Advances in layer specific fMRI for the study of language, cognition and directed brain networks

Daniel Sharoh

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Contents

1 General introduction

This thesis contains both methodological innovations in laminar resolution functional magnetic resonance imaging (lfMRI) and a study applying these innovations to a central topic in research on reading. The methodological contributions concern the expansion of lfMRI techniques to noninvasively measure directed connectivity throughout the human brain. By applying these techniques to the neurobiology of reading, we demonstrate the general applicability of lfMRI to the study of distributed brain networks which implement complex cognitive phenomena. In addition, this experiment provides the basis for further lfMRI experimentation on the neurobiology of language and likely generalizes to the study of other cognitive phenomena. The current chapter provides a general overview of lfMRI and the benefits and challenges of directed measurements. It also summarizes central questions in the study of brain function during reading that are relevant to the experiment performed in this thesis.

1.1 fMRI and BOLD

Functional Magnetic Resonance Imaging (fMRI) is a noninvasive method used to measure brain function with high spatial acuity. Since the discovery in 1990 that changes in the amount of deoxygenated blood could be used to image vasculature with MRI (**Ogawa et al., 1990**; **Ogawa and Lee, 1990**), fMRI has proven to be a reliable tool for researchers interested in brain function. The signal measured is called the blood oxygen level dependent (BOLD) signal, and it arises from differences in the magnetic properties of oxy- and de-oxyhemoglobin. The amount of de-oxyhemoglobin in a brain region varies by an indirect relationship to local synaptic activity. Typically, increased BOLD signal is associated with increased brain activity, and researchers can form inferences about brain function by observing characteristics of the BOLD signal.

In human fMRI studies investigating cognition, participants commonly perform tasks which are designed to investigate how cognitive phenomena are implemented in the brain. A researcher interested in language might ask a participant to read words while the researcher takes measurements of the participant's brain activity with an MR scanner. After analyzing the data, the researcher might conclude that many brain regions seemed to show increased BOLD signal in response to the task. In this scenario, it would be of interest to understand whether and how the observed regions interacted during the experiment. For this reason, there are various analysis tools available to assess connectivity among brain regions related to the task being performed by a participant, or task-dependent connectivity. A common approach which was

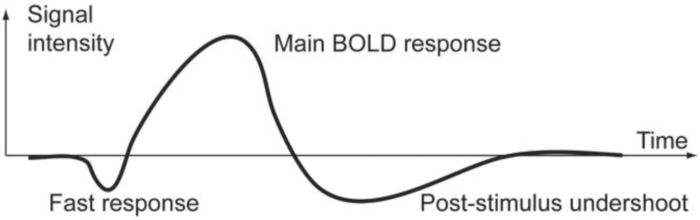

Figure 1.1. Canonical BOLD response to a stimulus. Early negative peak (2s) is followed by a positive peak (5s) before slowly returning to baseline. Figure from **Norris** (**2006**).

used in this work is generalized psychophysiological interaction analysis (gPPI, discussed in Chapter 3) (**McLaren et al., 2012**; **Friston et al., 1997**). It is possible with this method to identify networks of brain regions coordinated in the service of a cognitive task, but it is an outstanding challenge in fMRI research to establish the directed signal flow through these regions. The persistence of this challenge is ultimately attributable to the nature of the BOLD signal.

While the BOLD signal relates to brain activity, it has limitations which obscure the relationship among different brain regions. These limitations are inherent to how blood flow is regulated in the brain.

Changes in the amount of deoxyhemoglobin in a brain region are mediated largely through hemodynamics. Hemodynamics concerns changes in blood flow and blood volume, which lead to the main BOLD response identified in figure 1.1. Oxygen consumption in the brain also contributes to the BOLD response, and is thought to relate to the initial negative response. When changes in hemodynamic activity and oxygen consumption are in response to brain activity, they are referred to as the hemodynamic response. The metabolic and hemodynamic contributions to the BOLD response can be described in terms of a small set of parameters which characterize what is called the hemodynamic response function (HRF).

The first observable changes in blood-oxygenation occur several seconds following stimulus onset and peak roughly 5 seconds later (**Boynton et al., 1996**). Neurons by contrast do not wait for detectable changes in blood oxygenation before springing into action. Communication among neurons is rapid – with an upper limit on the order of 10s of milliseconds – and this rapid communication is not effectively represented in the sluggish changes of the blood oxygen level. This response latency cannot be practically quantified in most experiments, and so creates a number of challenges for the assessment of brain function. The response latency, and the hemodynamic response shape in general, varies by many factors, subject (**Aguirre et al., 1998**; **Kim et al., 1997**; **Lee**

et al., 1995; **Miezin et al., 2000**) and brain region (**Kruggel and von Cramon, 1999**; **Miezin et al., 2000**; **Thierry et al., 1999**) among them (see **de Zwart et al., 2005**, for review). Comparing BOLD response onsets in two brain regions therefore might not reliably indicate which brain region contained the neurons which first responded to an experimental task. The variable response function leads to further challenges when investigating how information is transmitted between two brain regions with correlated BOLD signals.

Another limiting property of the BOLD signal is in the different temporal scales of the measured BOLD response and of neuronal activity. Even allowing that the true BOLD response faithfully represents rapid neuronal activity, in practice most fMRI measurements are limited to a sampling rate far below the temporal scale of neuronal activity. Rapid activity thus becomes integrated over larger temporal windows, and information carried between brain regions at the high temporal resolution of the neuronal response is obscured in the measured BOLD response.

In response to these challenges, different methods have been proposed and applied to recover directed information from BOLD measurements. The majority of tools designed to measure directed connectivity achieve this through explicit modeling of hypothesized directed networks. In these approaches, the transmission of information from one region to another is predicted, or modeled, to explain brain activity. The most important limitation of model-based approaches is that they are restricted to confirming predicted interactions. They are thus impractical for exploratory work, limited to adjudicating among a small number of network arrangements, and heavily informed by theory. Notable model-based tools include Dynamic Causal Modeling (DCM) (**Friston et al., 2003**), Structural Equation Modeling (SEM) (**Büchel and Friston, 1997**, for SEM in fMRI analysis, see), and Granger Causal modeling (**Goebel et al., 2003**). Of these, DCM adjudicates among a limited number of pre-specified causal models; SEM, while not inherently a causal tool, can identify models describing the interaction among different experimental factors, though it is not well suited to identifying the factors themselves; and Granger causal methods suffer from poorly specified hemodynamic response functions across different brain regions. There is currently no model-free, consensus method to assess the directed effect of one region on another. It is hence a major challenge in fMRI to understand the dynamics of inter-regional interactions, and one which laminar resolution (lfMRI) may be able to address.

Laminar resolution imaging is thought to be sensitive to location encoded information indicative of the hierarchical origin of a given signal (see **Norris and Polimeni, in press**). If the interaction between two regions is largely driven

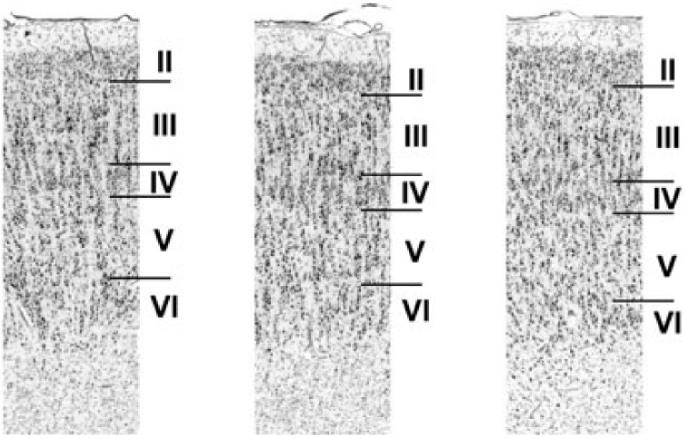

Figure 1.2. Cortical histology shown in silver stained sections from the fusiform gyri of three brains. Cell layers are designated by roman numerals. Layer I contains the fewest soma and is clearly visible contrasted against the more heavily stained layers (**Caspers et al., 2013**).

by forward- or back-propagating signal from one area to another, the directed nature of this signal could possibly be apparent in the cortical layers involved in the interaction. The principal motivation of this thesis was to explore this possibility. lfMRI can potentially be used to exploit this information to constrain the interpretation of functional measurements and expand inferences possible with fMRI. If successful, the lfMRI method would tie the hemodynamic consequences of neurocomputational properties to directed networks. This is in contrast to model-based approaches which explain brain activity in terms of predicted networks and incorporate assumptions about the underlying biology only for interpretation. One potential, but pivotal, strength of lfMRI is that it may thus be capable of measuring the information flow between brain regions directly and without the requirement that expected interactions be modeled *a priori*.

1.2 Laminar fMRI

MRI acquisitions in the submillimeter regime allow for the possibility of resolving the laminar structures which are thought to encode information related to a signal's hierarchical origin (see **Lawrence et al., in press**).

The 'laminar' in laminar fMRI refers to the layered structure of cells in the isocortex which emerges from organizational principles governing cell location and their connective tendencies (see figure 1.2). When considering cell populations within a volume of several cubic millimeters, topological features of the circuits formed by these cells become apparent, and the cortical laminae can be observed as components. Early tracing work by **Rockland and Pandya** (**1979**) in visual cortex showed that the incoming connections to a brain region clustered in specific cell layers according to their position in the processing hierarchy, relative to that brain region. This observation proved critical to our understanding of laminar circuits, and it is partly responsible for motivating research into the relationship of laminar circuits with the physiological parameters contributing to the BOLD response.

The laminae are organized with respect to depth, and so fMRI signal extracted from laminar resolution measurements can be referred to as 'depth dependent.' 'Depth dependent' perhaps more accurately characterizes what is also described as lfMRI, as it is not currently practical to resolve the functional signal of individual histological layers. In response to this limitation, the analysis of depth dependent fMRI measurements in humans is commonly analyzed or interpreted in terms of a simplified laminar structure comprising deep, middle and superficial compartments of the gray matter volume (**Polimeni et al., 2010a**; **Muckli et al., 2015**; **De Martino et al., 2015**; **Kok et al., 2016**; **Lawrence et al., 2018**; **Huber et al., 2015**, **2017**). These different depth bins correspond to the deep, middle and superficial cortical layers and contain the integrated signal of the cell layers they subsume. The deep, middle and superficial partition scheme is informed by the tendency of cells to synapse somewhat discretely at these depths according to their hierarchical relationship with the target region (**Felleman and Van Essen, 1991**).

The 'functional' in lfMRI refers to the use of a hemodynamic contrast to measure function at laminar resolution. This is possible because blood flow is regulated at the laminar level and can be localized to brain activity specific to individual cell layers (**Goense and Logothetis, 2006**; **O'Herron et al., 2016**). As discussed in the previous paragraph, it is currently impractical to measure individual laminae, but the spatial coupling of blood flow to the layers means that the signal integrated over each bin does track brain activity within the bin. A review of the work dealing with layer-dependent neurovascular coupling is given in Chapter 2.

Functional laminar work in humans suggests that signal measured at different depths varies as a function of its origin (**Huber et al., 2017**; **Kok et al., 2016**; **Muckli et al., 2015**; **Lawrence et al., 2018**). The perceived wisdom from these studies is that signal originating in a brain region which is superior in terms of processing hierarchy can be measured in the deep or superficial cortex; signal from an inferior source region can be measured in the middle portion of the cortex. A description of the work on human lfMRI and its use in assessing the top-down or bottom-up origin of an observed signal is also given in Chapter 2.

1.3 Importance of directed interactions, language

There are clear motivations to identify directed functional signal pathways throughout the brain. Directed connectivity methods are useful to discover brain networks, to inform parcellations of the brain, to increase the context of other parcellation methods, and, ultimately, to better understand the nature of large scale neural computation.

The identification of brain networks is a difficult theoretical problem, requiring assumptions about which properties are most appropriate for a given parcellation (**Hagoort, 2018**). Researchers may use architectonic based parcellation methods – cyto- myelo- and receptor – perhaps combining these in clever ways (**Schleicher et al., 2005**; **Glasser et al., 2016**), and they may also rely on functional measures to measure brain activity during rest or during experiments (see **Van Essen and Glasser, 2018**). Directed inter-regional measurements could greatly help to determine how brain regions interact to perform cognitive tasks and therefore inform functional parcellations. More immediately, task-based directed measurements could help resolve difficult questions in cognitive neuroscience. The study of language can be used as a concrete example.

fMRI based language research has the tendency to uncover widely distributed networks which interact in uncertain ways. In this thesis, I specifically focus on word reading. The process of reading draws on a diversity of brain regions which integrate visual and linguistic information. Full descriptions of this process are controversial though, with debate centered broadly around the division between orthographic and lexical-semantic processing (see **Carreiras et al., 2014**). Cognitively speaking, word reading requires that a string of characters be associated with orthographic, semantic, syntactic and phonological information. This information is not available in the visual input, and so must be retrieved from memory.

In the fMRI literature, it is known that a brain region called the left occipitotemporal cortex (lOTS), sometimes referred to as the "visual word form area (VWFA)," experiences functional hyperemia during word reading (**Dehaene et al., 2002**). lOTS does not express activation in isolation, however, as word reading involves an expansive network including the left middle temporal gyrus (lMTG) and the left inferior frontal gyrus (lIFG) (see **Price, 2012**). When considered in

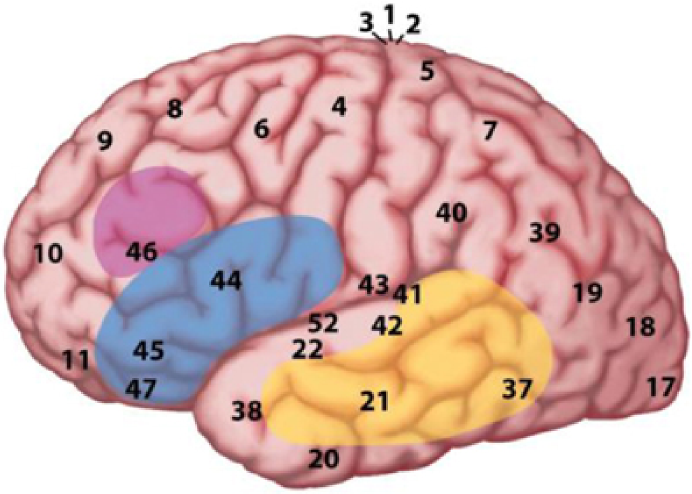

Figure 1.3. Figure shows core regions of the language network. Numbers indicate Brodmann areas. Temporal areas under discussion are shown in yellow. Blue and mauve overlays contain regions associated with different language processes. Figure from **Hagoort** (**2013**).

the context of language, these regions are part of what is often referred to as the language network (figure 1.3) (**Hagoort, 2013**).

Portions of the left temporal cortex are thought to critically support the retrieval of linguistic information associated with words, a process called lexical access. These regions haven been shown to express activation in a variety of modality independent tasks when participants access words (see **Price, 2012**). Unsurprisingly then, activation is observed in left temporal cortex during word reading in addition to the activation in lOTS. Based on the coactivation of these two regions with well-studied roles in language (lMTG) and reading (lOTS), it is relatively uncontroversial to argue that lexical access during reading is facilitated by means of communication between them. But because we currently lack noninvasive tools to measure directed connectivity between lOTS and lMTG, precise descriptions of their interaction remain controversial. It is not currently well understood how information related to reading is transmitted between the two regions, nor how the two regions interact to derive word meaning and other linguistic information from sensory input and memory. Consequently, implementation level descriptions of the integration of visual information with the language system have been challenging to provide.

One prominent account of the reading network argues that its regions act to integrate sensory information with past experience through bi-directional interaction between left occipital and left temporal regions (**Price and Devlin, 2011**). The interactive process results in lexical access. In the integrative interactive account, sensory information and knowledge of word meaning interact over a bi-directional circuit, with the intersection of the forward and back-propagating signal best observed in lOTS. Alternatively, it has been proposed that sensory information may be processed in a bottom-up manner such that lOTS modularly performs visuo-orthographic processing necessary to distill the visual representation of the word (the word-form) from the visual information stream, thence signaling the left temporal cortex for further processing (**Dehaene et al., 2002**). In this thesis, laminar fMRI was used to assess the directed relationship between these regions and determine if either theory of reading suitably explained the observed network structure.

Although this thesis aims to substantially contribute to knowledge of the reading network, its most consequential aspects are found in the development of methods for the use laminar imaging in cognitive contexts. The main foci of this work were to:

- assess the suitability of laminar imaging for investigating higher cognitive function
- assess the interaction between top-down and bottom-up signal streams within a single region
- assess the specificity of the depth dependent signal and its capacity to preserve distal interactions unique to particular depths
- identify directed whole-brain networks on the basis of the depth dependent signal.

In the service of these goals, we collected submillimeter whole-brain measurements and partitioned the brain into discrete depth bins. We analyzed the signal from these bins and identified distinct distributed networks on the basis of directed signal flow through constituent brain regions. Given the novelty of this work, it was necessary to develop and refine approaches to preprocess and analyze laminar resolution data. Image registration was particularly challenging given the high accuracy demanded by depth dependent analysis. It was also necessary to develop depth dependent gPPI methods to analyze depth dependent connectivity both within a region and across the entire brain. The development of the pipeline and analysis tools used in the present work are described in chapter 3.

The networks ultimately identified by the depth dependent connectivity methods corresponded to different signal pathways which targeted the left occipitotemporal sulcus (lOTS) during word reading. This finding is of critical importance to the neurobiology of reading. More generally, language processing is challenging to understand as it draws on regions throughout the brain which interact in complex, dynamic configurations. Our understanding of such networks, as well as similarly complex brain networks unrelated to language, would be greatly enhanced were it possible to elucidate the top-down and bottom-up influences on constituent regions. The work in this thesis addressed this challenge by showing that laminar fMRI can circumvent current methodological limitations which preclude direct noninvasive measurements of directed interaction.

By traversing the chapters of this thesis, in order, the reader will be presented cumulatively with information as to how this work was possible, why it was pursued, and what exactly was done. Chapter 2 provides the scientific introduction and background of the necessary neuroanatomical knowledge, state of the art with respect to laminar resolution imaging, and the language and reading background needed to understand the task paradigm. Chapter 3 describes the methods developed and adapted for laminar fMRI research. It also discusses the task paradigm and the new pipeline that was developed to analyze depth dependent data. Chapter 4 presents the results of the lfMRI analysis of a reading task and how it informs us about the nature of top-down and bottom-up interactions in a high level cognitive task. Chapter 5 concludes this thesis with a discussion of future possibilities stemming from the current work.

2 Background

This chapter describes the anatomy of cortical layers and their relationship to brain function. It addresses the relationship between cortical layers and hemodynamic phenomena, the current state of laminar fMRI and its application in the neurobiology of language.

2.1 Cortical histology

Anatomists have long been aware of the six layered structure of mammalian isocortex (**Campbell, 1905**; **Brodmann, 1909**; **von Economo and Koskinas, 1925**; **Ramon y Cajal, 1911**) and have considered its functional implications (see **Douglas and Martin, 2004**). As discussed in Chapter 1, isocortical histological structure expresses a layered configuration which emerges from organizational principles governing cell location and their connective tendencies. Throughout isocortex, a six layered structure is apparent. Variations in this structure have informed the well-known parcellations of the classical era and continue to influence modern research. The famous Brodmann map is one such parcellation scheme based on differences in the histological structure over the brain. The pioneering microphotographic work of Constantin von Economo documented the structure of the brain in meticulous detail, and culminated in another cytoarchitectonic parcellation scheme. The current state of knowledge would not be possible absent these pioneering efforts.

Although it was thought then that cytoarchitectonically distinct regions could fulfill distinct functional roles, an acute shortcoming of the era was the inability to reliably trace the connections among cells. This critically limited the understanding of how the evident cortical layers related to synaptic connection patterns. Sketches and microphotographs, as in figure 2.1, attributed to Ramón y Cajal command admiration even over the gulf of 100 years, and they underscore both the methodological limitations of their day and the standing challenge of accurately describing neuronal connectivity. It can be seen in figure 2.1 that although some cell bodies and their processes are apparent, methodologies of the day did not reveal the connection patterns among the cells and the cell layers. Hence, the relationship between cytoarchitectonics and synaptic targets remained to be explored.

The advent of retrograde tracers, notably horseradish peroxidase (HRP), contributed to the exploration of laminar structure in unprecedented detail, and, in combination with silver staining methods, **Rockland and Pandya** (**1979**) reported stunning laminar organization distinguishing efferent (outgoing) and afferent (incoming) pathways in macaque primary visual cortex (figure 2.2). The

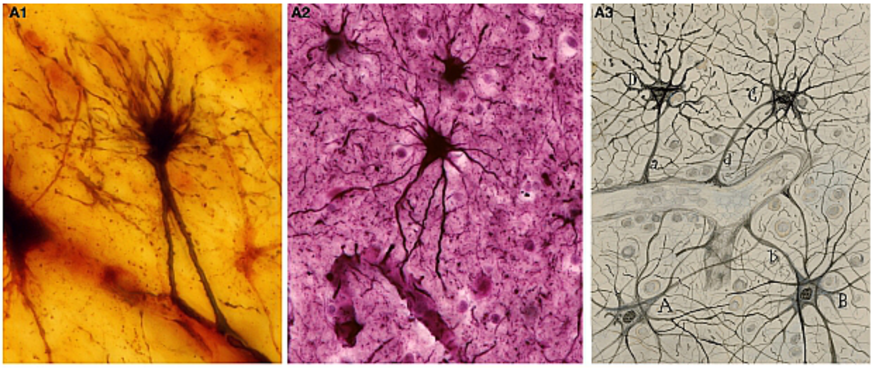

Figure 2.1. Drawings and preparations of Glia by Ramón y Cajal. Much of his work was recently cataloged by **Garcia-Lopez et al.** (**2010**) in a large archive project.

