Multimodal connectivity of the human basal forebrain

Sudesna Chakraborty, The University of Western Ontario

Supervisor: Khan, Ali R., The University of Western Ontario
: Schmitz, Taylor W., The University of Western Ontario
A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Neuroscience
© Sudesna Chakraborty 2023

Follow this and additional works at: https://ir.lib.uwo.ca/etd

Recommended Citation
https://ir.lib.uwo.ca/etd/9476

This Dissertation/Thesis is brought to you for free and open access by Scholarship@Western. It has been accepted for inclusion in Electronic Thesis and Dissertation Repository by an authorized administrator of Scholarship@Western. For more information, please contact wlswadmin@uwo.ca.
Abstract

The cholinergic innervation of the cortex originates almost entirely from populations of neurons in the basal forebrain (BF). This cholinergic signaling plays a crucial role in cognitive processing and failure of the circuitry causes cognitive impairment. Structurally, the ascending BF cholinergic projections are highly branched, with individual cells targeting multiple different cortical regions. However, much of our knowledge about cholinergic projection is based on non-human animal studies, and it is unclear how the BF cholinergic neurons are organized in the human brain and how it is related to their functional and structural integration with the cortex.

We used high-resolution 7 Tesla diffusion and resting state functional MRI in humans to examine multimodal forebrain cholinergic connectivity with the neocortex. First, discrete parcellation analysis was employed to examine if structural and functional data-driven approach can recapitulate the known nuclear subdivisions based on previous studies. Similar to the topography observed in mice, both structural and functional parcellation broadly differentiated the anteromedial from posterolateral nuclei of BF. Next, we used gradient estimation to capture a more fine-grained connectivity profile of the BF-cortical projectome. Similar topographical organization of BF cholinergic projection was observed, however, moving from anteromedial to posterolateral BF, structural and functional gradients became progressively detethered, with the most pronounced dissimilarity localized in the nucleus basalis of Meynert (NbM).

Structure-function tethering was shaped in part by the distance of cortical parcels from the BF and their myelin content. Functional but not structural connectivity with the BF grew stronger at shorter geodesic distances, with weakly myelinated transmodal cortical areas most strongly expressing this divergence. Additionally, an in vivo cell type-specific marker of the presynaptic cholinergic nerve terminals, $^{[18]F}$ FEOBV PET imaging was used to demonstrate that these transmodal cortical areas are also among the most densely innervated by its cholinergic projections. Altogether, multimodal gradients of BF connectivity revealed inhomogeneity in structure-function tethering which becomes most pronounced in the transition from anteromedial to posterolateral BF. Cortical cholinergic projections emanating from the NbM in particular exhibiting a broad repertoire of connections with key transmodal cortical areas associated with the ventral attention network.
Finally, the intrinsic BF cholinergic connectivity map of cortex created from these results was compared with meta-analytic connectivity map of cholinergic modulation on attention. The results demonstrate that patterns of brain activity evoked by directed attention are altered by pharmacological activation of acetylcholine (ACh) compared to placebo and these patterns spatially overlap with the intrinsic BF cholinergic connectivity map. This study is the first to examine human BF connectivity using both structural and functional MRI in combination with molecular imaging. Our findings imply that the BF provides cholinergic innervation to the cortex in a topography characterized by branch complexity. The most highly branched cholinergic neurons may originate from the nucleus basalis of Meynert and innervate hubs of the ventral attention network—consistent with the role of these areas in orienting attentional resources throughout the brain.

**Keywords:**
Basal Forebrain, Connectivity, Clustering, Gradient estimation, Human, Neuroimaging, Meta-analysis
Summary for Lay Audience

Cholinergic signaling is an essential process for how our brain thinks and processes information. If this signaling doesn't work properly, it can lead to cognitive issues. Cholinergic connections in the brain mostly come from specific neurons in a region called the basal forebrain (BF). However, we don't fully understand how these neurons are organized in the human brain and how they interact with the other areas of the brain.

