components of the spindle burst could be useful, as the fast component is generated via proprioceptive feedback while the slow delta component is generated via tactile feedback (Marcano-Reik and Blumberg, 2008). The second confounding factor would be external stimulation, for example from the mother or litter-mates. Recently, a study in human infants found that very slow cortical activity can precede bouts of
movements and the subsequent spindle bursts (Thornorsteinsson et al., 2010). The co-occurrence of this very slow activity along with the high-frequency component of the spindle burst would indicate that the ascending activity was internally generated. Perhaps under these conditions, the medical spread of activity towards the motor cortex would be particularly pronounced, strengthening connections between the regions.

5.5 Studying spontaneous activity to generate hypotheses

The results described in this thesis are largely observational. Observations, however, are valuable when they serve to motivate manipulations that test new hypotheses.

One strategy to test the hypotheses above about the functions of spindle burst activity would be to eliminate twitches in a subset of limbs in early life by injection of botulinum toxin, as described in the cat (Martin et al., 2004) in section 5.4.1. These experiments were done slightly after the peak of active sleep, and it would useful to do immediately after birth. An advantage of doing this in the mouse would be that sensorimotor function could later be directly assessed in adulthood via optogenetic stimulation (Ayling et al., 2009; Harrison et al., 2012).

A second strategy would be to take inspiration from the study of retinal waves and visual system development, in which genetic manipulation is used to disrupt the spontaneous peripheral inputs of early life (by knocking out receptor types necessary for coordination of retinal waves, for example (Cang et al., 2005; Feller, 2009)). Mice with aberrant spinal networks in which fibers crossing the midline cause simultaneous limb movements (Akay et al., 2006; Katayama et al., 2012) could be used to assess the
contribution of normal twitch patterns to cortical development. Normally, twitches occur in bouts but not necessarily simultaneously (see Figure 3.5 and (Robinson et al., 2000)). Depriving the cortex of isolated twitches (Figure 3.5 E and F) could result in abnormal segregation of inputs from right and left limbs, which could be assessed in adulthood via cortical imaging.

Particularly valuable could be knock-out mice lacking normal proprioceptors (Tourtellotte and Milbrandt, 1998). One would predict that the high-frequency, proprioceptive-driven component of spindle bursts would be reduced, while the delta-component, response to tactile inputs, would remain. In both cases, imaging of sensorimotor networks on the cortex as well as behavioural testing could be used to test the role of the normal twitch-feedback loop.

Finally, external manipulation of the twitch-feedback loop could be used, similarly to that used to test the role of twitching in the development of the withdrawal reflex ((Petersson et al., 2003), see also section 1.3.3.1). For example, ‘yoking’ or connecting limbs can be used to force two limbs to move together, as has been used in the prenatal rat to demonstrate short term motor learning (Robinson, 2005). By connecting the forelimb and the hindlimb during the first week of life, the nervous system would receive aberrant signals in which ascending sensory inputs did not match the twitch output. Even simpler would be to take advantage of the precise coordination that exists between barrel and motor cortices of the whisker system, in which each whisker projects to a slightly different motor region. Gluing two remote whiskers together chronically during the first week of life would force any movements of one to produce sensory inputs to the second. If the precise barrel to motor coordination failed to form in adulthood, it
would be good evidence that normal movements in early life, carried to the cortex via spindle bursts, are involved in the development of precise connections that underlie sensorimotor coordination.

These strategies would also serve to test the hypotheses, discussed in both chapters 3 and 4, that the correlated activity observed could promote cortico-cortico connectivity via Hebbian mechanisms. If yoking a hindlimb with a contralateral forelimb, for example, resulted in reciprocal activity between the corresponding cortical regions, rather than homologous ones, it would be strong evidence in favour of this hypothesis.

5.6 Concluding remarks

In the introduction to this thesis, I asked, “What does the brain do”? I have not, of course, answered that question, but I believe I have provided some insight into what the brain does not do. It does not passively wait for incoming sensory information, or for needed tasks to present themselves. Rather, it is active in and of itself, processing the past and preparing for the future. There are still more questions than answers about this activity and exciting times are ahead for those who study it.
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