"impenetrable thicket" (**Ramón y Cajal, 1981**) began to yield under the weight of modern scrutiny, bringing into relief the location of neuronal soma and the terminal reaches of their arbors. **Rockland and Pandya** (**1979**) demonstrated that for a given brain region in a network, input from higher order regions within the same network preferentially targeted nongranular cortex. By contrast, input from lower order regions preferentially targeted granular cortex. **Rockland and Pandya** (**1979**) also demonstrated that the distribution of termination sites in visual cortex was constrained by the rostral or caudal position of the efferent source region relative to the termination site. This important discovery enhanced the functional relevance of neuroanatomical research and linked synaptic topology with computation. At the time this led to the interpretation that rostrally directed caudal efferents may act to propagate sensory information to hierarchically superior regions rostral to the source; rostral efferents projecting caudally were interpreted as carrying feedback signal from higher to lower regions.

Although recent work (**Beul et al., 2017**) has shown that network hierarchy does not obey a rostral/caudal gradient throughout the brain, the laminar basis of neuronal organization and connectivity has persisted. We now know that connections among neurons are constrained on the basis of cortical depth, manifest in the layered, or laminar, structure observed by the classical anatomists. Tracing methods have improved dramatically since **Rockland and Pandya** (**1979**), and we have expanded our understanding of laminar structure considerably, at least with respect to determining a basic, if underspecified, schematic for laminar organization on the basis of soma location and arborization patterns (**Barbas, 2015**; **Beul et al., 2017**; **Rockland, in press**).

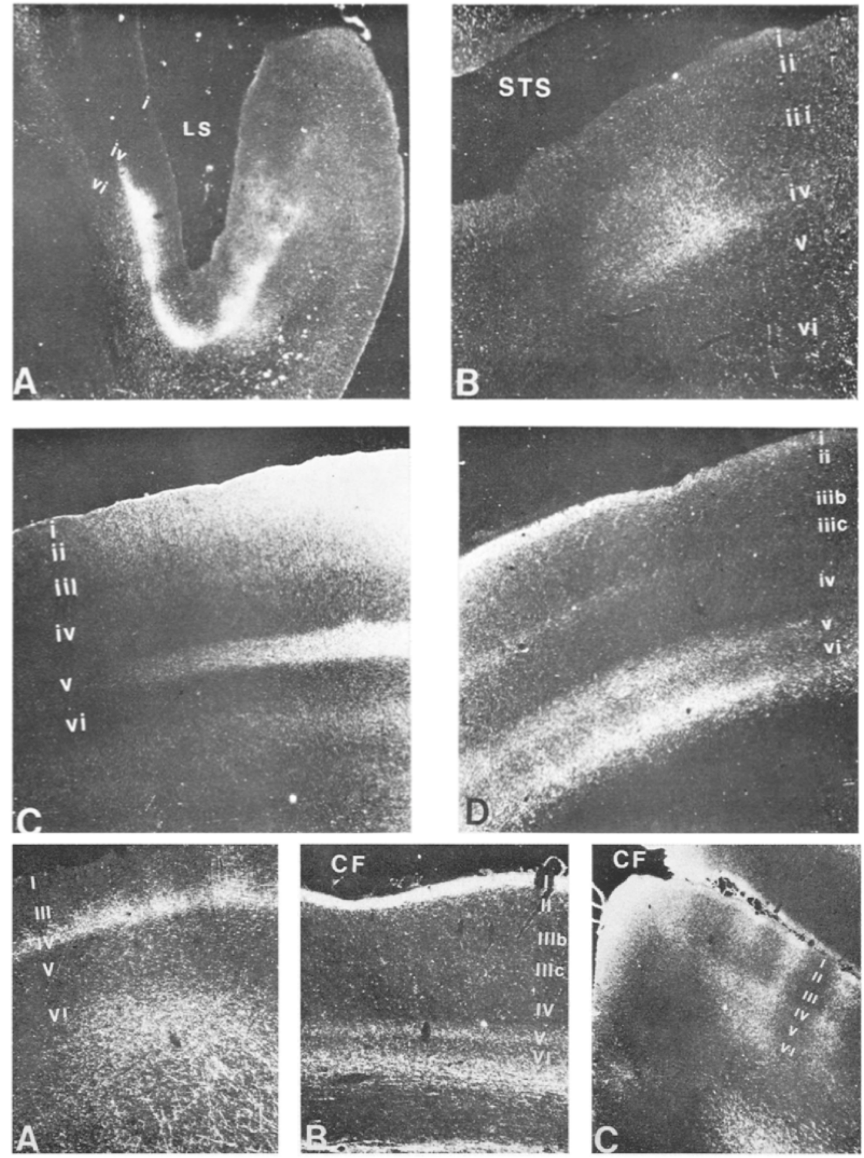

Figure 2.2. *Top four cells*: Silver grains shown in layer IV of (A) Brodmann area (BA) 18 and in (B) STS following isotope injection in BA 17. Silver grains were observed (C) concentrated in layer V within (C) area 17 following supragranular injection, and (D) throughout the nongranular layers within several mm of the injection site. Isotope uptake is along efferent nerve pathways away from the site of injection. The bottom three images show silver concentrations in (A) BA 19 and (B) 17 following injection in (C) BA 18. Figure from **Rockland and Pandya** (**1979**).

By reconsidering synaptic connectivity in the simple terms of an electrical circuit, **Douglas et al.** (**1989**) discovered that neuronal connectivity could be simplified to a "canonical microcircuit" (figure 2.3). It describes an excitatory circuit and was originally based largely on data from cat primary visual cortex (V1). The authors electrically stimulated the optic radiation above the lateral geniculate nucleus, while recording throughout the cortical layers of striate cortex. Neuronal activity was observed in layer IV, traced upward to layers II/III, down to layer V and finally to subcortical targets through layer VI. To account for connectivity among multiple brain regions, the canonical microcircuit also contains extrinsic (external) afferent connections. These are commonly categorized as targeting either granular (referring to layer IV; christened in **von Economo and Koskinas** (**1925**) for the granular appearance of the compressed pyramidal cells in this layer, particularly in V1) or nongranular cortex for termination. This circuit is foundational to the laminar fMRI research discussed in this thesis. It serves as the model to generate predictions of where signal originating in top-down or bottom-up source is expected to be observed. A more detailed schematic of the laminar connectivity model used in the present work can be see in figure 2.4.

Given the language focus of this thesis, it bears mentioning that one should resist the temptation to reflexively map the neurocomputational definition of feed-forward/back signal onto the cognitive usage of bottom-up and top-down information. Cognitive architecture interpretations of these concepts may not map predictably onto cortical network hierarchy, and so the easy analogy with the anatomical interpretation is problematic. In this thesis, the terms 'top-down/bottom-up,' 'feed-back/forward' and 'back/forward propagating' convey the anatomical meaning based on the relative hierarchical position of the regions involved as determined by synaptic terminations. The neurocomputational definition does, however, have a functional interpretation which underlies efforts to translate laminar work into the realm of cognition.

There is evidence that the configuration described by **Douglas et al.** (**1989**) is stereotypically repeated throughout isocortex, perhaps forming a fundamental computational element at the microarchitectural scale (see **Douglas and Martin, 2004**, **2007**). Importantly, it is thought to apply to cortico-cortical connections as well as the cortico-thalamic connections investigated in much of the neoclassical anatomical literature. It should be mentioned that a laminar basis for computation is an attractive alternative to cortical columns, which have been proposed to be a fundamental computational unit of the brain (reviewed in **Horton and Adams, 2005**). Columnar level components would have vertical arrangements of cells which perform computations locally within the column

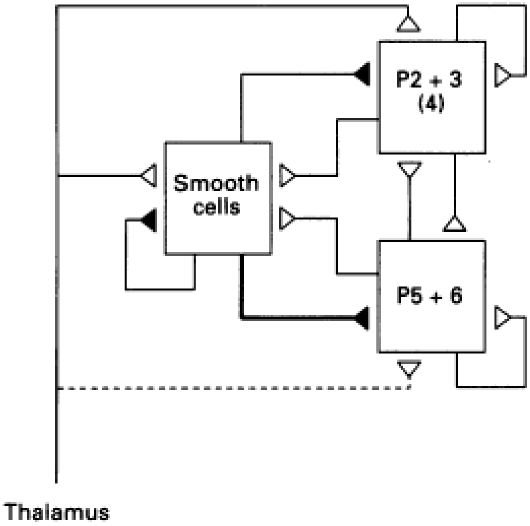

Figure 2.3. A generalized microcircuit of the neocortex. **Douglas et al.** (**1989**) discovered the that an extremely simple circuit principally limited to excitatory pyramidal cells could accurately model in vivo measurements if organized in a laminar-like hierarchy. Figure from **Douglas and Martin** (**1991**).

and communicate with a vast assemblage of columns throughout the brain. A difficulty in establishing columns as a computation unit is that they do not capture local circuits with anatomical fidelity, even in highly specialized cases such as rodent barrel cortex (see **Douglas and Martin, 2007**). By contrast, a stereotyped laminar microcircuit is an anatomically defined computational unit useful for the study of functional circuits in arbitrary cortical regions.

2.2 Laminar resolution functional magnetic resonance imaging

Top-down and bottom-up information streams are integral to brain function but notoriously difficult to measure noninvasively. Brain activity in a given region arises from signaling from both lower (bottom-up) and higher (top-down) order brain regions as well as activity intrinsic to the region itself. These signal streams contribute also therefore to the BOLD signal in the region.

Laminar resolution, functional magnetic resonance imaging (lfMRI) is a promising noninvasive technique with the potential to distinguish top-down and bottom-up signal contributions to the neurovascular response. One assumption made

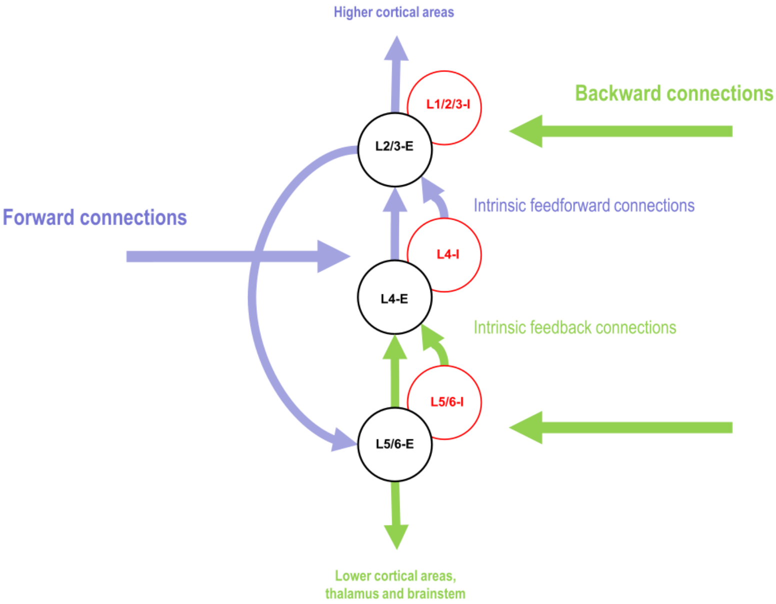

Figure 2.4. Schematic of intrinsic and extrinsic connections to an idealized cytoarchitectonic region. Extrinsic afferent connections terminate granularly or nongranularly with bottom-up and top-down sources respectively. Excitatory and inhibitory populations are both depicted. Figure from **Bastos et al.** (**2012**)

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when using the laminar method is that knowledge of the computational properties of cortical laminae can be used to constrain the interpretation of functional contrast measures on the basis of measurement depth. In principle, laminar resolution measurements explicitly capture information related to the hierarchical origin of the depth dependent signal components contributing to the overall response in a region.

This assumption requires that the depth dependent signal arises neuronally rather than strictly at the vascular level, and this fortunately appears to be the case. Local field potentials, related to synaptic activity, have been demonstrated to track the hemodynamics of cortical microvasculature at the level of the laminae (**Goense and Logothetis, 2006**). These hemodynamic effects relate to local changes in blood flow and blood volume which then affect draining veins that conduct blood unidirectionally toward the pial surface. The cortical vasculature in general is comprised of a randomly oriented microvasculature compartment and intracortical vessels oriented perpendicular to the pial surface **Duvernoy et al.** (**1981**). Isocortex can be further divided into four vascular layers which subsume the six histological layers (figure 2.5). The vascular properties of a region have implications for laminar resolution imaging, as the depth dependent variability of microvascular density and the linear increase in the diameter of ICVs toward the pial surface can affect the physiological parameters contributing to the BOLD contrast.

lfMRI relies on the same neurovascular coupling mechanisms exploited in standard BOLD imaging. A substantial body of work assessing the laminar sensitivity of neurovascular mechanisms (**Duong et al., 2000**; **Goense and Logothetis, 2006**; **Goense et al., 2012**; **Jin and Kim, 2008**, reviewed in **Norris and Polimeni, in press**), indicates that functional measures are indeed reasonably well localized to the site of activation. Furthermore, in recent years, there has been accumulating evidence that neurovascular coupling occurs at a sufficiently fine scale to make lfMRI feasible (**O'Herron et al., 2016**). This is good, because the laminar specificity of neurovascular coupling is critically important for researchers investigating the role of task related top-down and bottom-up signal. Specifically, evidence of laminar specific neurovascular coupling suggests that lfMRI is capable of distinguishing signal driven by neuronal activity in different cortical layers. In turn, signal in different cortical layers is thought to correspond to hierarchically distinct information streams.

Laminar fMRI in humans is relatively new, and the vast majority of this work has focused on primary sensory cortices, mainly visual cortex. Some of the earliest work showed that submillimeter measurements could capture depth dependent differences in the BOLD response detected in visual cortex when participants viewed a grating stimulus (**Koopmans et al., 2010**). After accounting for vascular artifacts, **Koopmans et al.** (**2010**) reported a peak BOLD response in depths corresponding to the middle layer of the visual cortex, the expected measurement site for BOLD signal related to bottom-up sensory input. **Polimeni et al.** (**2010a**) showed similarly that bottom-up sensory information was most clearly detected in the middle portion of the cortex, and also demonstrated the use of experiment condition contrasts to nullify responses at particular layers so to better observe task effects in the layers of interest. Later experiments in visual cortex (**Muckli et al., 2015**; **Kok et al., 2016**) have aimed to detect the presence of top-down signal by reducing the amount of bottom-up sensory information available while taking depth dependent measurements. In the absence of bottom-up information, these experiments have detected increased activation at depths associated with top-down signal – the deep or superficial layers. In motor cortex, **Huber et al.** (**2017**) reported depth dependent top-down effects in response to imagined finger tapping. These ex-

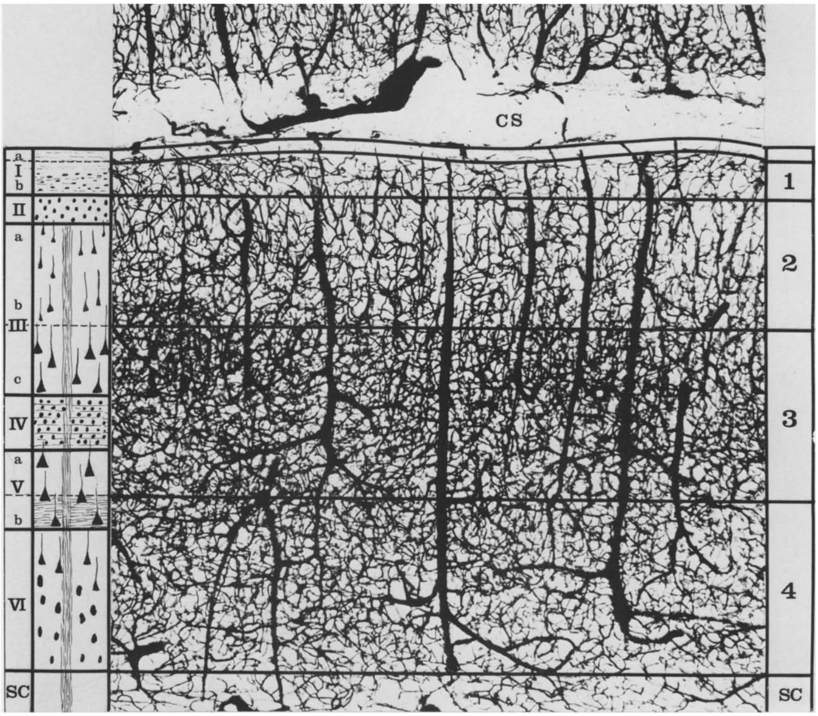

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Figure 2.5. Section showing vascular layers in human medial frontal gyrus with cellular layers illustrated on the left. The vascular density is noticeably higher in vascular layer 3. Figure from **Duvernoy et al.** (**1981**)

periments are evidence that depth dependent measurements indeed capture information related to the hierarchical origin of a signal. They are evidence that top-down and bottom-up signal contributions can be experimentally manipulated and measured at depths which correspond to hierarchically distinct termination points.

Despite these efforts to advance the frontier of laminar imaging, the top-down mediated phenomena studied have thus far been limited mainly to illusory percepts in sensory cortices. These experiments do not readily lend themselves to understanding the nature of top-down signal, its relationship to cognitive interpretations of 'top-down' and its effect on the bottom-up signal. Notably, these experiments have by design maximally distinguished top-down and bottom-up conditions by minimizing the bottom-up signal in the top-down condition. This is helpful to distinguish top-down from bottom-up signal, but precludes observations of top-down signal effects on the bottom-up signal. We therefore lack an empirically informed model of mesoarchitectural intrinsic connectivity as it relates to top-down signal.

2.3 Excitation and inhibition

One of the more immediate topics of investigation to follow from the ability to distinguish top-down and bottom-up BOLD signal is the functional purpose of the different signal streams. Without considering cognition, their purposes can be discussed in terms of excitatory or inhibitory effects on target populations.

It is generally agreed that afferent terminations in layer IV strongly excite their targets and drive spike rate increases throughout the layers of the target region (see **Bastos et al., 2012**). Nongranular afferent terminations, however, are more difficult to characterize, and have been shown to both excite and inhibit activity throughout the layers of the target region. Efferent cells are generally excitatory, at first blush suggesting that afferent signal should result in increased excitation throughout the target region, which is not the case. Efferents synapse on roughly equal proportions of excitatory and inhibitory cells irrespective of a granular or nongranular termination site. It is therefore not the case that nongranular terminations preferentially target inhibitory cells. As layer I is populated almost entirely with inhibitory cells, it is likely involved in extrinsic related inhibition, though it may also act to disinhibit deep layer cells to cause excitation. The net afferent effect throughout the layers of a given region is therefore likely intrinsically mediated, though the diversity of observations may relate to the inclusion of supragranular targets (see **Shipp, 2016**, for discussion).

While both excitatory and inhibitory effects have been associated with nongranular afferents, evidence from animal work suggests that feedback results in a net inhibitory effect when integrating signal over cortex adjacent to the site of termination (**Bullier et al., 1996**; **Hupé et al., 1998**, discussed in **Bastos et al., 2012**). Given the comparatively large volumes analyzed using fMRI and the small cell populations recorded in animal studies, the fMRI measurements should be expected to capture only net effects. Signal from nongranular afferents might therefore be reasonably expected to result in overall inhibition at the mesoarchitectural resolutions common in laminar fMRI.

2.4 Language and laminar fMRI

As stated in Chapter **??**, the work in this thesis concerns the processes and brain regions supporting word reading. The experimental question is whether connectivity during word reading between the left occipitotemporal sulcus (lOTS) and left temporal regions is best characterized by bottom-up or top-down signaling between the regions. To answer this question, we utilized a word reading paradigm in which participants read words, pseudo-words, and strings of characters taken from a fabricated false-font.

Processing differences between words and pseudo-words (orthographically and phonologically legal, referentless word-like items) are thoroughly studied in behavioral (**Reicher, 1969**; **Wheeler, 1970**; **Baron and Thurston, 1973**) and neuroimaging contexts (see **Price, 2012**, for an extensive review). Since **Dehaene et al.** (**2002**) observed that a region near the lOTS expressed greater activation when reading compared to listening to words (see figure 2.6), fMRI measurements have also shown larger BOLD responses when contrasting pseudo-words with words (see **Price, 2012**) in the same region. The interpretation of this signal difference has, however, been a long simmering matter of debate, with **Dehaene et al.** (**2002**) regarding the reading role of the region as a hub in a feedforward network and attributing the observed lexicality differences to imaging artifact or task confounds. An opposing view from **Price and Devlin** (**2011**) holds that lOTS acts to integrate visuospatial features derived during early visual processing with higher order information associated with previous experience such as semantic content and lexical statistics (figure 2.7). In this view, higher activation during pseudo-word reading reflects a failure to engage top-down information suitable for integration with visuospatial information, which would have a suppressive effect on the BOLD response in the region observed during word reading (**Price and Devlin, 2011**). Alternatively, higher activation during pseudo-word reading could be indicative of enhancement owing to longer reading times, task effects – as proposed in **Dehaene and Cohen** (**2011**) – or differences in frequency distributions of subsets of the graphemes within a word (n-gram distributions). Theories that support the enhancement or suppression account have testable laminar consequences.