To study this, we used high-resolution MRI images to examine the structural and functional connections of cholinergic BF with other areas, neocortex (the outer layer) of human brains. We discovered that the organization of these connections is similar to what is observed in mice, and monkeys and there are distinct differences between subregions of the BF. We found that the organization of the cholinergic projections can be broadly differentiated between anteromedial and posterolateral nuclei of the BF.

We used a technique called "gradients" to study these connections in more detail. As we moved from one part, anteromedial of the BF to posterolateral, we found that the structural and functional connection differences became more noticeable, especially in a specific subregion called nucleus basalis of Meynert. On the neocortex side, this dissimilarity in connection was most evident in the area called transmodal cortex, which is closely associated with the ventral attention network. Also, areas of the brain closer to the BF that receive dense cholinergic connections showed higher levels of divergence.

Additionally, we conducted a meta-analysis to study the relationship with the cognitive process of attention. We discovered that when acetylcholine (the neurotransmitter released by BF) is activated by certain drugs, it leads to diverse but functionally integrated patterns of brain activation that overlap with the ventral attention network.

In conclusion, this research sheds new light on how cholinergic signaling is organized in the human brain. It will be valuable for researchers studying the brain's connections and could potentially help in developing imaging-based methods for early detection of neurodegenerative diseases such as Alzheimer's disease before cognitive decline begins.
Co-Authorship Statement

Chapter 1:
S. Chakraborty – sole author
R. A.M. Haast, A.R. Khan, & T.W. Schmitz – reviewed the content and provided feedback

Chapter 2:
S. Chakraborty – designed research, preprocessed and analyzed data, wrote manuscript
R. A.M. Haast – preprocessing pipeline development, reviewed manuscript
A.R. Khan – designed research, preprocessing pipeline development, reviewed manuscript
T.W. Schmitz – designed research, reviewed manuscript

Chapter 3:
S. Chakraborty – designed research, preprocessed and analyzed data, wrote manuscript
R. A.M. Haast – preprocessing pipeline development, analyzed data, reviewed manuscript
P. Kanel – preprocessing of PET image, reviewed manuscript
A.R. Khan – designed research, preprocessing pipeline development, reviewed manuscript
T.W. Schmitz – designed research, reviewed manuscript

Chapter 4:
S. Chakraborty – analyzed data, wrote manuscript
S.K. Lee – literature review, methodology
S. Arnold – literature review, methodology
R. A.M. Haast & A.R. Khan – reviewed manuscript
T.W. Schmitz – designed research, wrote manuscript

Chapter 5:
S. Chakraborty – sole author
R. A.M. Haast, A.R. Khan, & T.W. Schmitz – reviewed the content and provided feedback
Acknowledgments

First, I would like to express my gratitude to Dr. Ali Khan for providing me with the opportunity to pursue PhD in his lab. Under your guidance, I have not only learned how to code, but also gained a deep appreciation for its usefulness and importance in scientific research. Your insightful feedback on methodology has been invaluable throughout my research journey. I am truly grateful for the six years of supervision and support you have provided me with. Thank you, Ali.

I would also like to express my deep appreciation to Dr. Taylor Schmitz for introducing me to the fascinating field of basal forebrain research. Your passion and perseverance have been truly inspiring throughout the years. Moreover, thank you for bringing a human element to our work - your empathy and concern, always asking me how I'm doing in every one-on-one meeting, definitely made my days better. Thank you, Taylor, for everything.

Special thanks to Dr. Roy Haast for generously sharing your time and offering countless valuable pieces of advice on both the general topic and specific analysis, and coding. Your detailed and constructive feedback on my writing has been invaluable. I would also like to extend my gratitude to Dr. Stefan Köhler. Thank you for your valuable guidance and for supporting my first two years in the PhD program. A big thank you to all former and present members of the Khan, Schmitz and Köhler labs for being exceptional friends and colleagues; special thanks to Dr. Haopei Yang and Dr. Loxlan Kasa for their friendship.