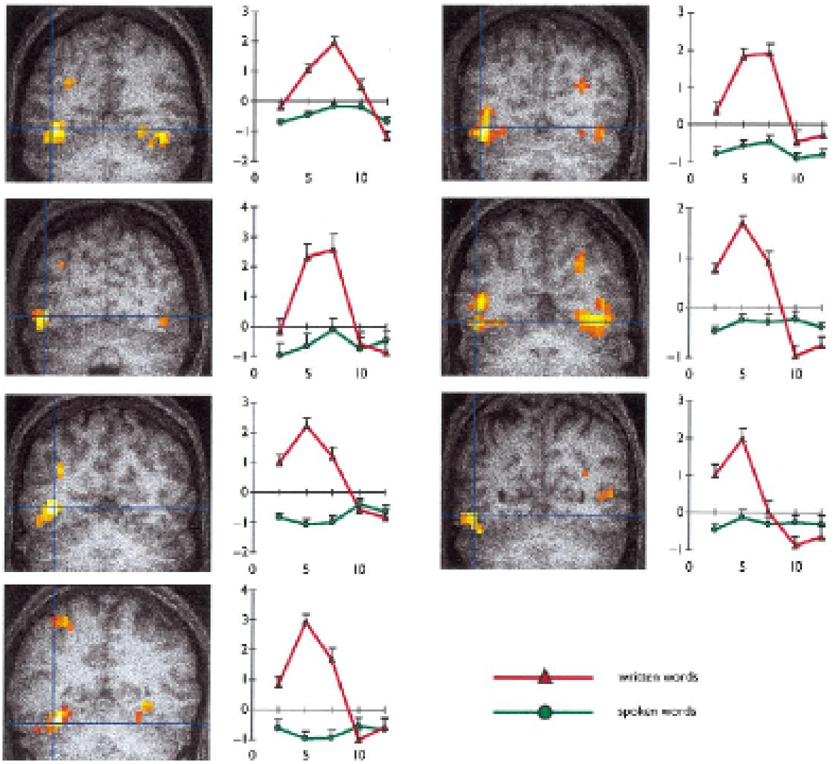

Figure 2.6. Early work by **Dehaene et al.** (**2002**) showing a portion of the left fusiform gyrus that responded preferentially to written over spoken words. This region is sometimes referred to as the visual word form area (VWFA).

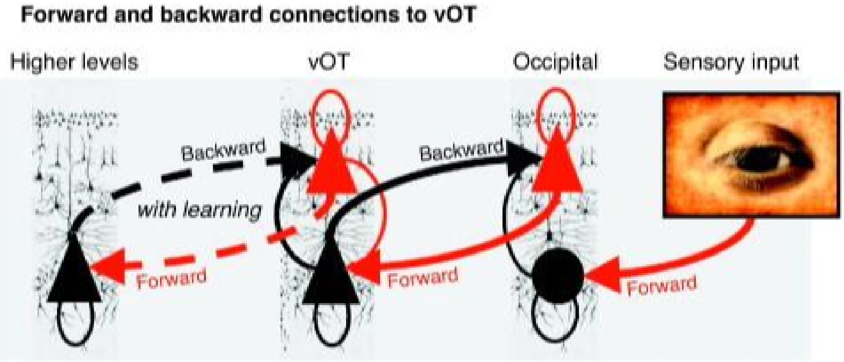

Figure 2.7. Schematic representation of an interactive account of reading. Top-down and sensory information interact across cortical regions during word reading. Laminar pathways are thought to mediate this interaction (**Price and Devlin, 2011**).

This debate then provides a vehicle to 1) extend laminar fMRI outside of primary sensory cortex, 2) assess the relative top-down contribution of each condition given the layer-signal distribution, and 3) observe interdepth effects of the changes in top-down signal, specifically suppression or enhancement effects observed at other depths. In light of findings demonstrating bi-directional signaling between regions identified with language processing (**Chu and Meltzer, 2018**), and findings demonstrating top-down influence on the occipitotemporal cortex within 100ms of exposure to visual word forms (**Chen et al., 2013**, **2015**), we hypothesized that if depth dependent signal is observable in the lOTS, differential top-down signal should be measured as an increased BOLD signal in deeper cortex.

We predicted that words would elicit a stronger top-down response relative to pseudo-words and that the overall signal would be reduced for words compared to pseudo-words. Because words are thought to elicit stronger lOTS directed top-down signal, the Lexicality effect, i.e., words against pseudo-words, was expected to target histological layers VI, V, or III/II/I. As discussed, these layers are known to contain extrinsic feed-back targets, and were subsumed within the deep (VI,V) or superficial (III/II/I) bins in our layering scheme (3). We restricted our predictions, however, to deeper cortex given that previous work with gradient-echo BOLD has discovered top-down effects which target the deep bin (**Kok et al., 2016**; **Lawrence et al., 2018**). Deep bin observations were thus expected to best express top-down signal effects whereas the middle bin was considered to express bottom-up effects.

This distinction was used in this thesis to demonstrate that these signal streams preserve source information and can be used to identify directed networks. In this work, we extracted depth dependent time-courses from submillimeter BOLD measurements. From these, we identified distinct distributed networks corresponding to top-down and bottom-up signal pathways which targeted the left occipitotemporal sulcus (lOTS) during word reading. We discovered:

- that reading words compared to pseudo-words increased the top-down BOLD signal observed in the deep layers of the lOTS
- that the deep bin signal increases accompanied signal decreases in the middle and superficial bins
- that increases in deep bin signal predicted decreases in the middle bin for words but not for pseudo-words

- that the depth dependent signal demonstrated unique connectivity patterns with other brain regions, thereby establishing directionality of interaction within the reading network.
- that signal in the deep bin showed a stronger interaction with language critical cortex than the middle bin.

These discoveries provide the first direct evidence that top-down signal to the lOTS is directly involved in reading and establish lfMRI for the noninvasive assessment of directed connectivity during task performance.

3 Development of analysis tools and pipeline for laminar resolution fMRI

3.1 Introduction

Laminar resolution imaging imposes unique methodological demands specific to the goals of extracting and analyzing depth dependent signal. Cortical laminae occupy brain regions of submillimeter spatial extent, and the submillimeter resolution necessary in laminar imaging creates challenges in acquisition and for image registration. The underlying topological properties of the laminae and the high correlation of signal generated in each layer furthermore require careful consideration of the appropriate functional MR contrast.

As discussed in Chapter 1, this thesis contains both methodological innovations in laminar resolution functional magnetic resonance imaging (lfMRI) and a study applying these innovations to a central topic in research on reading. The methodological aspects of this thesis are addressed in the current chapter. The methodological contributions concern the expansion of lfMRI techniques to the noninvasive measurement of directed connectivity throughout the human brain, as well as innovations arising from challenges in laminar resolution image registration and extraction of the depth dependent signal from the brain volumes. To this end, a collection of tools was developed or extended to correct image inhomogeneity, improve image registration, and the extended generalized psychophysiological interaction analysis (gPPI) (**McLaren et al., 2012**) for use with depth dependent data. This chapter also contains information about the experiment paradigm used in this thesis.

3.2 Task paradigm

Twenty-four native Dutch subjects (13 female, 18-30 years of age, 21 right handed) performed a single word reading task which presented words, pseudo-words and false-font items as task conditions. Subjects 2 and 19 were ultimately excluded from analysis owing to computer failure and signal dropout, leaving 22 datasets for analysis. Data was both lost and corrupted for subject 2 because of computer failure during image reconstruction. It was not possible to recover the lost data. Subject 19 experienced signal dropout in the left occipitotemporal cortex (lOTS) and so was excluded from analysis. Subjects had normal or corrected to normal vision and were screened for reading impairment. Left handed participants were included because regions of interest were determined through functional localization. Language function was observed to be left lateralized in all participants. Informed consent for all subjects was obtained in accordance with the Declaration of Helsinki and with ethical approval of the Donders Institute and the Erwin L. Hahn Institute. All subjects received monetary compensation for participation.

Initially, the experiment paradigm consisted of a 3 × 2 task design of Lexicality (words, pseudo-words, false-font items) × Length (short, long) with 120 items for each level, and where 'short' and 'long' designated the length of the words in terms of number of syllables (one or three). The inclusion of word and pseudo-word stimuli was to manipulate the availability of top-down information relevant to reading the items, and to thus manipulate the top-down signal directed toward lOTS with respect to item lexicality. The inclusion of false-font items was for the purpose of localizing the functional region of interest (fROI) used in the experiment. The localization procedure is described later in the chapter. The length manipulation was intended to parametrically modulate the bottom-up contribution to lOTS. It was determined through piloting, however, that the length manipulation was ineffective and was not analyzed as part of this study. The number of participants (24) was chosen on the basis of previous work using similar acquisition techniques (**Kok et al., 2016**). Our task thus included three relevant conditions, two of which (words, pseudo-words) were analyzed as conditions of interest, and one of which (false-font items) was used to localize the fROI for analysis of the depth dependent lexicality effect. The length manipulation was excluded from analysis, but short and long items remained in the stimulus set. Length was therefore modeled, but variance attributed to length was treated as a confounding factor. After collapsing across the length manipulation, the design as analyzed was effectively 3 × 1, with 240 items each for the word, pseudo-word and false-font conditions.

3.2.1 Item creation

Word items were selected from a list of high frequency, concrete Dutch nouns taken from the Celex database (**Baayen et al., 1995**). Words were selected to maximize frequency, minimize the standard deviation of word frequency, and minimize standard deviation of these values for short and long items.

Pseudo-words were generated using Wuggy (**Keuleers and Brysbaert, 2010**). Pseudo-word generation was constrained on the basis of phonemic neighborhood density, consonant/vowel structure and the number of characters of the word items. Parameters are included with the stimulus list in Appendix 1. False-font items were created from word items rendered in the false font. The false font (**Cohen et al., 2002**) was designed to preserve the low level features of familiar, orthographically legal characters, but to be visually distinct from letter shapes. These items are included in *Appendix 2*. Sample items can be seen in figure 3.1.

3.2.2 Stimulus presentation

Items were presented during fMRI measurements taken over 12 runs. Runs were delimited by breaks in data acquisition. Twenty items of each stimulus type (word, pseudo-word, false-font) were presented per run, with 60 items presented in total per run.

Individual stimuli were visually presented for 800ms in the center of the display. One item was presented per trial. Items were rendered in white on a black background, as shown in figure 3.1. Presentation onset was jittered around an effective TR of 3.9 seconds based on the design optimization calculations obtained using optseq (**Dale, 1999**).

Stimuli were presented in 5 item mini-blocks in which all 5 items were of the same condition type. Each mini-block was followed by a fixation cross presented for the duration of one trial. On three pseudorandom occasions per run, a question mark was presented that instructed the participant to indicate via button-box whether the previous mini-block contained existing Dutch words. Button-box responses were not analyzed and were considered only to ensure participant compliance. Runs were excluded from analysis if two of the three responses were incorrect or if no response was logged during the response window. Prior to the experiment, subjects were briefed on the type of items they were to see and instructed to silently read the items on the screen.

The experiment was performed using Presentation® software (Version 16.1, Neurobehavioral Systems, Inc., Berkeley, CA, www.neurobs.com). Two versions of the experiment were created with approximately half of the participants assigned to each version. The versions differed in block and item order. The different experiment versions were intended to capture latent, unintended effects inherent in presentation order or other version specific properties.

3.2.3 Task design model

The task design matrix included condition regressors, temporal and spatial dispersion derivatives, physiologic regressors, motion regressors produced by using SPM version 12 (**Penny et al., 2011**), drift terms, frequency filters, outlier censors, and constant terms modeling the mean signal per run. Outlier time points were determined using *3dToutcount* in AFNI (**Cox, 1996**). Voxels were considered outliers if the probability of the distance of the voxel's intensity value from the trend exceeded p = 0.001 as defined by its location within a Gaussian probability distribution. Time points were excluded from analysis if 2% or more of the voxels at that time point were categorized as outliers.

Stimulus onsets were modeled as instantaneous events with zero duration and convolved with the canonical hemodynamic response function. Condition regressors were created separately for word, pseudo-word and false-font items; and for the long and short items within each condition. In total six condition types were modeled, but only three were analyzed after collapsing across length. The additional factor of Depth was not modeled in the first level analysis, but was analyzed following the extraction of the depth dependent signal from the lOTS. This procedure is described later in the current chapter. Depth was modeled in a group level, 3-way ANOVA of Depth × Lexicality with Subject modeled as a random factor.

3.3 Acquisition

Acquisitions for laminar resolution fMRI must have sufficient spatial acuity to resolve functional activation at distinct cortical depths and to reliably localize the functional activation to its source depth. lfMRI acquisitions are therefore limited to submillimeter resolution. It is also important to consider how the functional and anatomical images will be aligned. Functional T2*-weighted images are often acquired with the echo planar imaging technique which is known to induce geometric distortions that reduce registration accuracy and, ultimately, the interpretability of the results.

Scan protocols were selected after balancing the competing demands imposed by the experiment. It was necessary to prioritize submillimeter acquisition and coverage extent. Furthermore, it was important that the acquisition time was not excessively long.

3.3.1 Functional acquisition

Near whole brain, submillimeter (0.943, 0.900 slice direction) resolution T2*-weighted GE-BOLD data were acquired using a GRAPPA accelerated (acceleration factor 8 × 1) 3D-EPI acquisition protocol (**Poser et al., 2010**) with CAIPI shift kz=0, ky=4 (**Breuer et al., 2005**; **Setsompop et al., 2012**); effective TE = 20ms; TR = 44ms; effective TR = 3960ms; BW = 1044Hz/Px; FoV = 215mm × 215mm × 215mm with 112 phase encode steps in the slice direction (100.8mm); $\alpha = 13°$; partial Fourier factor=6/8 in both slice and phase-encoding directions. The first phase encoding gradient was applied in the posterior to anterior direction. An axial slab was collected in each subject and positioned to include the occipitotemporal sulcus. The 10cm slab was sufficient to allow complete brain cov-

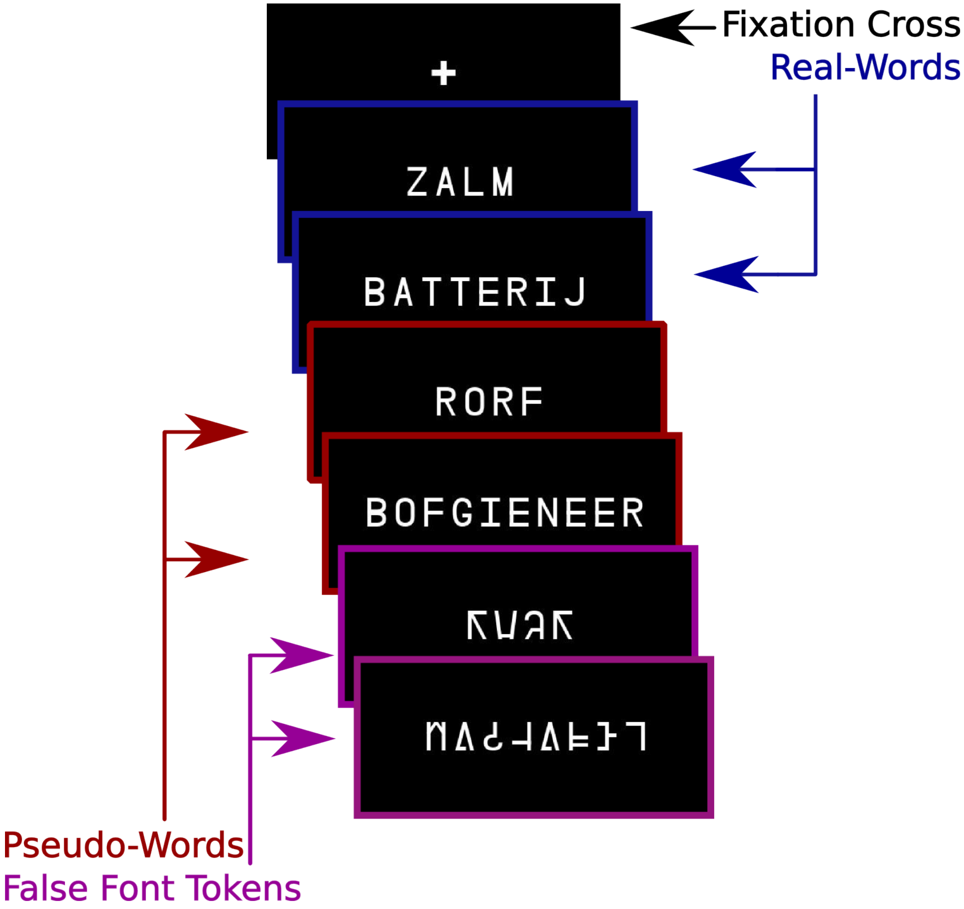

Figure 3.1. Sample stimuli from each task condition. Word stimuli are in Dutch. English translations of the Dutch word stimuli examples are 'salmon' and 'battery.' Stimuli were presented in 5 item mini-blocks with the items from the same condition presented in each block. The false-font condition was used to determine the functional Region Of Interest (fROI) to test the word and pseudo-word contrast.

erage in several subjects and nearly complete coverage in the remaining subjects. In subjects with partial coverage, superior portions of the brain were excluded from measurement. Data were acquired on a Siemens Magnetom 7 Tesla scanner (Siemens Healthineers, Erlangen, Germany) with a 32-channel head coil (Nova Medical, Wilmington, USA) at The Erwin L. Hahn Institute in Essen, Germany. Functional data consisted per subject of 12 3D-EPI datasets of 77 volumes each, though some sessions were incomplete owing to time constraints or other difficulties. No session contained fewer than 10 analyzed 3D-EPI datasets. Example images from each type of acquisition can be seen in figure 3.2

3.3.2 Anatomy acquisition

Two anatomic images were acquired in each subject using the MP2RAGE (**Marques et al., 2010**) acquisition protocol (voxel resolution = 0.75mm × 0.75mm × 0.75mm, TR = 6000ms, TE = 3.06ms, $T1_1$ = 800ms, $T1_2$ = 2700ms, α_1 = 4°, α_2 = 5°, BW = 240Hz/Px, FoV = 240mm × 240mm with 192 slices (144mm) and a T1-weighted inversion recovery EPI (IR-EPI) based on the parameters used in the 3D-EPI protocol. To create the T1 contrast, the following parameters were modified from the functional acquisition: α = 90°, T1 = 800ms, TR = 200ms, TE = 20ms. It was necessary to increase the number of phase encode steps in the slice direction from 112 to 160 and to expand the FoV in the slice direction to ensure fully overlapping coverage with the functional data. The IR-EPI images were used for image registration as they are known to provide high tissue contrast while preserving the geometric distortions of the functional images (**Dumoulin et al., 2018**). High accuracy, cross-modal registration is challenging, particularly with high resolution acquisitions known to exaggerate geometric distortions. Performing coregistration taking the IR-EPI as the source image mitigated the challenges caused by these distortions.

3.4 Choice of acquisition for laminar resolution imaging

Although lfMRI relies on the same neurovascular coupling mechanisms exploited in standard BOLD imaging (Chapter 2), the requirement for submillimeter resolution has led to considerable discussion as to the best MR-contrast for interrogating the hemodynamic response. The work in this thesis bears critically on this debate, but this section describes the initial rationale underlying the choice of acquisition protocol.

The benefits and drawbacks of common acquisition techniques are well understood. The standard gradient echo BOLD contrast is highly sensitive to functional activation, but is known to have a considerable contribution from vessels downstream from the site of activation. The less commonly used spin-echo BOLD sequence only acquires data from a subset of the contrast mechanisms that contribute to gradient-echo BOLD, but is believed to have a superior intrinsic spatial localization at high static magnetic field strengths (**Yacoub et al., 2003**; **Lee et al., 1999**; **Duong et al., 2003**). In addition to acquisitions using these most common contrasts, contrasts based on cerebral blood flow and volume (CBF, CBV) should also be considered.

It is technically far easier to test and compare these contrasts in animal models, and historically such experiments largely preceded human lfMRI (**Goense and Logothetis, 2006**; **Harel et al., 2006**; **Kim et al., 2007**; **Kim and Kim, 2010**; **Lu et al., 2004**; **Silva and Koretsky, 2002**; **Smirnakis et al., 2007**; **Zappe et al., 2008**). The conclusion drawn from these was that CBV was consistently found to have the superior characteristics in terms of spatial resolution, and gradient-echo BOLD the poorest. Spin-echo BOLD and CBF are somewhere between these two extremes. This hierarchy may be explained in terms of the current view that blood volume changes occur in the arterioles and capillaries (**Kim et al., 2007**; **Behzadi and Liu, 2005**; **Devor et al., 2007**; **Hillman et al., 2007**), and hence CBV contrast should not be a downstream contrast as is BOLD.

The first laminar fMRI studies in humans are comparatively recent (**Koopmans et al., 2010**; **Ress et al., 2007**; **Polimeni et al., 2010a**), and utilized gradient-echo BOLD contrast. Since then, the VASO technique for measuring CBV noninvasively (**Lu et al., 2004**) has been further developed for application for laminar fMRI at high static magnetic field strengths (**Huber et al., 2014**, **2015**, **2017**), and a number of spin echo (**Olman et al., 2012**; **De Martino et al., 2013**; **Kemper et al., 2016**) and combined spin-echo and gradient echo studies (**Muckli et al., 2015**; **Moerel et al., 2018**) have been performed. Laminar CBF has to date not been published for human studies.

Our rationale for selecting gradient-echo BOLD for the current study was based primarily on its exclusive ability to acquire high spatial resolution data from large volumes within an acceptable acquisition time. The two main alternatives – CBV and spin-echo – are currently techniques that are restricted in their volume coverage and suffer from comparatively long acquisition times (**Huber et al., 2017**; **Kemper et al., 2016**). While GE acquisitions are known to be sensitive to the effects of draining vasculature, these are of minimal concern to measurements taken in the deeper portions of the gray matter volume.

3.5 Image registration

Image registration is a multistep process designed to bring two images into alignment. In fMRI, image registration is critical to remove artifact related to subject motion and to map functional activation to anatomically meaningful coordinates. In laminar resolution imaging, registration is both more challenging and more crucial because high resolution cortical volumes must be accurately aligned with the gray and white matter tissue boundaries to give meaningful depth dependent results. Given the complications inherent in the registration of submillimeter data, different combinations of tools were necessary to achieve accurate registrations in different participants. The criteria for success were constant, however, across all participants, and were confined only to anatomy. In all participants, alignment quality was determined by visual inspection of brain edges and the left occipitotemporal sulcus. In addition, different registration approaches were necessary in the event that a given tool experienced failure. These conditions are discussed below. The current section describes how this high accuracy registration was achieved.

3.5.1 Skull removal

Skull removal is a preprocessing step performed to improve registration accuracy. Image registration often relies on boundary detection and tissue intensity normalization algorithms, which can give inaccurate results if the skull is not removed. Skull removal was performed on functional and anatomic data prior to crossmodal image registration.

Different skull removal procedures were used depending on image modality. The FreeSurfer (**Fischl, 2012**) watershed function was applied to the IR-EPI datasets, sometimes following a first pass B1 bias field correction developed during this analysis (discussed in *B1 correction*). Nearly all processed brains required manual intervention to remove voxels containing skull or unwanted tissue, or to reintroduce voxels removed in error.

For the purposes of group analysis, skull removal was performed on all MP2RAGE images in the same manner. These were aligned to the mean functional images produced during motion correction. Prior to this alignment, skull removal was performed on the mean functional images.

For mean functional images, voxels containing skull or unwanted tissue were first removed with a first pass application of *3dAutomask*. *3dAutomask* is an AFNI tool typically used to remove the skull in images with poor tissue contrast, such as with T2*-weighted images.

Parameters for this program were optimized on a per subject basis, and all results were manually edited to ensure that only voxels containing skull were removed. These results were found to be adequate on the basis of visual inspection following manual intervention, where 'adequate' describes results which did not contain residual skull or exclude voxels containing brain-matter. *3dAutomask* parameters were iteratively optimized until a result was obtained which reasonably limited the necessary manual intervention.

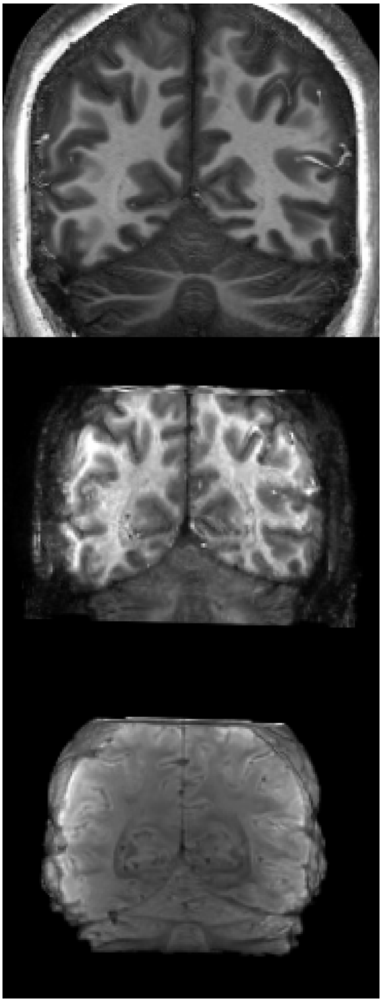

Figure 3.2. Different acquisitions used in this experiment. From top to bottom: MP2RAGE, IR-EPI, 3D-EPI. The left hemisphere is shown on the right in the image.

3.5.2 B1 Correction

During acquisition, inhomogeneity in the B1 field leads to intensity gradients which affect normalization algorithms critical to skull removal, image registration and tissue segmentation. B1 correction on the IR-EPI data was unsuccessful on 5 datasets using the standard tools available in the FreeSurfer suite, resulting in failed skull removal and inaccurate segmentations. It was possible to reduce B1 inhomogeneity by applying an additional B1 correction before applying FreeSurfer tools.

A first-pass transform for the mean functional and IR-EPI images (with the skull) was first calculated before the transformation was applied to the mean functional image. The B1 bias captured in the mean functional image was then lutilized to correct the IR-EPI anatomic images. Following the initial coregistration, the mean functional image was smoothed and voxel-wise inten-

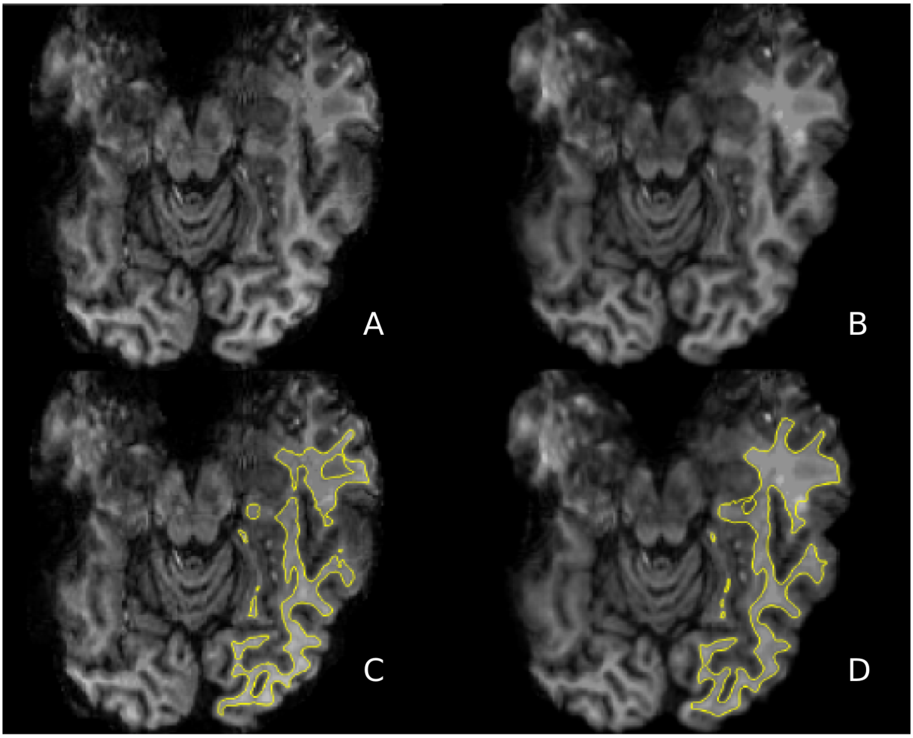

Figure 3.3. Comparison of FreeSurfer white matter surface generation on the left hemisphere before and after supplemental B1 correction. (A) uncorrected; (B) corrected; (C) surfaces generated from A; (D) surfaces generated from B. The left hemisphere is shown on image right.

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sity scaled between 0.3*v* and 0.9*v* of its intensity value *v* to prevent extreme values from unduly influencing bias correction. The IR-EPI was then divided by the scaled image, and the result was taken as the corrected image. The corrected image could then be coregistered to the original mean functional image and used as the input dataset for the standard FreeSurfer processing pipeline. We observed a marked improvement in both the coregistration results and the results of the FreeSurfer segmentation and surface generation after performing this correction (figure 3.3). The computation time required to run the FreeSurfer processing pipeline was drastically reduced as well, in some cases by up to 15 hours.

3.5.3 Coregistration

Coregistration is the process of calculating a transformation which maps one image into the space of another and brings the two images into alignment. The images generally are of different image modalities, and the typical case is to align an anatomical T1-weighted image with T2 or T2*-weighted functional data. Coregistration is an important step in fMRI preprocessing because functional images often have poor tissue contrast which makes it difficult to identify the brain regions responding to an experimenter's task. By aligning the functional and anatomical images, it is possible to determine where in the brain activation is observed. Although coregistration is a common step in fMRI preprocessing, it is not trivially performed on submillimeter, partial volume data. Furthermore, the requirement that registrations be accurate with respect to the boundaries of the gray matter volume is a complicating factor. This section describes the unique approach taken to achieve accurate registration solutions challenging submillimeter data.

Within-subject coregistration was performed using the skull-removed mean functional image and the skull- removed IR-EPI image. Using this image set mitigated registration error owing to image distortion typically observed in EPI acquisitions. High quality coregistration was crucial to the laminar analysis featured in this experiment, as the accurate definition of tissue boundaries in functional space follows only from a highly accurate coregistration of the structural and functional images. Note also that the transformation computed in this step was applied to the structural image to avoid introducing interpolation errors.

Several coregistration tools were used to calculate optimal image alignment. For a given subject, multiple transforms were calculated and visually inspected. The best alignment as determined by visual inspection was taken for further analysis. Volume coregistration was performed using FreeSurfer's robust, outlier insensitive registration cost function as implemented in *mri_robust_register*. If the resulting transformation resulted in poor registration, we then used the NMI cost function implemented in *mri_robust_register* and finally the NMI cost function implemented in AFNI's *3dAllineate*. If necessary, manual improvements were applied to the best transformations generated by these tools. Registration quality was assessed by visual inspection of alignment along the left occipitotemporal sulcus and brain edges.

In 11 subjects, failure to reconstruct surfaces from the IR-EPI image made it necessary to perform surface reconstruction on MP2RAGE data, and therefore to bring the MP2RAGE surfaces into register with the functional data. Following the initial coregistration described above for the IR-EPI images, the IR-EPI images were generally in good alignment with the task data and could serve as the source image for this purpose. In this case, MP2RAGE surfaces were aligned to the IR-EPI volumes using FreeSurfer's boundary based registration program. If the IR-EPI volume was not in good alignment with the functional data, an initial alignment between the MP2RAGE and functional data was first computed using the tools described in the previous paragraph before performing the boundary based registration. The boundary based registration procedure is described below in a dedicated section.

3.5.4 Normalization for group analysis

Functional data for each subject were mapped into MNI space for group analysis. The skull-removed MP2RAGE image was first brought into alignment with the skull-removed mean functional image. Alignment was performed using *3dAllineate* in AFNI (**Cox, 1996**) After inspecting the quality of the registration, the functionally aligned MP2RAGE images were aligned to the MNI128 template available in standard FreeSurfer (**Fischl, 2012**) installations. This transformation was concatenated with the inverted transformation mapping the MP2RAGE to the functional space, and then applied to the motion corrected functional data. Alignment quality of task critical regions in temporal cortex was verified by visually inspecting the alignment of brain edges and the middle temporal gyrus. In addition, first level *t*-statistic maps (described in Depth dependent signal extraction) were used to verify the alignment of functionally critical cortex, though only for the purpose of the depth dependent group analysis. Inaccuracies in subject registrations were addressed with a manually created, secondary transformation containing small translations intended to improve task critical region alignment without introducing large global inaccuracies. Crucially, the experimental question related to the depth dependent connectivity with brain regions that responded to the word/pseudo-word contrast. The use of the non-laminar first level maps to facilitate alignment was therefore independent of the laminar analyses. The result of this spatial normalization was MNI mapped functional data for each subject. A 3mm Gaussian filter was applied to the spatially normalized data to be used for group analysis.

3.5.5 Tissue segmentation and surface generation

Tissue segmentation and surface generation are essential to the extraction of the depth dependent signal. Tissue segmentation involves labeling voxels as containing gray or white matter. The categorization can then be used to calculate the location of the gray and white matter surfaces in the brain volumes of each participant. The surfaces were used in this work to guide the creation of the depth dependent bins from which the depth dependent signal was later extracted.

Tissue segmentation was performed in FreeSurfer using the skull-removed, functionally aligned IR-EPI image. An example of surfaces generated from an aligned IR-EPI dataset are shown in figure 3.4 overlaid on the IR-EPI image used to generate them as well as the functional data to be analyzed. Failures to properly reconstruct subject surfaces were addressed by inserting control points, applying additional normalization as described in a previous section, or disabling the correction of defects in surface topology, if they did not occur in experiment critical regions. The IR-EPI images commonly included artifacts in noncritical locations that would result in discontiguities in the surface and unsuccessful surface generation. As these defects did not often occur near lOTS, it was possible to generate accurate surfaces even after bypassing correction. If surface reconstruction failed following these interventions, the MP2RAGE dataset was used in place of the IR-EPI, and additional registration steps were applied (discussed in *Boundary based registration*).

3.5.6 Boundary based registration

Surfaces reconstructed from the IR-EPI image did not require additional alignment to the functional data beyond resampling the FreeSurfer generated surfaces from “conformed space” to functional space. “Conformed space,” native to FreeSurfer, is a 1mm isotropic 256^3 grid in the RAS coordinate system.

Surfaces reconstructed from MP2RAGE images underwent an additional registration step using *bbregister*, FreeSurfer’s boundary based registration (BBR) tool. The goal of this procedure was to produce surfaces in register with the functional data. As the IR-EPI and MP2RAGE datasets were generally well aligned from the coregistration procedure described previously, the main purpose of the BBR was to find a solution accommodating the distortions affecting surface placement along key anatomy. Using a boundary based cost function, IR-EPI images that were unable to be used for surface generation were aligned with the boundaries generated from the MP2RAGE images.

The inverse of this transformation was then applied to the surfaces to align the boundaries to the IR-EPI image. If the BBR produced alignment proved inaccurate, simple solutions to improve accuracy involved optimizing the registration for the fROI through a weighting mask, manual intervention, and improving the alignment of the two images prior to the boundary based registration. Failing a simple solution, we also computed a nonlinear boundary based registration (**van Mourik et al., 2018**). In this approach, the registration algorithm recursively divided and aligned surface segments to increase registration accuracy.

The importance of highly accurate image alignment in laminar resolution imaging cannot be overstated. In the present work, registration inaccuracies in excess of 1mm had the potential to displace the cortical layers, leading to meaningless results. Great care was taken to ensure accurate registrations and alignment of the surfaces with the functional images. As in the other registration procedures, registration quality was assessed through visual inspection. Alignment quality in lOTS and left temporal cortex was prioritized over regions that were less experiment critical.

3.6 Procedure to define functional region of interest

The first level, non-laminar analysis was used to identify the lOTS fROI. Minimally smoothed (1mm Gaussian filter) native space data were used to define the lOTS region used for the extraction of the depth dependent time-courses. The task design was fitted using the generalized least squares regression implemented in the OpenFmri analysis suite (https://github.com/TimVanMourik/OpenFmriAnalysis).

Two tailed *t*-statistics were calculated in MATLAB (The MathWorks Inc.) for condition and contrast effects in each subject individually. Anatomically, the region of interest was located proximal to the fundus of the occipitotemporal sulcus. It was functionally defined as a cluster of voxels which responded to visually presented words and pseudo-words, but preferentially to pseudo-words. In addition, this region is known to express reduced BOLD amplitude to false-font items compared to items composed of orthographically legal characters.

The region was defined in each subject through a series of masking operations implemented with AFNI's voxel-wise dataset calculator *3dcalc*. These operations were performed on the t-statistics from the 1mm smoothed, native space analysis. First, voxels were removed if they did not reach threshold in both the word and pseudo-word conditions. This was defined as a *t*-statistic $1 \leq t \leq 3$, depending on the number of voxels which survived the fROI masking. *T* was initially set to $t = 2$ and was increased or reduced if the number of surviving voxels fell outside of the desired range (see below). All voxels with a larger *t*-statistic for the false-font condition than for either the word or pseudo-word conditions were then removed. Finally, voxels were excluded if the difference between *t*-statistics of words and pseudo-words was larger than the original *t*-statistic value of each individual condition. Clusters were considered for inclu-

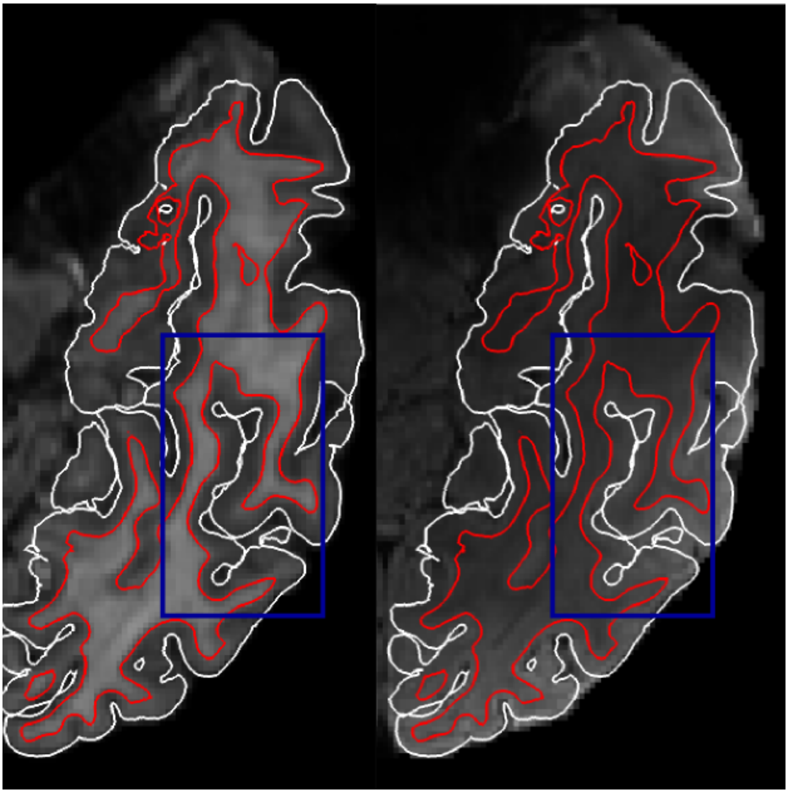


Figure 3.4. Single subject white (red) and pial (white) surfaces overlaid with the (left) IR-EPI and (right) task images. Blue box shows the region used to assess surface quality

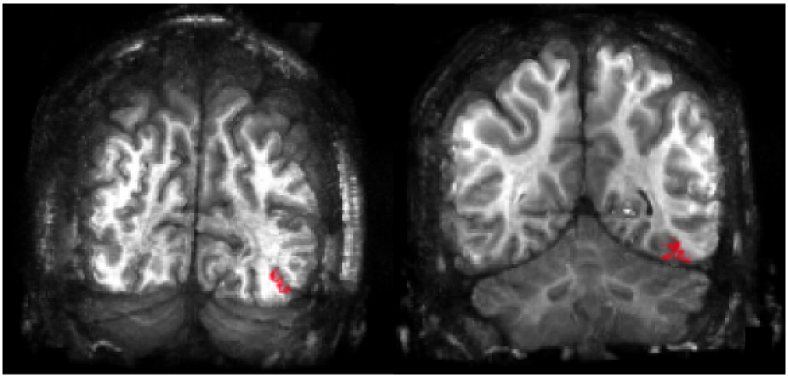


Figure 3.5. Functional ROI as defined in two subjects on IR-EPI.

sion if they were 1) located within the extent of the occipitotemporal sulcus, if 2) cluster size was between 100 and 400 voxels, if 3) 30-50% of the voxels responded preferentially to the word condition over the pseudo-word condition, and if 4) the total response was comparable between the word and pseudo-word preferred voxels when considering the proportion of voxels preferring each condition. These criteria were selected to isolate a functional region which is known to respond preferentially to both words and pseudo-words compared to false-font items, prefer pseudo-words to words, and contain a mixture of individual voxels which prefer each condition. The fROI selection procedure was biased by design toward pseudo-word activation because stronger pseudo-word activation is a functional feature of the region (**Price, 2012**).

Further consideration was given with respect to the importance of cluster contiguity. Given the high spatial resolution of our data, it was possible to distinguish populations of active voxels spanning the cerebrospinal fluid (CSF) boundary bridging the occipitotemporal sulcus. Following the removal of the voxels located in CSF in some subjects, formerly contiguous clusters became distinguishable. We determined that the most reasonable approach was to include formerly contiguous voxels in the laminar analysis.

Given that this region is often functionally defined and generally identified near the fundus of the occipitotemporal sulcus, partial volume effects have almost certainly influenced earlier fMRI measurements at standard resolutions. The decision to exclude populations of voxels stranded on either side of the chasm would have proven arbitrary in that standard resolution studies investigating this region often fail to distinguish fusiform and inferior temporal cortex. We concluded that allowing for discontiguities in the left OTS fROI more faithfully adhered to the literature definition of the region than an ad hoc justification for voxel removal. Functional ROIs as defined in two participants can be seen in figure 3.5.

3.7 Equivolume contouring

The gray matter volume of each subject was partitioned into equivolume bins using the OpenFmri (https://github.com/TimVanMourik/OpenFmriAnalysis) implementation of the equivolume contouring approach described in **Waehnert et al.** (**2014**). In this approach, a boundary, i.e a FreeSurfer surface, is represented as a level set function. The level set function is then evolved in fixed intervals weighted by local curvature. Each evolution of the function yields an intermediate boundary where the total volume between two adjacent boundaries is interpreted as containing a fixed, curvature invariant volume of gray matter. Applying the equivolume parcellation increases the likelihood that the histological profile of each bin is consistent throughout the region (**Bok, 1929**). The equivolume method increases the likelihood that the histological profile of each bin is consistent throughout the given region.

Bok, a 20th century Dutch neurologist, discovered a principle of histological structure essential to equivolume contouring: although cortical thickness varies with curvature, the volume of individual histological layers is maintained in proportion to the overall thickness. Put differently, if layer IV accounts for 20% of the cortical volume of a given cytoarchitectonic region, it will invariantly account for 20% of the cortical volume irrespective of changes in the thickness of the region. Even without knowledge of the precise histological composition of a given bin, the composition can be assumed to hold the same relative volume through a cytoarchitectonic region. The Bok principle is visualized in figure 3.6.

The Bok principle and equivolume contouring help address a stubborn difficulty arising in lfMRI. Isocortex typically comprises six histological layers, but current methodological limitations do not allow histologically accurate contours. While producing an arbitrary number of bins, whether 6 or 100, is mathematically possible, the number of biologically interpretable bins is constrained by cortical thickness, voxel size, and the topic of research. As a rule of thumb: the fewer bins used, the more distinguishable the signal across the bins. It stands to reason that the number of bins should be limited by the ratio of the voxel edges to the cortical thickness in the volume of interest. Thus, measurements taken with isotropic 0.9mm voxels in a volume that is 2.8mm thick would impose a limit of three cortical bins to minimize the likelihood of each voxels to subsume multiple depth-bins. The cortical thickness in the region used in this work was determined by FreeSurfer to be 2.68mm thick. This calculation was performed using the thickness estimates of the MP2RAGE datasets which are known to show a systematic reduction in cortical thickness estimates relative to MPRAGE (**Fujimoto et al., 2014**). The thickness estimate should therefore be regarded as a conservative estimate.