I also want to express my sincere gratitude to my friends outside of work. Thank you, Kristy, for providing a place to exercise, relax, and improve my physical and mental health. I have met some truly amazing people in your studio, and I want to give a special thanks to all my Pilates friends, especially Carolyn, for being an incredible role model and always inspiring me, and Kerry, for brightening my toughest days with laughter. I cannot think of London without you all!

Lastly, I want to express my deep gratitude to my family for their unwavering support throughout this journey. I owe a great deal to my mother, without whom I would not have chosen this path, and to my father, who has always been the best teacher for any math question I had. I also want to thank my smart (no-longer-little) brother, Sujoy, for always cheering me up and being there to listen whenever needed.
Table of Contents

Abstract.................................................................................................................................................. ii
Summary for Lay Audience.................................................................................................................. iv
Co-Authorship Statement..................................................................................................................... v
Acknowledgments.................................................................................................................................. vi
Table of Contents................................................................................................................................. vii
List of Tables.......................................................................................................................................... ix
List of Figures.......................................................................................................................................... x
List of Abbreviations............................................................................................................................. xii
Chapter 1.............................................................................................................................................. 1
  1. Introduction...................................................................................................................................... 1
    1.1 Motivation..................................................................................................................................... 1
    1.2 Basal forebrain function and anatomical organization................................................................. 2
    1.3 Neuroimaging of basal forebrain.................................................................................................. 9
    1.4 Connectivity-based parcellation................................................................................................. 16
    1.5 Goals of this thesis and overview of projects.............................................................................. 20
    1.6 References................................................................................................................................... 22
Chapter 2............................................................................................................................................. 27
  2 Connectivity based discrete parcellation of the human basal forebrain................................. 27
    2.1 Goals of this chapter...................................................................................................................... 27
    2.2 Introduction.................................................................................................................................. 27
    2.3 Materials and Methods............................................................................................................... 31
    2.4 Results.......................................................................................................................................... 36
    2.5 Discussion..................................................................................................................................... 43
    2.7 Reference....................................................................................................................................... 46
Chapter 3.............................................................................................................................................. 51
  3 Multimodal gradients of human basal forebrain connectivity...................................................... 51
List of Tables

Table 1.1: Acetylcholine receptor subtypes and their functions.................................................. 2
Table 1.2: BF cholinergic cell groups and their projections based on rodent and monkey brain (Liu et al., 2015; M. M. Mesulam, Mufson, Wainer, et al., 1983).......................................................... 4
Table 1.3: NbM (Ch4) cholinergic cell groups and their predicted cortical projections in the human brain as suggested by Mesulam (Liu et al., 2015)................................................................. 6
Table 1.4: Currently available human BF atlases based on postmortem brain(s)...................... 7
Table 1.5: Major clustering algorithms.......................................................................................... 18
Table 2.1: Summary of functional connectivity-based parcellation of the basal forebrain..... 30
List of Figures