The gray matter volume of each participant was partitioned into three bins: the smallest number of bins which allowed for the dissociation of the deep, middle and superficial contributions to the overall BOLD signal. For the purpose of the spatial GLM, it was necessary to include two additional non-cortical bins representing white matter and CSF volumes respectively. The inclusion of these additional bins was due to partial volume effects caused by voxels extending outside the cortical strip. Voxels observed within these boundaries were assigned a value representing the fractional volume observed within a particular set of boundaries. The result of the complete process – surface generation, contouring, and fROI identification is shown for one subject in figure 3.8.

The ultimate output of this procedure was a 4D dataset whose first three dimensions represented spatial coordinates and whose fourth dimension represented the different bins. Incrementing over the fourth dimension indices gave the fractional volume of each voxel found in that particular bin. This dataset is referred to as the layer-volume distribution.

At the mesoarchitectural level of lfMRI, it is not practical to measure individual histological layers. One common approach to this challenge has been to consider a simplified model of layer interactions which merges supragranular (layers I,II,III), granular (layer IV) and infragranular (layers V,VI) histological layers into three logical layers based on shared connection tendencies (**Koopmans et al., 2010**; **Muckli et al., 2015**; **De Martino et al., 2015**; **Kok et al., 2016**; **Lawrence et al., 2018**). This model is based in large part on patterns of laminar connectivity discovered in **Rockland and Pandya** (**1979**) when exploring the link between anatomy and functional hierarchy, and schematized in the Felleman and Van Essen hierarchy (1991). The laminar simplification has proven valuable when constrained by functional data, and has to date informed efforts in lfMRI. This is the approach that was taken in the present work, as it was sufficient to distinguish top-down from bottom-up contributions to the BOLD signal.

3.8 Depth dependent signal extraction

Following equivolume contouring, the fROIs were used to mask the layer-volume distribution. This resulted in a layer-volume distribution within the extent of the fROI. By treating this distribution as a design matrix (schematic shown in figure 3.7) with voxels defined along the rows and depth-bins along the columns, it was possible to regress it against the signal observed in the fROI for each time point in the experiment (**van Mourik et al., 2019**; **Polimeni et al., 2010b**).

Fitting the voxel-volume distribution thusly in a spatial GLM to each time point in the experiment yielded the relative contribution of each bin to the overall signal at each time point, thereby representing a depth dependent time-series for each depth bin. These were treated similarly to voxel time-courses and used to fit the task design. The analysis was performed using the generalized least squares spatial GLM implemented in the OpenFmri

The task design described in *Task paradigm* was fitted to the extracted depth dependent time courses. Percent signal change was calculated as a division be-

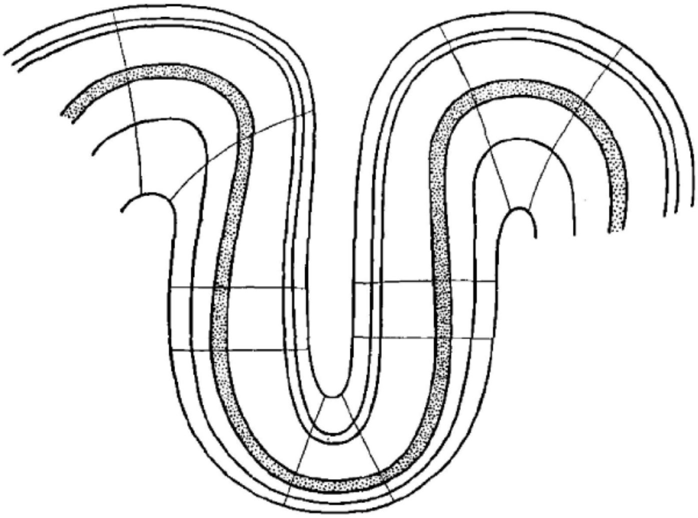
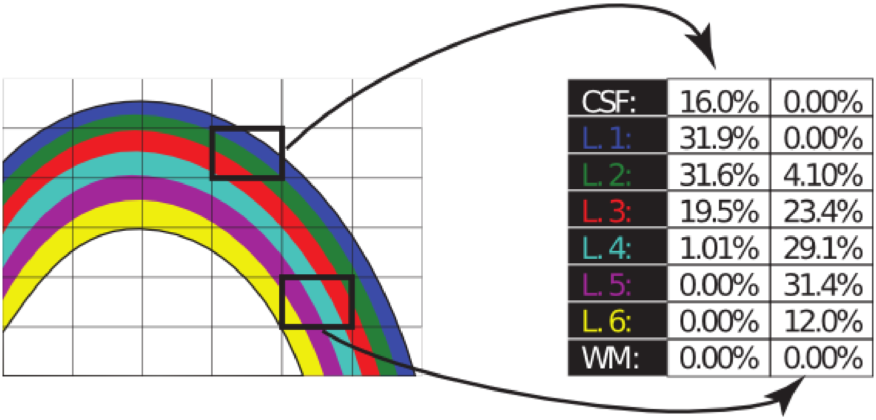


Figure 3.6. Sketch from **Bok** (**1929**) illustrating the constant histological proportionality through a consistent cytoarchitectonic section. This is the foundational principle of the equivolume layering method.



CSF:	16.0%	0.00%
L. 1:	31.9%	0.00%
L. 2:	31.6%	4.10%
L. 3:	19.5%	23.4%
L. 4:	1.01%	29.1%
L. 5:	0.00%	31.4%
L. 6:	0.00%	12.0%
WM:	0.00%	0.00%

Figure 3.7. Simulated layer-volume distribution. Idealized gyrus with six equivolume layers shown in different colors. The voxel-volume in each layer is determined accounting for the full volume of each voxel. Figure from van Mourik (2015).

tween β-weights assigned to each condition and the average weight assigned to the constant terms. The percent signal change values were analyzed at the group level in an ANOVA, and subsequent two-tailed *t*-tests were performed to examine depth dependent responses to the word and pseudo-word contrast. ANOVA results were considered significant at $p \leq 0.01$. *T*-statistics were considered significant at $t \leq 0.01$. These analyses were implemented in MATLAB.

3.9 Physiologic noise removal

During image acquisition, signal variance related to cardiac and respiratory activity introduces confounds which reduce the ability to detect signal of interest. By recording cardiac and pneumatic activity in participants during an experiment, the signal variance introduced by this activity can be modeled.

Cardiac and respiration data were collected concurrently with the functional data using a pulse oximeter and pneumatic belt. Physiologic regressor estimation up to the 6th (cardiac) and 8th (respiration) order was performed using a modified version of the PhysIO toolbox of the TAPAS suite (**Kasper et al., 2017**). The modifications were necessary to account for unique log file formats produced by our equipment. Regressors were then included in the design as nuisance regressors. Regressor quality was assessed with a partial F-test.

3.10 Intra-regional gPPI

Generalized psychophysiological interaction (gPPI) analysis is commonly used to assess the interaction between brain regions in the context of an experimental task (**McLaren et al., 2012**). It is commonly performed on fMRI data to fit terms modeling the interaction between tasks conditions (i.e. words and pseudo-words in the present analysis) and brain regions in a GLM framework. The goal of gPPI is typically to assess the interaction between brain regions in the context of a task. In gPPI analysis, the first level model is extended by including the time-course of a seed region in addition to interaction terms of the seed region with each task condition regressor. In the generalized form, the analysis can model interactions between a brain region and each task condition in a given experiment, allowing interactions to be assessed for arbitrarily large experiment designs. In the intra-regional analysis, gPPI was used to assess the task-related interactions between the depth bins within the lOTS. gPPI models were created and fitted for each participant, and a group level, two-tailed, paired *t*-statistic was then computed to assess the contrast between the word and pseudo-word interaction terms. Results were considered significant at $p \leq 0.01$

As the goal of gPPI is to observe the effect of the interaction between the task and the neuronal response of a seed region, a deconvolution is typically applied to the seed time-course before computing the interaction term. Given the problems associated with deconvolution (**O'Reilly et al., 2012**) and the novel nature of this work, we omitted deconvolution from our gPPI analysis. **O'Reilly et al.** (**2012**) have shown that this omission is not expected to greatly affect the outcome in block designs such as that used in the present study.

There is no known precedent for laminar specific gPPI. The gPPI design was created by adding seed region time-courses and interaction terms to the original design. Interaction terms were calculated as the product of the detrended depth dependent time-series and binary condition vectors (1 when a condition response was expected, 0 when it was not) derived from the task regressors. A time point was included in the interaction term if the task regressor diverged from 0 by 0.0001.

Different models were created for each intra-regional analysis to assess the interaction between two depth-bins while alternating seed/target assignment. We did not model the third remaining bin as it was expected to exhibit high collinearity with the bins of interest. Six models were created in total, each containing six interaction terms (each of the six conditions multiplied by the seed-region time-course), the seed-region itself, and the full design as discussed previously in *Task paradigm*. Group effects were assessed using AFNI's *3dANOVA3*.

3.11 Whole brain task-dependent connectivity analysis

As commonly applied, gPPI analysis is not suited to the study of directed interactions between brain regions. By performing gPPI on depth dependent data, it is, however, possible to leverage knowledge of inter-regional laminar connectivity patterns to obtain directional information. Thus, gPPI on depth dependent data can be regarded as a directed measure, capable of capturing the hierarchical relationship between distinct regions in a distributed network.

To assess the depth dependent connectivity of the lOTS to the whole-brain during word and pseudo-word reading, we performed a group gPPI analysis. Interaction terms for each depth bin with each task regressor were created using the same procedure as the intra-regional analysis. This extended the first level model by 15 terms. The final model included the six original task regressors, two terms modeling the time courses in the deep and middle bins, and 12 interaction terms – six modeling the interaction of the deep bin with each task regressor, and six modeling the interaction of the middle bin with each task regressor. The interaction of the deep and middle bin timecourses was also modeled to account for depth dependent interactions within the lOTS.

The gPPI models were then fitted to the MNI normalized functional data in each participant to identify task based, depth dependent networks. Importantly, signal extracted from the depth bins using the spatial GLM was not subject to spatial smoothing through the cortical depth. Thus, the depth dependent specificity of the signal extracted from each depth bin was preserved. The depth bin time-courses were analyzed as well to assess task-independent connectivity for each depth bin. The task-independent connectivity from the deep and middle bins was assessed using the deep and middle bin time-courses included in the gPPI model. Significance testing was performed using AFNI's *3dANOVA3*, as in the intra-regional gPPI analysis. Following family-wise error correction, results were considered significant at $p_{uncorr} = 0.001$, $\alpha = 0.05$. The family-wise error correction procedure is described in the following paragraph. The final results were visualized using the rendering plugin in AFNI. In addition to subjects 2 and 19, subject 4 was excluded from this analysis. It was not possible to successfully bring subject 4 into MNI space. Large inaccuracies in the registration resulted in the exclusion of this subject from the whole brain analysis.

The parameters of the spatial autocorrelation function (ACF) representing the smoothness of the data were computed using AFNI's *3dFWHMx* on the residual time-series of first level analysis performed on the normalized functional data. The ACF parameters were used by the AFNI program *3dClustSim* to compute the likelihood of random clusters given the ACF parameters 0.4747, 3.2569, and 7.8772, in a volume of the dimensions 63 × 82 × 55 with 2mm isotropic voxels. The dimensions of the volume used for permutation testing were determined through a group level functional data mask.

3.12 Conclusion

This thesis instantiates the first whole brain, laminar level fMRI study on higher cognitive function (i.e reading). To pursue this work, it was necessary to develop or extended a suite of analysis techniques which included novel approaches to image registration, B1 inhomogeneity correction, and depth dependent gPPI analysis. The developed analysis pipeline is well suited to the rapid analysis of laminar resolution data in relatively high-*n* studies. Future improvements in automaticity of this analysis pipeline will further support the use of laminar resolution fMRI in cognitive neuroscience.

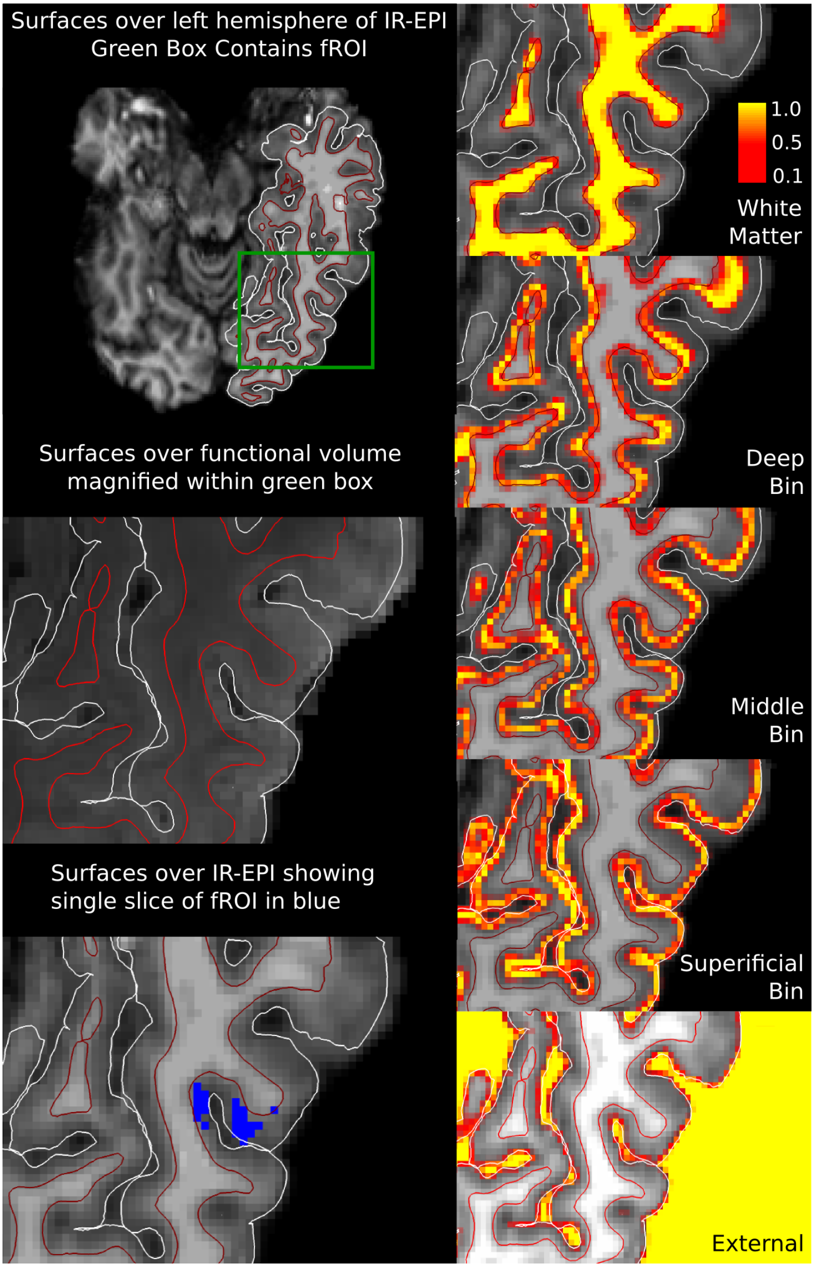


Figure 3.8. Surfaces and volumetric layering in one subject. Left column: White matter (shown in red) and pial (shown in white) surfaces are shown overlaid on anatomical and T2*- weighted images. Slice through fROI is shown in blue. Magnification within the green box highlights fROI location. Right column: Equivolume depth bins shown in volume space. Each voxel was assigned 5 values between 0-1 according to the proportion of its volume in each of the depth bins. A value of 1 (yellow) indicates that the voxel is fully contained within a given depth bin, 0 (black) that it is not within the depth bin. Intermediate values indicate the fractional volume within a given bin.

4 Directed connectivity in distributed reading network identified through laminar resolution fMRI

4.1 Top-down task effects in occipitotemporal sulcus

We manipulated the top-down signal directed toward left occipitotemporal sulcus (lOTS) by visually presenting words and pseudo-words in Dutch (explained in figure 3.1). As visual representations of words are related to information unavailable from bottom-up sensory information, we expected to observe a relative increase in top-down signal during word reading owing to the linguistic information that could be retrieved from memory (**Jackendoff, 2002**; **Price and Devlin, 2011**). The top-down signal was expected to be observed in the deep bin of the lOTS and to originate in the left temporal cortex (see **Hagoort, 2013**).

A three-way analysis of variance (ANOVA) was performed to assess the main effects of Depth (deep, middle, superficial) and Condition (words, pseudo-words) as well as their interaction. Participants were modeled as a random factor. Significant main effects were found for both Depth (F (2,105) = 84.37, $p < 0.001$) and Condition (F (1,105) = 10.98, $p = 0.0013$). The Depth × Condition interaction was also significant (F (2,105) = 12.24, $p < 0.001$). The nature of this interaction was further tested by t-statistics on the word and pseudo-word response contrast at each depth bin (figure 4.1). In addition to the contrast shown in figure 4.1, figure 4.2 shows the percent signal change BOLD response for both conditions in all subjects across the three depth bins.

The depth dependent t-statistics were reduced in the middle and superficial bins for words relative to pseudo-words. T-statistics in the deep bin were, however, greater for the word condition (figure 4.1). The greater t-statistic in the deep bin is evidence for elevated top-down related signal in the lOTS for word compared to pseudo-word reading. The concurrent reduction of the t-statistics within the middle and superficial bins suggests that the increased top-down signal acted to facilitate word reading and was related to the globally attenuated BOLD response throughout the lOTS, as proposed in (**Price and Devlin, 2011**).

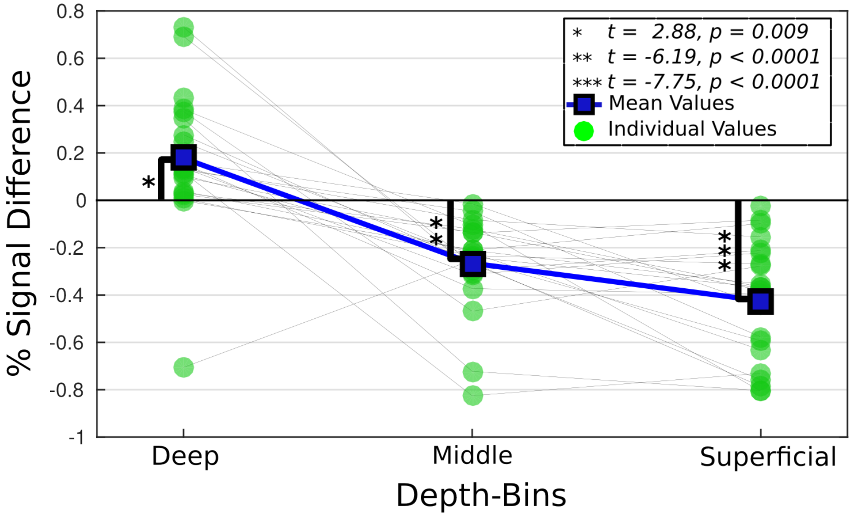


Figure 4.1. The difference in percent signal change between words and pseudo-words is shown by depth for all participants. Significance bars relate to *t*-statistics computed on the difference from 0 (n = 22)

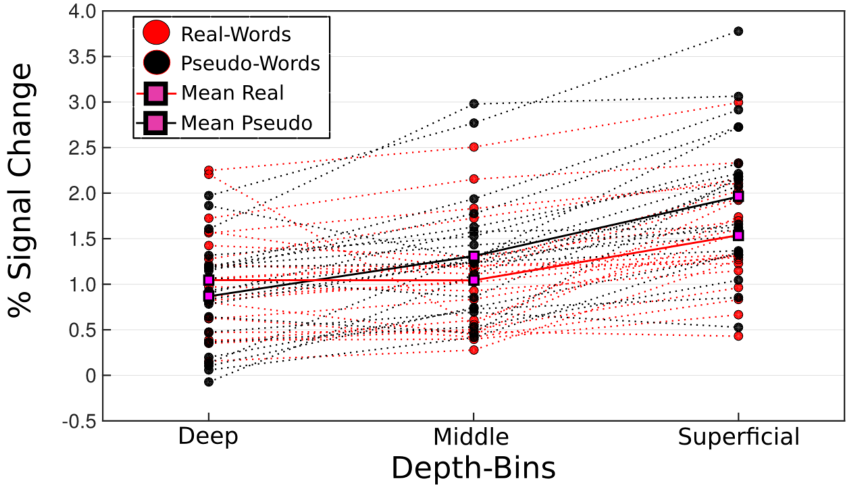


Figure 4.2. BOLD responses shown as percent signal change in all subjects for both word and pseudo-word conditions. Mean percent signal change for word and pseudo-word conditions is plotted above individual subject responses. (*n*=22)

While the relative top-down information content was varied through the word/pseudo-word manipulation, the experiment did not include a bottom-up manipulation. The bottom-up information content was therefore held constant across conditions, and the middle bin effect was unlikely to be the result of differential bottom-up stimulation.

We propose that the middle bin effect might have related to intrinsic inhibitory connections which originated in neurons located within the deep layers and targeted neurons in the middle layer. This interpretation is supported by an intra-regional connectivity analysis showing that the deep bin predicted the diminished response of the middle bin to the word and pseudo-word contrast.

4.2 Within region bin to bin interaction effects during task

Generalized psychophysiological interaction analysis (gPPI) is commonly performed on fMRI data to fit terms in a GLM framework which model the interaction between task conditions (i.e. words and pseudo-words in our experiment) and brain regions (**McLaren et al., 2012**). The goal is typically to assess the interaction between brain regions in the context of an experimental task. One interpretation of the gPPI analysis is that the strength of the interaction relates to the tendency of the seed region to enhance the response of the target region to a task condition. We used this technique to assess the task-related interactions between the depth bins within the lOTS.

Seed and Target	*t*-value	*p*-value
Deep to Middle	-3.429	0.003$*$
Deep to Superficial	-2.194	0.04
Middle to Deep	-0.9677	0.35
Middle to Superficial	-0.3773	0.71
Superficial to Deep	-1.655	0.11
Superficial to Middle	-0.8662	0.34

Table 1. Bin to bin intra-regional gPPI. Table shows *t*-statistics for contrast of the word compared to pseudo-word conditions. The deep bin shows reduced interaction with the middle bin during word reading. $* p \leq 0.01, n = 22$

The gPPI model contained all terms used in the first level model with the addition of terms modeling the interaction between the time-courses of the individual depth bins and the task conditions. These were fitted for each participant, and group level *t*-statistics were then computed from the fitted interaction term parameters. The *t*-statistics represent the pairwise effect of each bin on every other bin, and contrasted the word and pseudo-word conditions to determine if inter-bin signal modulation contributed to the lexicality effect shown in figure 4.1.

Activity in the deep bin was shown to predict decreased activity in the middle bin during word reading compared to pseudo-word reading. This is observed as a negative interaction effect during word reading, indicating that activation in the deep bin predicted a reduced response in the middle bin during word compared to pseudo-word reading, or equivalently, an increased response for pseudo-words compared words. Taken together with the increased deep bin and reduced middle bin *t*-statistics observed during word reading in figure 4.1, the gPPI results suggest that increased top-down signal during word reading had a suppressive effect on the middle bin, and perhaps on the global signal in the region (table 1).

4.3 Task independent bin to whole brain interactions

A second gPPI analysis was performed to examine the effect of the lOTS depth bins on the BOLD response throughout the entire brain. As commonly applied, gPPI analysis is not suited to the study of directed interactions between brain regions. By performing gPPI on depth dependent data, it is, however, possible to leverage knowledge of inter-regional laminar connectivity patterns to obtain directional information. Thus, gPPI on depth dependent data can be regarded as a directed measure, capable of capturing the hierarchical relationship between distinct regions in a distributed network.

The whole brain gPPI model included the interaction terms for the depth bins (deep and middle) and the task conditions (words and pseudo-words). These were then fitted to spatially normalized data in each participant to identify task based, depth dependent networks at the group level. No spatial normalization was applied to the depth bins. The depth bin time-courses (deep and middle) and their interaction (deep × middle) were also modeled and used to assess task-independent connectivity for each depth bin. The interaction between the seeds was included to model intra-regional interactions of one bin on another. GPPI targets predicted by the deep bin terms were interpreted as being components of a top-down network related through lOTS. Those predicted by middle bin terms were considered components of a bottom-up network.

Strikingly, the deep and middle bins did not interact with similar brain regions. In the task-independent analysis, we calculated *t*-statistics for the deep and middle bin time-course estimates to obtain a pseudo resting state connectivity measure. Notably, the middle bin time-course experienced strong interactions with bilateral regions posterior to the lOTS, indicative of a feed-forward network including early visual regions. The deep bin interacted with left lateralized gPPI targets anterior to the left OTS, an expected source of top-down

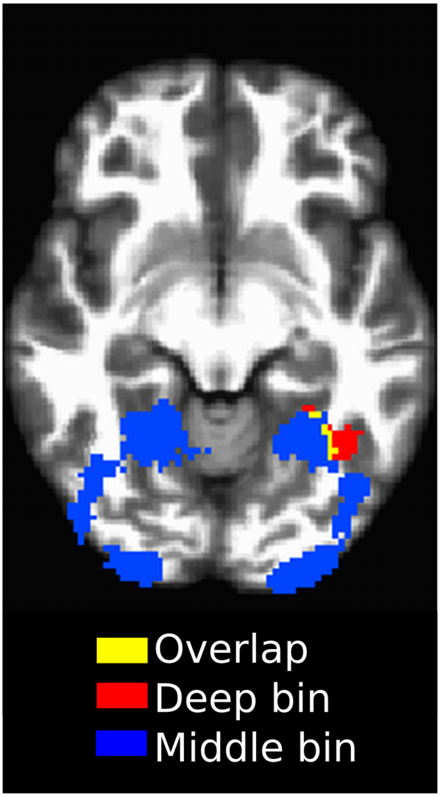


Figure 4.3. Task-independent connectivity. Colors indicate clusters targeted by the deep bin, middle bin or the overlap. The wide bilateral and posterior targets of the middle bin point to its role as a target of bottom-up input. The left hemisphere is shown on the right of the image.
$p_{uncorr} = 0.001, \alpha = 0.05, n = 21$

afferents to the region. The difference in regional specificity between the two bins is noteworthy. The deep bin interacted with regions within a small volume. By comparison, middle bin interactions were distributed throughout a large bilateral volume (figure 4.3).

4.4 Task dependent bin to whole brain interactions

The depth dependent interaction terms of the task dependent analysis identified a top-down network sensitive to the lexicality contrast. The identified network included language critical regions in left temporal cortex and responded preferentially to word reading over pseudo-word reading. When corrected for multiple comparisons ($p_{\text{uncorr}} = 0.001$, $\alpha = 0.05$), the deep bin exclusively targeted left middle temporal gyrus (lMTG) and lpMTG, regions commonly associated with lexical retrieval and semantic processing (**Hagoort, 2013**; **Price, 2012**;

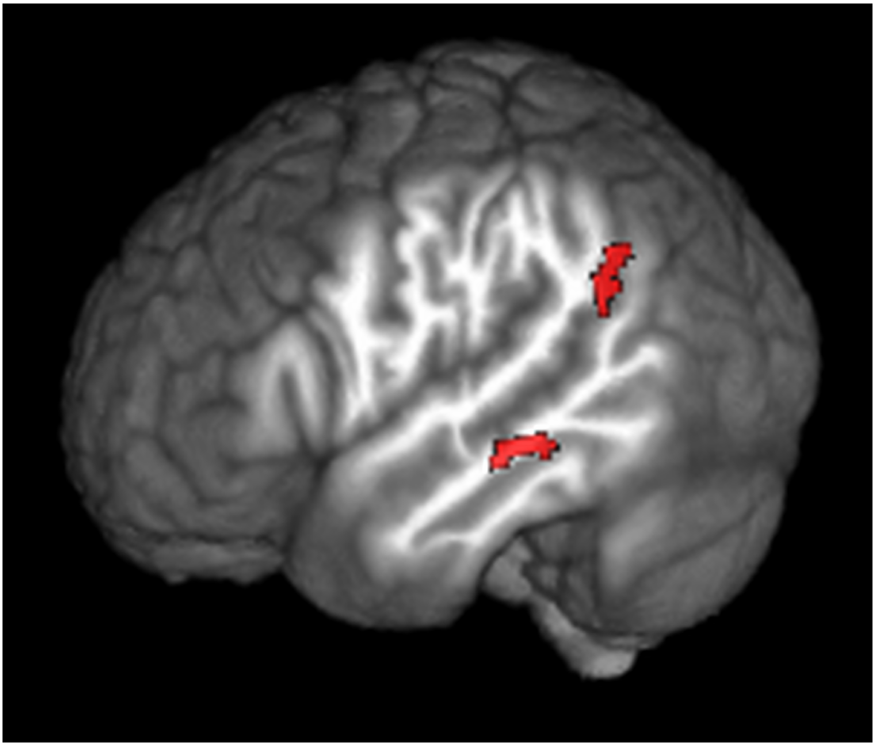


Figure 4.4. Words against pseudo-words gPPI for the deep bin. Shown in red: the deep bin preferentially targets left lateralized, language critical regions during word reading. $p_{uncorr} = 0.001, \alpha = 0.05, n = 21$

Snijders et al., 2009) (figure 4.4). The middle bin showed reduced interaction generally, with no clusters surviving correction.

4.5 Discussion

4.5.1 Depth dependent responses to word reading

That the deep bin showed a greater response to words than pseudo-words is evidence that words elicited stronger a top-down contribution to the observed BOLD signal in lOTS than pseudo-words. This is remarkable given that the overall response was greater for pseudo-words than it was for words.

The reading paradigm used in this work manipulated the top-down information load while holding the sensory input constant across task conditions. The low level visual features of words and pseudo-words did not differ, and both map to phonological knowledge (**Reicher, 1969**). Effects resulting from the contrast of these two items should therefore relate to differences in top-down information, information that was not available from the visual stimulus itself. Words differ most saliently from pseudo-words in semantic content –pseudo-words have no meaning whereas words do. They also lack other properties of words such as frequency and syntactic class. Top-down information was expected to relate to top-down signal contributions to lOTS. These were expected to be observed outside of the middle bin (**Douglas and Martin, 2004**), and thus to result in signal changes in the deep or superficial bins. No contrast effect was predicted in the middle bin. With respect to the word/pseudo-word contrast, it can be seen in figure 4.1 & 4.2 that:

- the deep bin response is increased for words
- words show an overall reduced response
- the middle and superficial bin responses are increased for pseudo-words

These results should not be interpreted without considering the biophysical properties of the BOLD signal and of the cortical laminae. It is well known that the BOLD response is observed downstream of the source of activation (reviewed in **Norris, 2006**). Gradient-echo acquisitions are furthermore sensitive to venous blood draining from the site of activation (ibid). Some signal contamination from the deep toward the superficial bin was thus expected (**Markuerkiaga et al., 2016**).

The deep bin, however, is not affected by signal contamination and is therefore expected to contain only signal unique to that bin. The preference for word reading in the deep bin is therefore most likely the result of greater top-down signal contribution to the lOTS. Taken together with the overall reduced signal in lOTS for word reading, it is strong evidence that depth dependent time courses can vary independently of the overall signal integrated over the three depth bins. It follows from these results that signal observed within specific depths relates to different cognitive processes. In this experiment, word and pseudo-word reading effects were found in different depths. This finding illustrates the sensitivity of lfMRI to a nuance which would have been missed by conventional fMRI.

More fundamentally, this result shows that the top-down manipulation was detectable without the reduction of the bottom-up signal. The top-down effects were robust enough to detect in the presence of bottom-up signal. It is generally agreed that synaptic terminations in layer IV strongly excite their targets and drive spike rate increases throughout the layers of the target region

(**Bastos et al., 2012**). It was hitherto unknown, however, whether comparatively subtle changes in top-down related responses would be detectable in the BOLD signal in the presence of bottom-up responses. The reading paradigm used in this work manipulated the top-down information load while holding the sensory input constant across task conditions. The robust deep bin effect during the word condition (figures 4.1 & 4.2) indicates that top-down manipulations could indeed be detected without experimentally altering the bottom-up signal. Many lfMRI experiments pertaining to human cognition reduce bottom-up signal sources to improve sensitivity to top-down effects (**Muckli et al., 2015**; **Kok et al., 2016**; **Huber et al., 2017**). This common approach is not suitable for measuring and characterizing the modulatory effect of the top-down signal. The results in this thesis suggests that lfMRI may be used in cognitive paradigms where both top-down and bottom-up signal is present. Although it is relatively simple to reduce the bottom-up stream in low-level sensory paradigms, such a restriction would constrain the designs available to researchers investigating higher order cognitive phenomena. This result suggests that lfMRI may be of more general use to cognitive neuroscience than hitherto demonstrated.

Although the signal decrease in the middle and superficial bins during word reading supports the independence of the deep bin effect with respect to the other bins, the mechanism of this reduction is presently unknown. BOLD signal decreases in left occipitotemporal cortex when contrasting word with pseudo-word reading are well documented (**Price, 2012**). It has been proposed that a facilitative effect on lOTS from top-down linguistic information results BOLD in suppression (**Price and Devlin, 2011**; **Chen et al., 2013**, **2015**). In this interpretation, BOLD suppression arises from less effortful processing for words. Alternatively, the difficulty of pseudo-word reading might result in BOLD enhancement, perhaps related to learning or attention (**Dehaene and Cohen, 2011**). In the enhancement account the differential responses in the middle and superficial bins would be interpreted as signal increases for pseudo-words compared to words. Both accounts also rely on top-down mechanisms, with the enhancement account arguing for increased top-down activation in the superficial bin rather than the deep bin of the suppression account. Although the present work predicts that the deep bin receive top-down related signal, there have been some reports of top-down related measurements in the superficial bin (**Muckli et al., 2015**; **De Martino et al., 2015**). Considering the possibility of both the suppression and enhancement accounts, the results reported in the current chapter are best explained by suppression, although they do not explicitly exclude the possibility that enhancement during pseudo-word reading might occur in addition to suppression during word reading.

The presence of increased top-down signal to the superficial bin during pseudo-word reading would not explain the deep bin effect during word reading. Thus, two distinct accounts would be necessary to explain the deep and superficial responses. The enhancement interpretation would also need to account for the increased middle bin response during pseudo-word reading. Two possible explanations might be that 1) increased activation in the superficial bin resulted in increased middle bin activation through intrinsic connectivity within the lOTS or that 2) supragranular layers targeted by top-down signal were included in the middle bin. Findings from the two gPPI analyses do not support either of these possibilities.

In the suppression interpretation, signal decrease instead of increase in the middle and superficial bins would need to be explained. The most likely mechanism for this would be suppression through increased activation in the deep bin mediated by intrinsic connectivity within lOTS. In this parsimonious interpretation, the increased response in the deep bin related to the decreased responses in the middle and superficial bins. The nature of the inter-bin interactions was further explored in an intra-regional gPPI analysis.

4.5.2 Intra-regional interaction between bins

A bin to bin generalized psychophysiological interaction (gPPI) analysis (**McLaren et al., 2012**) analysis within the lOTS investigated the task-dependent interaction between depth bins. This analysis was performed to further investigate whether signal increases in the deep bin related to signal decreases in the middle and superficial bins. The suppression account generates specific predictions of the connectivity profiles extracted by the intra-regional gPPI analysis. Signal increases in the deep bin would be expected to relate to signal decreases in the middle bin. In gPPI terms, increased activation in the deep bin would reduce the word and pseudo-word contrast effect in the middle bin, resulting in the reduction of the *t*-statistic for words when compared to pseudo-words. This is what was observed in the reported analysis. When the reduced interaction (table 1) is considered together with the depth dependent *t*-statistics, it is most plausible that the overall reduction in the condition effect for words is attributable to the increased deep bin effect. In summary, our results provide evidence in favor of top-down facilitation during word compared to pseudo-word reading, and suggest that activity in the deep layers can act to suppress activation in the middle layers. This mechanistic, inter-laminar description of suppression suggests new possibilities in the use of functional measures to investigate intrinsic connectivity at the mesoarchitectural scales.

4.5.3 Task independent connectivity

A bin to whole-brain task independent gPPI analysis was performed using the depth bin seed regions included in the gPPI model to explore differences in baseline connectivity to external brain regions. The middle bin contained histological layers targeted by feed-forward connections, and was expected to express higher connectivity with the early visual pathway. The deep bin was expected to show higher connectivity posterior to the seed region related to top-down targets.

The connectivity patterns observed in the task-independent gPPI results (figure 4.3) are in agreement with the expected organization of bottom-up and top-down efferent signal streams which target lOTS. This result indicates that the deep and middle bin formed distinct connections and that these connections agreed with the functional role of the histological layers they subsumed. The relatively large extent of the middle bin connectivity is clear from the figure. Relative to a single region, the bottom-up signal is by necessity uninterrupted throughout the processing hierarchy. The top-down stream by comparison does not have these constraints and may be restricted to fewer regions. The larger volume of the middle bin gPPI targets and the smaller volume of the deep bin gPPI targets are consistent with these constraints. The anterior/posterior spatial distribution of the targets is consistent with the top-down/bottom-up signal origins. Bottom up signal originates in lower regions relative to efferent targets, primarily in visual cortex for the lOTS. Top-down signal originates in higher regions, often anterior to target populations. Finally, the bilateral middle bin interactions are consistent with findings that the lOTS receives input from bilateral sources (**Chu and Meltzer, 2018**).

It is currently thought that the BOLD signal is physiologically most related to local field potentials (LFPs), which in turn relate to synaptic activity, or input to a brain region (**Einevoll et al., 2013**; **Logothetis et al., 2001**). Seeding from the lOTS, we therefore expected our results to most closely relate to brain connectivity between lOTS and external regions which generate input to the lOTS. I do not therefore interpret these results to indicate that the bottom-up information stream terminates in its entirety at the middle bin of the lOTS. Rather, these results indicate that the baseline signal of the middle bin, which is expected to relate to input to the lOTS, expresses the strongest correlation with posterior occipital regions lower in the visual processing hierarchy. It is expected, though not addressed in these data, that the bottom-up information stream continues through the brain. It would thus be predicted that similar results would be obtained were the analysis to be repeated using the middle bin of a slightly more anterior seed region.

4.5.4 Task based connectivity

The sensitivity of adjacent depth bins to distinct regions in distributed networks is our most important finding. It is remarkable that the different depth-bins expressed this degree of specificity and that they could be used to investigate directed interactions instantiating high level cognitive phenomena. The unique connectivity profiles associated with each depth bin empirically demonstrate the ability of laminar fMRI to noninvasively identify directed networks. The increased connectivity to the left posterior middle temporal gyrus (lpMTG) and left MTG (lMTG) during word reading was only observed when seeding from the deep bin (figure 4.4), which we interpret as direct evidence of a top-down interaction between language critical regions in the left temporal cortex and the lOTS during word reading. Information flow between the temporal and occipital regions during word reading is therefore best characterized in terms of top-down rather than bottom-up signaling, a finding which provides insight into the functional role of these regions during word reading

The experiment discussed here was designed to determine if it was more likely that top-down modulation of the lOTS or bottom-up signaling through the lOTS came to bear more heavily on the BOLD response in the lOTS during word reading. The experiment did not trace the bottom-up signal through the reading network, though it is uncontroversial that bottom-up signal contributes to the lOTS, and the l(p)MTG regions presumably do not initiate the response in lOTS. Rather, the experiment tracked whether the bottom-up or top-down streams most affected the lOTS. As a consequence of the observed top-down interaction, it can be claimed that the top-down stream originated in left temporal cortex. With respect to the bottom-up signal, one possibility is that bottom-up signal from the lOTS targeted left temporal regions directly, or perhaps also through a dorsal network implicated in relating orthographic to phonological information (**Price, 2012**). Top-down modulatory signal could thence target the lOTS. It would be possible to examine this possibility by repeating the task based gPPI analysis seeding from the temporal regions targeted by the lOTS seed. If the bottom-up stream in fact targeted the left temporal regions, the middle bin of the left temporal regions would be expected to interact with the lOTS.

What emerges from the top-down interaction is a reading network in which bottom up signal related to lower level visual and orthographic information is received in the lOTS and modulated by top-down signal originating in left temporal cortex. This does not preclude the continuation of the bottom-up stream beyond the lOTS, but indicates that the modulatory signal from left temporal cortex is the most functionally consequential to word reading. The impact on dominant theories of reading is discussed below in *Neurobiology of reading*. Beyond the unique contribution to the neurobiology of language, the ability to measure directed connectivity has far reaching consequences for expanding our knowledge of brain networks and the processes they implement.

4.5.5 Signal localization with gPPI and gradient-echo BOLD

It was not previously known that the commonly used GE-BOLD contrast would be capable of resolving spatially adjacent BOLD responses with sufficient accuracy to interrogate the depth dependent connectivity of distributed networks, or to observe interactions among bins within a region. We attribute these findings to a combination of factors. First among these is the fact that the gPPI analysis regressed out the main effects of the task conditions and returned a value related to the temporal correlation between regions.

Furthermore, previous theoretical work has demonstrated that the GE-BOLD response has a peak in the layer in which it originates and a flat tail of far lower intensity up to the pial surface (**Markuerkiaga et al., 2016**). **Markuerkiaga et al.** (**2016**) reported a depth dependent peak to tail response ratio of at least 5:1 in all cortical depths at 7 T, which would reduce the detectability of unique variance downstream from its source. The gPPI results suggest that this ratio may be conservative, or perhaps influenced by task properties. Our reading experiment presented stimuli at a high frequency relative to presentation rates discussed in **Markuerkiaga et al.** (**2016**), which should have produced relatively higher frequency task signal. The vasculature attributed to downstream BOLD effects consists of post-capillary vessels draining into larger vessels, whereby differences in the vessel length and flow velocity will act to reduce the coherence of the signal leading the vasculature bed to act as a low-pass filter. High frequency task signal components would therefore be expected to undergo greater attenuation than lower frequency components, and would experience a larger depth dependent peak-to-tail response ratio. It is to be expected that some amount of signal contamination will occur from deeper to more superficial layers, but that it should be best represented in the main effects of the task conditions. In light of our results, it seems clear that unique variance related to each bin was well localized, and that signal contamination was isolated to the main task effects, where it could be removed during the gPPI analysis. While it was not the focus of this study, these results further support the notion that the neurovascular response is highly linear and spatially tightly coupled, as previously reported for optical imaging techniques (**O'Herron et al., 2016**).

4.5.6 Neurobiology of reading

Laminar fMRI in humans is relatively new, and its application to the study of cognition even more so. Work to date has been restricted to primary cortices or early visual regions. There is no precedent of laminar fMRI in the study of uniquely human capacities, such as language. Recent work from **Lawrence et al.** (**in press**) has studied top-down memory effects on visual regions, and at least one experiment has recently been performed investigating working memory in higher cortical areas (**Finn et al., 2018**), but there have not been any efforts to investigate capacities as complex as language. The results presented in this work are encouraging to researchers who are interested in using laminar fMRI in the context of cognitive experiments. The reported findings are evidence that assumptions about the canonical microcircuit (**Douglas and Martin, 2004**) and its hemodynamic properties may generalize throughout the cortex. More specifically, the reported findings are informative of the neurobiology of reading. They provide an unprecedented implementation level view of how the brain integrates visual information with the language system.