Figure 1.1: Cortical and subcortical projection map of basal forebrain in mice brain.......... 5
Figure 1.2: Cortical projection map of NbM (Ch 4) in the human brain as suggested by
Mesulam.................................................................................................................................. 6
Figure 1.3: Schematic representation of magnetic resonance imaging principles............. 10
Figure 1.4: Schematic illustration of water diffusion in a neuron...................................... 11
Figure 1.5: Diffusion ellipsoid example of isotropic (left) and anisotropic (right) diffusion.... 13
Figure 1.6: Simplified demonstration of basic tractography process.................................. 14
Figure 1.7: Schematic representation of BOLD response measured in fMRI paradigms........ 15
Figure 2.1: A 3D view of labeled BF subdivisions defined by (Zaborszky et al., 2008) projected
on glass brain (left) and scatter plot of the same labeled BF (right; A: anterior, P: posterior).... 33
Figure 2.2: Intrinsic validation of diffusion and functional clustering solutions.................. 37
Figure 2.3: BF spectral clustering results up to k=5, chosen cluster solution for each modality
is highlighted with a red rectangle (A: anterior, P: posterior)............................................... 38
Figure 2.4: Spatial overlap between the probabilistic atlas (Zaborszky et al., 2008) divided in
Ch123 and Ch4a/Ch4p subregions.......................................................................................... 39
Figure 2.5: Cortical projection of each cluster subdivisions in structural (left) and functional
(right) parcellation.................................................................................................................. 40
Figure 2.6: Spider plots showing the Yeo network (Byrge & Kennedy, 2019; Yeo et al., 2011)
correspondence of each cluster subdivision in structural (top) and functional (bottom)
parcellation (chosen cluster solution for each modality is highlighted with a red rectangle). 42
Figure 3.1: Structural and functional gradients across BF.................................................. 62
Figure 3.2: Structural and functional gradient-weighted cortical maps and their relationship... 65
Figure 3.3: Multimodal connectivity in relation to cortical geodesic distance and myelination. 68
Figure 3.4: Cortical cholinergic innervation in relation to cortical residual map and geodesic
distance........................................................................................................................................ 71
Figure 3.5: Summary of findings...........................................................................................................72
Supplemental Figure 3.1: Gradient-weighted cortical mapping method........................................86
Supplemental Figure 3.2: A seven–network parcellation based on resting-state fMRI of the
human cerebral cortex by Byrge & Kennedy (2019), and Yeo et al., (2011).........................86
Supplemental Figure 3.3: Cortical cholinergic innervation in relation to cortical maps of
weighted residuals and geodesic distance......................................................................................87
Figure 4.1: Homologous midcingulo-insular hubs of the ventral attention/salience networks
in the human and mouse brain.......................................................................................................91
Figure 4.2: Neuroimaging meta-analysis strategies........................................................................94
Figure 4.3: Correlation of BF task co-activation with the multimodal gradient of cortical
cholinergic innervation.................................................................................................................100
Figure 4.4: Meta-analyses of task activations under ACh and Attentional demand........102
Figure 4.5: Correlation of ACh cortical co-activation with the multimodal gradient of cortical
cholinergic innervation.................................................................................................................104
Figure 4.6: Behavioral meta-analyses for main effects of ACh on response latency and
accuracy in pharmacological neuroimaging studies.................................................................105
Figure 5.1: Summary of our findings on BF cortical connectivities (from Chapter 3)........121
# List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3D</td>
<td>3 dimension</td>
</tr>
<tr>
<td>7T</td>
<td>7 Tesla</td>
</tr>
<tr>
<td>ACC</td>
<td>anterior cingulate cortex</td>
</tr>
<tr>
<td>Ach</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>ALE</td>
<td>Activation Likelihood Estimation</td>
</tr>
<tr>
<td>BF</td>
<td>Basal Forebrain</td>
</tr>
<tr>
<td>BOLD</td>
<td>Blood-oxygen-level-dependent</td>
</tr>
<tr>
<td>CB</td>
<td>cingulum bundle</td>
</tr>
<tr>
<td>ChAT</td>
<td>choline-o-acetyltransferase</td>
</tr>
<tr>
<td>CHI</td>
<td>Calinski-Harabasz index</td>
</tr>
<tr>
<td>CoV</td>
<td>coefficients of variation</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebro-Spinal Fluid</td>
</tr>
<tr>
<td>DB</td>
<td>diagonal band of Broca</td>
</tr>
<tr>
<td>DBI</td>
<td>Davies-Boulddin index</td>
</tr>
<tr>
<td>DLPFC</td>
<td>dorsolateral prefrontal cortices</td>
</tr>
<tr>
<td>dMRI</td>
<td>diffusion MRI (structural data)</td>
</tr>
<tr>
<td>DSI</td>
<td>Dice Similarity Index</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion Tensor Imaging</td>
</tr>
<tr>
<td>DWI</td>
<td>Diffusion-weighted imaging</td>
</tr>
<tr>
<td>FA</td>
<td>fractional anisotropy</td>
</tr>
<tr>
<td>FEOBV</td>
<td>fluoroethoxybenzovesamicol</td>
</tr>
<tr>
<td>fG1</td>
<td>primary functional gradient</td>
</tr>
<tr>
<td>fG1ctx</td>
<td>gradient-weighted cortical map corresponding to fG1</td>
</tr>
<tr>
<td>FID</td>
<td>Free Induction Decay</td>
</tr>
<tr>
<td>fMRI</td>
<td>functional MRI</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
</tr>
<tr>
<td>HARDI</td>
<td>high angular resolution diffusion imaging</td>
</tr>
<tr>
<td>HCP</td>
<td>Human Connectome Project</td>
</tr>
</tbody>
</table>
$k$  partition (number of clusters dataset has to be divided) in a clustering algorithm