The reported whole-brain gPPI results show that the deep bin of the lOTS is predictive of an increased contrast effect of words compared to pseudo-words in left temporal cortex. This is direct evidence that the left temporal cortex regions in the language network relate to the lOTS during word reading through top-down rather than bottom-up signaling. The reported evidence of top-down signaling is not derived from qualities of the stimuli, but rather from the location of the measurement. This independence from experimental manipulations is a valuable and convincing property of depth dependent measurements.

As discussed above, two dominant accounts of word reading propose to explain how visual information might be incorporated into language processes. **Price and Devlin** (**2011**) argue for a top-down suppression effect in lOTS during word reading whereas **Dehaene and Cohen** (**2011**) argue instead for enhancement during pseudo-word reading.

The connectivity results provide strong support for the suppression account and do not support the enhancement account. The whole brain gPPI analysis shows that the deep bin exclusively received top-down modulatory signal from lMTG and lpMTG, and only during word reading. This is interpreted as evidence of left temporal cortex exerting top-down influence over lOTS.

There were no interactions observed to be stronger during pseudo-word compared to word reading, and no interactions were observed to reach significance with the middle bin for either condition type. Connectivity to the superficial bin was not tested because superficial signal was expected to be contaminated by the other two depth bins. However, were the differential effects in the middle and superficial bins to arise from enhancement during pseudo-word reading, the middle bin would be expected to express connectivity to brain regions responsible for the enhancement. As the middle bin did not express any connectivity related to word or pseudo-word reading, it is unlikely that signal increases in the middle and superficial bins resulted from enhancement. Future work will attempt to address signal contamination problems in the superficial bin for its full inclusion in the depth dependent analysis.

To conclude, our results are direct evidence that left temporal cortex interacts with lOTS during word reading in a top-down manner. This finding best supports an interactive account of reading (**Price and Devlin, 2011**) whereby word reading is distinguished from pseudo-word reading though increased top-down connectivity between left temporal cortex and lOTS. When considered together with reports of early occipital top-down effects (**Chen et al., 2013**, **2015**), the reported results agree with an account of word reading in which lexical retrieval is achieved by interaction between left temporal cortex and lOTS.

5 Outlook

This thesis introduced unprecedented possibilities to extract directionality in brain networks crucial to higher cognitive function. It also demonstrated non-invasive measurements of interaction between brain regions at a spatial scale previously limited to invasive recordings. The benefits of directed connectivity measurements were demonstrated in this thesis in the reading network, but are potentially applicable to the study of inter-regional systems throughout the brain. It is this potential application that most beckons the speculative, though I will also discuss the more immediate research suggested by the findings in this thesis.

5.1 Expansion of depth dependent connectivity methods

The clearest path to future work lies in expanding the whole brain laminar connectivity analysis to explore depth dependent connectivity over multiple regions. Presently, the whole brain analysis was restricted to depth dependency in the seed regions of the gPPI analysis, but not the targets. To expand the analysis, it would be necessary to perform the same methodological procedure on target regions to extract the depth dependent signal, and significance testing of the fully depth dependent gPPI results would need to account for collinearity of the target regions.

The results of the reported whole-brain gPPI analysis could be used to inform predictions for the expanded analysis. As reported in chapter 4, the deep bin of the left occipitotemporal sulcus (lOTS) showed the strongest connectivity with regions in the left middle temporal cortex during word reading. This was interpreted as greater top-down connectivity to the lOTS from left temporal cortex during word reading. Efferent top-down signal has been shown to originate outside of the granular layer (see **Douglas and Martin, 2004**), and it might be expected that the deep bin of the lOTS would therefore show greatest connectivity with either the deep or superficial bin of its left temporal cortex targets, at the exclusion of the middle bin. One potential complication of this analysis is that the BOLD response is thought to relate more directly synaptic input than to the output of cell populations (**Logothetis et al., 2001**). It may be challenging then to establish depth dependent, directed links between regions using BOLD measurements with networks consisting of one input region and one output region. With such a network, the analysis might be biased toward the input layers of the output region.

Further experimentation with depth dependent spatial deconvolution would be of use to better understand the mechanisms underlying the signal localization evident in the whole-brain gPPI results. In discussing these results, it was proposed that the signal localization necessary to identify the depth dependent connectivity profiles might be attributable to a low-pass filtering mechanism implemented through the vasculature. It was proposed that because downstream BOLD effects relate to post-capillary and larger vessels, which introduce differences in vessel length and flow velocity, they acted as a low pass filter relative to the site of activation. By applying spatial deconvolution (**Markuerkiaga et al., 2016**) to the measurements obtained for this analysis, it might be possible to evaluate presence of such a filtering mechanism. Depth dependent spatial deconvolution is intended to account for downstream BOLD effects by modeling the spatial spread of the responses localized to particular cortical layers (see **Markuerkiaga et al., 2016**). From the gPPI results, however, it appears that signal variance arising from downstream BOLD effects was captured by the regressors modeling the main task conditions. If the gPPI model was able to account for the downstream effects because of the proposed vascular filtering mechanism, then it would not be expected that deconvolution would appreciably impact the gPPI results, but it would be expected to unbias the activation profiles.

To expand the applicability of the depth dependent gPPI it is necessary to also address temporal deconvolution, and perhaps ultimately model the spatiotemporal depth dependent BOLD response. Temporal deconvolution in gPPI is necessary to accommodate event-related designs (**O'Reilly et al., 2012**). The whole brain gPPI analysis discussed in this thesis excluded deconvolution of the seed region timecourses because 1) it was not clear how the deconvolution would affect the interpretation of depth dependent responses, and 2) the experiment paradigm was such that it could be analyzed as a block design, minimizing the need to apply deconvolution to address connectivity at the level of the neuronal response (ibid). Most modern fMRI experiments, however, use event-related designs, and it is important to study how temporal deconvolution could be sensibly applied to event-related designs used in depth dependent experiments. The goal of this line of inquiry should ultimately be spatiotemporal models of the depth dependent BOLD response so that downstream effects can be modeled relative to stimulus onset, ideally taking brain region into account. How critical spatial deconvolution is to the depth dependent gPPI analysis is, however, unknown, but accurate spatiotemporal models would be of use for different analysis methods.

5.2 Future work in language

The findings in this thesis indicate that future work can fruitfully incorporate lfMRI methods into the study of language and reading. Two areas of immediate interest are the study of the combinatorial processes which allow for boundless novel utterances; and the further development of priming methodologies. Priming experiments are often used to study language processing and other cognitive phenomena, but priming effects are thought to arise from a diversity of neurobiological phenomena and are not fully understood.

Priming effects are observed when the presentation of one stimulus affects the measurement of how a subsequently presented stimulus is processed (see **Grill-Spector et al., 2006**, for review). This measurement could be of the BOLD signal, the time it takes a participant in an experiment to react to the stimulus, or other measurements of brain activity or behavior. In behavioral studies of language, priming is often observed as decreased reaction time when two similar words are presented in succession (**Wheatley et al., 2005**). In fMRI experiments it has often been observed as a decreased BOLD response in brain regions associated with language, such as the left MTG, although increased responses have also been reported in certain experiments (see **Segaert et al., 2013**). Priming in neuroimaging is often used as a means of identifying which brain regions are most sensitive to specific experimental manipulations and can be used to study linguistic processes in the brain (**Gagnepain et al., 2008**; **Glezer et al., 2009**). To give an example, if syntactic structure is used as the means of priming, it would be expected that the largest effect on the BOLD response would be observed in brain regions most crucial to syntactic processing, as demonstrated in **Weber and Indefrey** (**2009**); **Weber et al.** (**2016**). There are different types of priming, however, and It is currently unclear what mechanisms drive different priming effects. It is possible, for example, that presenting two identical stimuli in succession results in a diminished BOLD response because of reduced input signal driving the region in question, reduced activation in the region independent of the input, or if top-down signal from elsewhere in the brain facilitates the processing of the primed item. Laminar fMRI can potentially investigate different priming effects and the mechanisms underlying suppression or enhancement as discussed in **Segaert et al.** (**2013**). This understanding would enhance the explanatory power of commonly used priming paradigms.

Beyond the exploration of the BOLD dynamics underlying priming phenomena, directed priming can be used as a tool to study combinatorial processes responsible for generating compositional meaning within different levels of linguistic structure, and, ultimately, at the final level of the utterance. Combinatorial processes perfuse language and minimally require the means to store sensory input, identify relevant units within the input and recombine them according to rules unavailable by virtue of the input alone. At the level of phonology, speech sounds are identified as such and combined into meaningful units, meaningful speech sounds are combined into morphemes, morphemes are combined into words, words are sorted according to syntactic rules and semantic knowledge of the known word meanings. When the utterance is fully decoded, a meaning derived not from the words themselves but from their meaning in combination with each other is present in the brain of the listener because of these iterative combinatorial processes.

As discussed in **Grill-Spector et al., 2006**, the repetition of a process generally results in effects whereby a behavioral readout of the brain process becomes faster or the BOLD response to the process is attenuated (**McDonald et al., 2010**). The phenomenon is not limited only to the repetition of syntactic patterns, or of individual words or pictures; it is also possible to evoke priming responses by repetition of conceptual or other information (see **Heyman et al., 2015**). For example, if a person is shown a cat, they might be expected to recognize a dog or a mouse more quickly than a wheelbarrow because of relatedness of the concepts.

As mentioned, priming effects can be used to identify brain regions related to the processing of specific types of information. Left MTG has been discussed at length in this thesis as critical to lexical retrieval and knowledge of word meaning. Support for the role of lMTG in reading can be found also in priming experiments where reading two identical words in succession has been shown to affect the BOLD response in the left MTG when reading the repeated word (see **Segaert et al., 2013**). Similarly, effects in lMTG have been observed after reading semantically similar words, like cat and dog as described earlier (ibid). It is not known, however, what is causing the suppression in either case. It is proposed that repetition suppression relates to activity within or downstream of the target region and may arise from response sharpening (fewer neurons; **Li et al., 1993**), neuronal fatigue (diminished response amplitude; **Miller and Desimone, 1994**) or facilitation effects (reduced response latency; **Sobotka and Ringo, 1996**) (see **Grill-Spector et al., 2006**). Semantic priming in lMTG may draw more heavily on top-down sources, but may also relate to similar bottom-up mechanisms proposed to account for repetition suppression effects (**Matsumoto et al., 2005**). Laminar fMRI is a potential method to examine these priming effects in greater detail.

Developing a better mechanistic model of the priming effects related to semantic knowledge has implications for understanding how semantic knowledge is represented in the brain. Laminar fMRI may be useful in this endeavor by providing the means to resolve the top-down or bottom-up signal components arising from different manipulations. If for example, knowledge of the concepts which amount to ‘cats’ and ‘dogs’ were supported largely by processes within the lMTG, signal reductions should be observed to be distributed over the gray matter volume in lMTG. If instead signal reduction in lMTG was caused primarily by reduced input to the region, the sharpest reduction might be observed in granular cortex. It is also possible that top-down influences could contribute to the suppression, in which case elevated BOLD might be observed in top-down termination sites accompanied by global signal reduction over the region. These results would be informative as to the basic understanding of priming phenomena as well as the organization of semantic knowledge in the brain.

Similarly, laminar fMRI might be used to explore accounts of repetition suppression. This is challenging in humans given that the proposed theories describe phenomena at the neuronal level. Laminar fMRI measurements are based on large populations of neurons, but the neuronal level accounts can generate depth dependent predictions which differ in terms of expected activation patterns. The fatigue (**Miller and Desimone, 1994**) and facilitation (**Sobotka and Ringo, 1996**) accounts might predict a global signal reduction with no depth dependent effect. In this event, the two accounts might be discriminated by empirically determining the hemodynamic response function to determine if the observed suppression was merely the result of a mis-modeled BOLD response for the lower latency, facilitative response. The sharpening account argues that repetition reduces activity in cells extraneous to processing the repeated stimulus (**Li et al., 1993**). This would seem to predict that the strongest suppression would be observed in granular cortex as the two most likely mechanisms for signal reduction are decreased signaling to granular cortex or increased intrinsic suppression mediated by nongranular cortex. Laminar fMRI is unique in providing a direct measure of these basic properties of neuronal computation. Other means of exploring these possibilities would require invasive recordings or models utilizing tuning curves (**Grill-Spector et al., 2006**).

While lexical semantics is critical to the study of language, the larger combinatorial processes are arguably more central. They are also more challenging to measure as they, by nature, rely on iterative processing inclusive of all linguistic units. It has been proposed by **Hagoort** (**2013**) that the left inferior frontal gyrus (lIFG) is critical to the processes which support combining abstract units of meaning according to known rules. Through these processes, words can be arranged according to the rules of syntax, and compositional meaning of a full utterance can be derived, though it is not represented by any individual word in the utterance. Crucially, it is proposed that compositionality would not be possible through processes within the lMTG or lIFG individually, but only through the interaction between lMTG and lIFG.

To test this theory, one could develop an experiment which compared the impact of different types of priming on the BOLD signal. By extracting directed measurements of the interaction of critical brain regions, it may be possible to observe the centrality of individual brain regions in combinatorial processes, and to observe how compositional meaning is represented in the brain. For example, if a word is primed by compositional knowledge, it might be expected that the priming effect would involve the transmission of top-down information from the lIFG to the lMTG. This potential finding would support the critical role of lIFG in compositionality and also the interactive nature of combinatorial processes in language.

It is of note that lIFG is not exclusively dedicated to language processing, and has, for example, been shown to be important to the processing of musical patterns (**Kunert et al., 2015**). This point underscores an important theme in cognitive neuroscience: brain function should be understood in terms of interactive task dependent networks. The study of such networks requires the means to measure the directed transmission of information through them, in addition to simply identifying network topologies. lIFG might be critical to derive compositional meaning in combination with lMTG, but critical to knowledge of musical scales when combined with a different brain region. It is therefore meaningless to consider lIFG independently, and impossible to understand the exact processes involved in a cognitive phenomenon without knowledge of how information is transmitted in functionally dependent, emergent brain networks. It is in this general context that the contributions made in this thesis to develop directed connectivity analysis using laminar fMRI may have the greatest impact.

5.3 Laminar fMRI and neuroanatomy

Increased capabilities and maturity of the laminar method may lead to a reevaluation of the generally unidirectional relationship between fMRI and neuroanatomical research. It is not difficult to find research in the fMRI literature predicated on knowledge of brain anatomy, though there is a scarcity of neuroanatomical work motivated by findings in functional MRI. This is not a fault of fMRI, but it indicates that fMRI is not considered to be well suited to the questions asked by the modern day anatomist. The advances reported in this thesis may well indicate a seachange in this arrangement.

The approach taken to laminar fMRI in this thesis is fundamentally based on assumptions of a depth dependent connectivity regime. By observing distinct top-down and bottom-up pathways, the data from the reading experiment confirmed that laminar anatomy could be used to meaningfully interpret functional data. Knowing this, it may be possible to instead infer knowledge of anatomy by observing depth dependent functional responses.

It is well known that knowledge of anatomy and knowledge of function rarely correspond in obvious ways. lfMRI is distinct from other functional measures, however, as interpretations of top-down and bottom-up contributions can only be made by considering laminar structure. It may therefore be possible to simultaneously explore functional and anatomical connectivity using a functional measure. Brain regions such as the middle temporal gyrus (MTG), discussed at length in this thesis, are particularly interesting in this respect. MTG is proposed to be unique to humans (Zilles, *personal communication*), which, although extremely interesting, inhibits access to animal work on the region. This evolutionary twist has effectively dashed the possibility of exploring connectivity to and within MTG with methods common in animal work. Because invasive methods were, to date, required to measure top-down and bottom-up contributions to MTG at anatomically useful spatial scales, research involving directed measurements in MTG has often been carried out in small populations undergoing surgical interventions. Laminar fMRI could, in a sense, democratize access to directed measurements in brain regions such as the MTG.

A second area for potential collaboration follows from a recent work by **Beul et al.** (**2017**). **Beul et al.** (**2017**) show that the likelihood of interareal connections between two regions can be predicted by comparing the cytoarchitechtonic similarity of the regions. The authors show additionally that brain regions with reduced neuronal density and cytoarchitechtonic complexity have a higher number of connections to other brain regions. **Barbas et al.** (**2018**) discuss this property in the context of working memory, and illustrate the tendency of top-down signal to originate in comparatively less specialized cortical regions. Thus, brain networks were shown to exhibit the property that more structurally complex regions were shown to be hierarchically inferior to less complex regions. By combining the complexity measures of **Beul et al.** (**2017**) with knowledge of cortical layers and depth dependent functional measures, it may be possible to explore the functional relevance of complexity based cortical hierarchy and derive new methods inclusive of these measures. Also, given the incipient challenge of applying the laminar method to association cortex without clear sensory input, additional and independent assessments of network hierarchy are welcome.

To return to the warning offered in Chapter 1, cognitive notions of top-down and bottom-up do not map clearly onto the homonymic anatomical terms. It is worth unpacking this warning as it can be used both to understand the past and to guide future work.

Evolutionarily older brain regions are generally better conserved across species than newer ones. Perhaps for this reason alone, the vast majority of work in animals has been performed in primary visual cortex. Cat visual cortex, in particular, is perhaps one of the most thoroughly studied brain regions to date (**Douglas et al., 1989**; **Douglas and Martin, 2007**). For reasons that are likely related to evolution, older brain regions, like primary visual cortex, form fairly well defined hierarchies with other brain regions, whereby visual input proceeds from more primitive to more recent areas.

Cognitive neuroscience, and visual neuroscience, in specific, has extensively researched how visual information is processed by the brain, and how what is eventually perceived relates to the interaction of different brain regions and to top-down and bottom-up information (see **Teufel and Nanay, 2017**). In the visual context, ’bottom-up’ often relates to bottom-up sensory input, which aligns with the anatomical interpretation. In association cortex, however, there are often no clear bottom-up or top-down regions from an anatomical perspective. Regions furthermore are associated with a diversity of functions and express highly variable behavior in response to different experimental tasks (see **Sporns, 2013**). It will therefore be challenging to effectively manipulate bottom-up signal directed to a region when the bottom-up signal is non-sensory and its source is unknown. Absent the transmission of sensory information, it is unclear how a bottom-up region should be identified.

With careful experimentation, it is possible that laminar fMRI might lead to a better understanding of how the structure of associative brain regions supports higher cognition. These findings would stand to contribute both to functional and anatomical knowledge of the brain regions under study, and perhaps accelerate the convergence of cognitive science and neurobiology. It would be interesting to investigate, for example, if brain regions in higher cortex can accommodate multiple configurations. Can the hierarchical relationship between two regions be reversed? If so, could the different connectivity profiles be observed as a corresponding reversal of the depth dependent connectivity between the two regions? Such a finding would represent an enormous achievement for neuroscience.

5.4 The functional role of 'top-down' connections

An interesting property of isocortical histology is the bifurcation of top-down terminations to either deep or superficial cortex (see **Shipp, 2016**). Notably, this does not occur within individual afferent neurons. Individual neurons will synapse on deep or superficial neurons, but not both (ibid). It is possible then that the deep and superficial pathways support different functions.

It may be possible to use lfMRI to explore differences in the functionality of deep and superficial top-down signal. **Shipp** (**2016**) has proposed that the superficial pathway may mediate attention whereas the deep pathway may relate to predictions arising from past experience. An experiment might attempt to dissociate multiple top-down information sources in the same region and determine if there is an observed preference certain phenomena to evoke BOLD responses in the deep or superficial bins. To compare top-down effects related to word knowledge with top-down effects related to attention, the simple addition of an attention cue to the paradigm used in this thesis might be sufficient to dissociate these effects. Knowledge of the functional role of the divergent top-down targets is not easily accessible from the study of anatomy alone, and it is not clear whether investigations of the functional consequences of top-down and bottom-up signal streams readily translate from animals to humans (**Vinken et al., 2016**). Laminar fMRI is thus uniquely suited to examine the functional consequences of the distinct top-down pathways.

5.5 Conclusion

Laminar fMRI has many potential avenues of contribution to a number of research fields. It is shown in this thesis that the laminar method can be used to identify the directed structure of networked regions distributed over the brain. Although informative in its own right, It is difficult to not train one's attention on the future work all but demanded by the observations reported in this thesis. I am hopeful that a substantial increase in the knowledge of directed brain networks and a deeper understanding of the relationship between structure and brain function will follow from the work in this thesis.

I have discussed a number of potential research directions that could benefit from the unique capabilities of lfMRI, but the greatest potential for discovery lies in expanding the accessibility of the laminar method. With further development of the analysis techniques described in this thesis, depth dependent connectivity may become a standard analysis method. If so, the outlook for laminar fMRI research will be enriched by the imagination of anyone with interest and with access to an MRI scanner.

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6 Summary

This thesis describes research into laminar specific functional magnetic resonance imaging (lfMRI), and details the development and adaptation of analysis tools which use lfMRI measurements to extract previously inaccessible information from the laminar resolution blood oxygen level dependent (BOLD) signal. This information relates to distinctive functional properties of specific depth-compartments as well as to directed connectivity patterns between specific depth-compartments spanning the multiple distal brain regions.

The mammalian isocortex is comprised of at most six cortical cell layers which have been shown to relate to brain function in systematic ways. It is thought that functional MR contrasts, such as give rise to the BOLD signal, are sensitive to layer specific signal differences, in turn encoding a signature trace of the synaptic activity specific to these cell layers. This is of interest because brain activity in different layers is thought to arise from distinctive layer-dependent connectivity patterns with distal brain regions. One of the principal motivations for the work in this thesis was to assess whether layer-dependent measurements might be used to identify layer-dependent networks during language processing, and demonstrate the directionality of information flow through these networks based on these layer-dependent patterns.

This undertaking was not without its share of hurdles. It was necessary to overcome a multitude of challenges related to image registration and validation, and to create the tools needed to perform task-based connectivity analysis at the laminar level.

Numerous brain regions – described within – are known to be involved in reading. The exact configuration of these regions is contentious, however. The description of how linguistic information is represented and processed throughout these regions is controversial, and a complete neurobiological theory of reading hinges on understanding how the visual information encoding the words we read relates to our knowledge of these words. In this thesis, it is specifically shown that information related to word meaning is communicated backward to a lower order brain region which is not critical for knowledge of word meaning. This was shown by examining the connectivity patterns uniquely related to different cortical depths and relied wholly on noninvasive methods. This finding is evidence for the importance of feedback connections to earlier visual areas during word reading, and also evidence that unique information is contained in the depth-dependent BOLD signal.

As a result of this work, there is now a foundation to perform similar investigations in the future, and it is clear that research based on laminar-specific connectivity can be informative as to the nature of brain networks.

7 Nederlandse samenvatting

Dit proefschrift beschrijft onderzoek naar laminaire functionele kernspintomografie (laminar functional magnetic resonance imaging, lfMRI). Specifiek gaat het over de ontwikkeling en aanpassing van analysetools, waarbij lfMRI-metingen gebruikt worden om informatie die hiervoor niet toegankelijk was, te extraheren uit het laminaire BOLD-signaal. Deze informatie heeft betrekking op onderscheidende functionele eigenschappen van specifieke diepte-compartimenten en op gerichte connectiviteitspatronen tussen specifieke diepte-compartimenten die de verschillende distale hersengebieden omspannen.

De isocortex van zoogdieren bestaat uit hoogstens zes corticale cellagen waarvan is aangetoond dat ze op systematische manieren verband houden met de hersenfunctie. Er wordt gedacht dat functionele MR-contrasten, zoals het BOLD-signaal, gevoelig zijn voor laag-specifieke signaalverschillen, die op hun beurt coderen voor een kenmerkend spoor van de synaptische activiteit die specifiek is voor deze cellagen. Dit is van belang omdat gedacht wordt dat hersenactiviteit in verschillende lagen voortkomt uit verschillende laag-afhankelijke verbindingspatronen met distale hersengebieden. Een belangrijke motivatie voor het werk in dit proefschrift was om te beoordelen of laag-afhankelijke metingen kunnen worden gebruikt om laag-afhankelijke netwerken te identificeren tijdens de taalverwerking, en om de richting van de informatiestroom door die netwerken aan te tonen op basis van laag-afhankelijke patronen.

Deze exercitie was niet zonder hindernissen. Onderdeel van dit onderzoek was het overwinnen van een groot aantal uitdagingen met betrekking tot beeldregistratie en -validatie, en het creëren van tools die nodig zijn om taakgebaseerde connectiviteitsanalyse op laminair niveau uit te voeren.

Van verschillende hersengebieden - die in dit proefschrift zijn beschreven - is bekend dat ze bij het lezen betrokken zijn. De exacte configuratie van deze gebieden is echter omstreden: er is discussie over de beschrijving van hoe taalkundige informatie in deze regio's wordt weergegeven en verwerkt. Om een complete neurobiologische theorie van lezen op te kunnen stellen, moet eerst begrepen worden hoe de visuele informatie die gelezen woorden codeert, verband houdt met onze kennis van deze woorden.

In dit proefschrift werd specifiek aangetoond dat informatie gerelateerd aan woordbetekenis achterwaarts wordt gecommuniceerd naar een hersengebied van lagere orde dat niet kritisch is voor kennis van woordbetekenis. Dit werd aangetoond door het onderzoeken van de connectiviteitspatronen die specifiek gerelateerd zijn aan verschillende corticale diepten en volledig afhankelijk zijn van niet-invasieve methoden. Deze bevinding is bewijs voor het belang van feedbackverbindingen met eerdere visuele gebieden tijdens het lezen van woorden, en ook bewijs dat unieke informatie is vervat in het diepte-afhankelijke BOLD-signaal.

Resultaat van dit werk is een fundament om in de toekomst soortgelijke onderzoeken uit te voeren. Ook is door dit werk duidelijk geworden dat onderzoek op basis van laminair-specifieke connectiviteit inzicht kan geven in de aard van hersennetwerken.

8 Biography

Daniel Sharoh was born in New Haven, Connecticut on October 8th, 1987. This event was followed some days later by the largest single day loss in the history of the Dow Jones Industrial Average.

He attended Live Oaks Elementary School, which was a ten-minute walk from his childhood home in Milford, Connecticut (small city with a big heart). His mother delighted in dressing him in propellered hats and suspenders, and his father radiated silence when faced with this indulgence. At the age of nine, he was permitted to take this ten-minute walk unsupervised, a decision of questionable wisdom. His brother, three years his junior, or two, depending on the time of year and which of them was asked, did not have to wear these propellored hats, an enduring mystery.

Later, and perhaps more consequentially, he finished high school and embarked on an ill-fated stint in Boston as a volunteer worker. This was followed by a better-fated cycling tour spanning from Greece to Belgium, which convincingly established, to his own satisfaction, that the Netherlands was well adapted to the needs of cyclists.

For the next several years there were cycles of adventure and university, always in new places with respect to both. After graduating from a university (Southern Connecticut State) in 2009, Daniel no longer needed to attend classes or drive to campus every morning. His degree was in Anthropology with a focus on language.

With this degree, he began an improbable tenure as a research assistant in New Haven. This work concerned programming fMRI analysis pipelines, collecting EEG and fMRI measurements, data analysis and assorted lab klusjes. This improbable job was the improbable link to his new home in the Netherlands and his new life at the Donders Centre.

After five years in the RA position, he met a woman name Merel van Goch who was a visiting Fulbright fellow. They became quite close, and she informed, or reminded, him that there was a world over the ocean which merited exploration. Overcome with its sagacity, he took this advice to heart and applied for what he would later come to learn was an "ambitious" PhD project. Everything worked out fine, and he continues on today as a postdoctoral fellow with Peter Hagoort investigating laminar connectivity and language.

9 Acknowledgments

Individual achievement is only the lack of attribution.
Please note that all mispellings are intensional and all omissions accidental.
Netherlands

Peter and David, thank you for your collective faith in me and for your interest in this work. It has been clear to me, in my time here, that you have both done well to support an excellent scientific community for people like me to learn and to explore ideas. Perhaps owing in part to your own abundance of vision, there is no place in the world like the Donders, and I am grateful to both of you for inviting me to experience it. I will stop writing now before I earn corrections on this acknowledgment.
Kirsten, there is a latency separating the times you've learned me the lessons peppering the Donders years, and my realizing that I might finally understand both these lessons and their source. I wonder what epiphanies you've already left.
Katrien, our walks in the forest always reminded me of a more ancient tradition of scholarship.
Tim, thank you for your generosity with your time and knowledge. When I first moved here, I was surprised to learn how eager you were to just chat without expecting anything in return. I was happier still to learn that this wasn't a first impression, but a characteristic of yours. It goes without saying (and with saying) that you have been a force behind this work and behind many of the things I've learned along the way.
Tildie, you have been instrumental to my good luck here. There may well be a religion based on you one day, and the perceptually gifted might even sniff out its beginnings in our own time.
Daniel, I knew you were one of the smart ones early on. It's not possible to be as naturally unpretentious and as fearlessly curious as you are without being one of the smart ones. In the scheme of things though, it isn't being one of the smart ones that is truly important – thank Christ – but what matters is making every space that you're in better for having you in it, and the people you know happier for having met you. We're both fortunate that you understand this too. Please enjoy this opportunity to repay me for whatever traumas I inflicted on you in September. (Paranymph, for posterity.)
Dario, I thought I had sworn off my own kind until there you were. And besides, there is no wisdom in running away from your feelings. You of all people should understand this variety of inevitability because you are peerless in matters of

honesty and realism. But these qualities, however charming, aren't why you are in my Pantheon of Friendship, and you're definitely not there only so that I can have someone who I can outbench. You are as solid as they come, fresh eyes for any problem, and a pleasure to be around, and, it doesn't hurt that you're not one to leave me at the table by myself. Thanks for all of this, for being an essential part of today, and if you see a tip drill, point her out. (Paranymph, for posterity.)

Abele, nothing on cognitive scales is truly random, and honest stochasticity, if it's even out there, can't be measured by intentional beasts.

Cecilia, maybe my first actual and probably the longest lasting friendship I've enjoyed since coming here. I don't see you enough, and when I do it's not for long enough.

Ashley, you are my bridge between the old and new Donders, my living reminder of a time when the center was smaller and the friendships were closer. I'm glad you decided to come back before we all expand beyond the particle horizon.

Bart, I use your tin-foil-to-clean-the-grill trick every time I grill. Also, I'm still finding glass all over my patio from you.

Vitória and Kris, Do you know how hard it is to find another couple you can spend a week in the middle of nowhere with without it ever becoming awkward? With your love of lawn games, disdain for socializing outside of home environments, and hopeless mixing of research with pleasure, I have confirmed what I knew even before I moved here: you guys are absolutely worth knowing.

Lauren Bains of my existence, You inspire me to be a more selfless, adventurous and generous person. Our long talks on the autobahn left a mark, and when I think about the person I want to become, that person is a little more like you.

Irati, you crack me up. The world is never so small as when a new colleague in Nijmegen mediates a single degree of separation between yourself and childhood.

Marcel, you are a trouble maker and a lot of fun to argue with.

Flora, hey it's my turn now!

Laura, give me a call when you want to start a traveling accordion group.

Adrian and Sofi, when I see you guys there's nothing but fun times and good energy. You're kind and generous people, and I'm happy to know you. Maybe sometimes I spill an entire bottle of Cachaça into your sofa, but generally, things go alright.

Giel, I am pumped about my first real carnaval in Limburg. I hope there are no catastrofen. By the way, you were right about the print shop.

Gwilym and Jenny, my first and most cherished house guests, thank you for repaying my hospitality with stomach flu. I look forward to virulently honoring your non-wedding.

Mao, Lieke, Joey, Jasper, Sophie, I've been with some of you longer than others, but I have enjoyed all of your company immensely over the years. Thank you for the laughs, good vibes, and thought provoking discussions.

Tom and Iske, before you even knew me you helped get me into shape with mock interviews, and then, helped me turn a bunch of my crazy formless ideas into useful ones. Tom was my first ever pity question (PPM) in Nijmegen! I hope to see you both much sooner than the last time.

Christienne, because of your generous dressings-down I am now aware that listening is actually a part of conversation, and that a story isn't always told in search of advice. Sometimes, probably most of the time even, people just want to be heard and felt like they are understood.

Jill, you've got enough life in you for all of us. I think I'll be able to find you in your Mediterranean dream-world sooner than even you might think.

Ken, two people who are flakes never hang out enough.

Renaud, it's wonderful to meet hippies who like billiards, darts, chess and pastis.

René, we should stay motivated. I've heard of a great big laminar project in the sky, in the world of never ending positive results. You can always find low p values there – day, or night.

René, the way you play that guitar so smooth, you've got no business in Science. It's a colossal waste of your talent. Get a real job in a rock band.

Hartmut, it is almost enjoyable to listen to you grumble about the state of things, but it is definitely enjoyable to hang out with you while you do it. Maybe we will actually make it to the Doornroosje one day.

Emma, my mysterious friend and bathrobe lady. It's always a nice surprise to find myself sitting across from you at whatever work event I forced myself to go to for social reasons.

Lenno, if you've learned even a portion of what I've learned from you, then our project was well worth all the trouble. I couldn't be prouder of your success.

Armin, Qapla' loDnI'! Wo' batlhvaD heghlu'meH QaQ jajvam.

Fabian, you are the only German I have ever known who has been late to something – birding of all things!! I was terribly disappointed to see you leave when you did, but such is the life of nomads. You were one of my first friends in Nijmegen, and a delight to spend time with, even in a meeting environment.

Laura and Andrea, there probably isn't one of us who expected to be here today, and who isn't surprised to find us here after, but these are good days, and I'm glad you're in them.

To my group, current and former, and specifically Sophie, Ksenija, Valeria, Kris, Richard, Julia, Marvin, Joost, Anne, Alexis and others, I have found our chats and interactions to be extremely useful for both content and motivation. You are without a doubt memorable and irreplaceable characters. Thank you from the bottom of my heart.

My Language in Interaction community, with special emphasis on my PhD group, specifically Linda, James, Joe, Merel, Iris and Arnold, I am lucky to have been able to meet and learn from all of you. Thank you for your friendly discussion and new perspectives.

Pascal, this was written on the old Mac you donated. I don't know where you've gone off to, but you should come back. You're a lot of fun.

Joanne and Marten, the two of you are examples of people to be. Marten has to be the most empathetic skull-cracking former bartender I know, and Joanne radiates a Dalai lama-like aura! Plus, the two of you like a good time, which never hurts. I feel lucky to know you both.

Dorine and Sid, you guys are great. Sid's got to be the nicest sarcastic bastard out there, and bumping into Dorine out there in wild Nijmegen always makes my night.

Marek, Mike, Ed, Hong, Try to keep a position in the TG open for me, and keep quiet about TG related PhD jobs. If everyone knew how **REDACTED**, nothing would get done around the center, and we'd all be screwed.

Mijn schoonouders, ik vind jullie zo grappig. Als ik praat met iemand over mijn Nederlands en hoe ik heb het geleerd, zeg ik, mijn schoonouders. Ik kan over PSV en formule 1 praten! Ik ben zo blij met mijn fijne sokken, misschien wel 10 paar, en mijn mooie collectie handgemaakte kaarten. Mijn voeten zijn lekker warm.

Jan en Josien, zonder jullie, ik zou nergens geschreven hebben! Geen bureau, geen tafel, niks.

Macky, I write with complete certainty that no one else has ever written their thesis using a Jacquard loom. Thank you.

Merel, I vacillate among different versions of the exact same story. In my story, you are equally likely to have saved me, or maybe inspired me instead. It's just as likely that I moved here for love or because you suggested it in the first place, but it's all the same story. I am here because I met you and because I knew that not coming here would have been very, very stupid. So stupid, that I might as well have been the kind of person who walks out of his house with his pants on backward. Exactly how I was coming back with you was just details, but those worked out nicely too. Sometimes, I talk with people who have relocated here like I did, and I am surprised to hear that it's even possible for them to feel isolated or like they're missing something. It's because I have you though, that's why I've passed through all of this trauma without interacting with it, without even being aware of it, like a neutrino!! In concrete terms, this thesis wouldn't have happened without you. More importantly to me, it's impossible that the rest of the insensible changes that have come to shape my world through the passage of 2014 into 2020 would have happened at all without you. When I look back on how different my life is now from then, I can only marvel at your central and irreplaceable hand in all of it.

United States

My mother, you are a happy ray of sunshine, and you have taught me to avoid haircuts. From the money that I've saved from avoiding haircuts, I should have put a special fund together for you and bought you a cottage here so that you could have come to live in the Netherlands too. I miss you all the time, and if there is something nasty to say about my new home, it's that you aren't here.
My father, I attribute to you my fascination with electricity and its unintended impact on the body. Because of you, I have learned never to shy away from unsafe working conditions, unstable ladders, frayed wires or large drops. I have learned that manufacturers always include extra parts, but for some reason hide them behind the onerous steps of disassembly and reassembly. I have learned that cracks in the windshield do indeed go away eventually, if you just wait long enough; it is important to wipe down the walls in the shower after use; turning up the thermostat while the heat is already on does not make it warmer in the sense that people think it does. Finally, I understand now that a merry Christmas can be most effectively measured in kilowatt hours.
Grandma, you're a funny lady with a dark sense of humor and deep affection for caffeine. I love you dearly, and not a day goes by that I don't think of you. Sometimes, I make a nice big pot of coffee, put on my Dean Martin records and pretend that we're sitting together doing nothing in particular.
Poppy, thank you for all the sling shots and fire making devices. I miss you.
Jayne, can't wait to see you later in the year.
Nicholas, easily my favorite brother. You are a pleasure to be around when carving a path through Hell, but then also on the rare and sunny blood-free days. You should know that no one else wants to play Bushido Wiffle Ball ^{TM} with me. I remain sorry that I made fun of you for your fire building tactics.

They paid dividends in both amount of fire present and servings of crow eaten by me. Looking back, in fact, I see a steady increase in servings of crow and humble pie in my diet.

Liz, there is a remarkable depth to you that comes clearer into focus whenever we talk. I'll catch you on the air at the Italian House Party this Sunday.

Shawn, Ryan, Nina and Wayne, don't worry, I will make it a point to come see you in the Summer. Ryan, maybe you can put in **now** for the time off?

Miss Lynn, you've been a family member now for years, and a wonderful addition! I love when you're around because you don't take nothing from no one, and you're not shy about saying so. Sometimes I wish there wasn't so much medical knowledge present at the dinner table, but it is a small price that I am willing to pay.

Gayle Emerling, Bob Emerling, you will have a spot at table two, but feel free to come over to the dais. It may have been because you were so sick of your boys' other friends, but you have always welcomed me into your home. Even when you have been angry with me! And Bob too, he's yelled at me before... Irrespective of the volume of a given moment, I've always known that I was welcome and even encouraged to share your home and your table. Everyone deserves a family, but because of the Emerlings, I have two of them.

Jared, Mr. Moxy, humor is the most powerful form of communication there is. I can't examine anything without understanding it through humor in some respect. And without you, I wouldn't see nearly as much humor in the world. I'm not trying to be hokey about it, but that's the truth. Now, I don't care where you go, that's the dullest acknowledgment you're ever going to read for the rest of your life.

Rook, I know you're out there.

Charli, from our first strange and long talk during my still stranger time in Brighton, I knew I had found someone just as explosive and out there as I was. There is no one I'd rather smuggle in and out of my house or whose car I'd rather sleep in.

Pete Cunningham, I find that you are usually right about most things and still somehow nice to talk to. How you accomplish this, and that you do at all, is at the core of your charm.

Eben, I recoil the strongest from myself when I think of the times you've pointed out to me that I should be embarrassed, which I do appreciate. I have nicer memories though of what I still think of as the adventures of that age, and of the way you smashed my world wide open.

Charlie, you can climb in through my hotel window any time you like.

Monica, I can’t believe you’re a square now. Don’t you remember when you picked me up all decked out in a bloody apron for a big night out? You’re in the same small group that Eben is in, filled with the people who stole me from the nest.

Katie, you’re a force of nature, and knowing you has been a singular experience. I can’t look out into cold days without thinking of you.

Magnus, stroll through the candle aisle on your next shopping trip, I might just be there waiting for you.

Sean-man, I think about what you’re up to nowadays, and I’m thrilled that you somehow managed to get paid to do what you love. When I think back on all the dumb stuff we used to do, I think it turns out that it might have been dumb, but not *stupid*. How do you imagine we would have turned out if we didn’t waste all that time together? I don’t think we’d be as smart as we are today if we weren’t so dumb back then.

Ian, even though you’ve never said it, I know that you love me too. I think about you every time I put on my L.L. Bean boots. Maine is a weird town, and you’re pretty weird too. But I’m also weird, and I don’t think I would have had half the fun I did if I hadn’t met you in that glorified paper mill that passes for a university up there in the void.

Einar, my time with you continues to mark one of the most pleasant working relationships I’ve ever had. Apart from this, I never would have guessed that you were quietly and effectively laying the foundation for what has grown into my love of fusion back in the MRI cave some years ago.

Rob, I shake my head in disbelief and laugh when I look back on our adventures. You’ve been a fantastic friend and really just a lot of fun to be around, talk with, or even just to play chess over skype with or through the mail (while it lasted). These later activities may seem a little more tame in comparison to some of our earlier shenanigans, but you can’t **always** leave your foot on the gas, I guess!

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Jaco Pastorius, Robert Smith, Prince, Thelonious Monk, Eric Dolphy, and Charles Mingus, according to my accounting, you lot have contributed by far the most to my writing sessions. Thank you for your support.

Those who do not read this book or this section, please know that you are well represented here.

-29A, the hexadecimal of the Beast.

Donders Graduate School for Cognitive Neuroscience

For a successful research Institute, it is vital to train the next generation of young scientists. To achieve this goal, the Donders Institute for Brain, Cognition and Behaviour established the Donders Graduate School for Cognitive Neuroscience (DGCN), which was officially recognized as a national graduate school in 2009. The Graduate School covers training at both Master's and PhD level and provides an excellent educational context fully aligned with the research program of the Donders Institute. The school successfully attracts highly talented national and international students in biology, physics, psycholinguistics, psychology, behavioral science, medicine and related disciplines. Selective admission and assessment centers guarantee the enrollment of the best and most motivated students. The DGCN tracks the career of PhD graduates carefully. More than 50 percent of PhD alumni show a continuation in academia with postdoc positions at top institutes worldwide, e.g. Stanford University, University of Oxford, University of Cambridge, UCL London, MPI Leipzig, Hanyang University in South Korea, NTNU Norway, University of Illinois, North Western University, Northeastern University in Boston, ETH Zürich, University of Vienna etc.. Positions outside academia spread among the following sectors: specialists in a medical environment, mainly in genetics, geriatrics, psychiatry and neurology. Specialists in a psychological environment, e.g. as specialist in neuropsychology, psychological diagnostics or therapy. Positions in higher education as coordinators or lecturers. A smaller percentage enters business as research consultants, analysts or head of research and development. Fewer graduates stay in a research environment as lab coordinators, technical support or policy advisers. Upcoming possibilities are positions in the IT sector and management position in pharmaceutical industry. In general, the PhD graduates almost invariably continue with high-quality positions that play an important role in our knowledge economy. For more information on the DGCN as well as past and upcoming defenses please visit: http://www.ru.nl/donders/graduate-school/phd/