MA  modelled activation

mACHRs  muscarinic acetylcholine receptors

MACM  Meta-Analytic Connectivity Mapping

MD  mean diffusivity

MNI  Montreal Neurological Institute

MPRAGE  magnetization-prepared rapid gradient-echo

MRI  Magnetic Resonance Imaging

MS  Medial Septal nucleus

nAChRs  nicotinic acetylcholine receptors

NbM  nucleus basalis of Meynert

NREM  non-REM sleep

NSP  nucleus subabdominalis

PET  Positron Emission Tomography

PFC  prefrontal cortex

REM  rapid eye movement (sleep)

ROI  region of interest

rsfMRI  resting-state functional MRI

sG1  primary structural gradient

sG1ctx  gradient-weighted cortical map corresponding to sG1

SI  substantia innominata

SN  septal nucleus

T1w/T1w  $T_1$-weighted

T2w/T1w  $T_2$-weighted

UF  uncinate fasciculus

VACHT  vesicular acetylcholine transporter

WM  white matter
Chapter 1
1. Introduction
1.1 Motivation
1.1.1 Why study the cholinergic basal forebrain in humans?

The basal forebrain contains a population of large-projection neurons that release the neurotransmitter acetylcholine. Research has shown that acetylcholine is involved in the regulation of cortical activity essential for cognitive processes such as attention, sensory processing, and memory (Muñoz & Rudy, 2014). Moreover, the cholinergic basal forebrain has been implicated in the pathology of several neurological disorders, including Alzheimer’s disease (Liu et al., 2015; Nemy et al., 2022; S. J. Teipel et al., 2005). In Alzheimer’s disease, the cholinergic neurons in the basal forebrain degenerate, leading to a decrease in acetylcholine levels in the brain, which contributes to the cognitive decline seen in this disorder. Understanding the cholinergic basal forebrain and its role in cognitive function and disease can provide insights into the underlying mechanisms of several neurological disorders and inform the development of potential treatments for these conditions. Therefore, studying this brain region is essential for understanding the neural basis of cognition and for developing effective interventions for neurological disorders.

However, cholinergic neurons are challenging to study in living humans for several reasons. One major obstacle is their location deep in the brain, which makes them difficult to access using non-invasive techniques. The basal forebrain, where cholinergic neurons are located, is buried beneath the cortex. Another challenge is the small size of the cholinergic neurons in the basal forebrain. These neurons are relatively sparse, and their axons project widely throughout the brain, making it difficult to isolate their specific contributions to neural activity. Finally, cholinergic neurons in the basal forebrain are also highly interconnected with other brain regions, which makes it difficult to tease apart their specific contributions to neural activity from the activity of other regions in the brain.

Taken together, these factors make it challenging to study cholinergic neurons in living humans. However, advances in brain imaging techniques, including structural and functional magnetic resonance imaging (MRI) and positron emission tomography (PET), have made it possible to gain insights into the role of cholinergic neurons in cognition and to investigate their dysfunctioning in neurological disorders. In this Chapter 1, I will review our current, limited understanding of the organization of the basal forebrain cholinergic system.
in non-human primates and in humans, and the emerging role of basal forebrain cholinergic neurons as a potential but a relatively under-explored early site of dysfunction in multiple neurodegenerative diseases of aging including Alzheimer’s and Parkinson’s disease. I will then discuss how the integration of multimodal in vivo imaging techniques may help to (1) increase our understanding of the basal forebrain cholinergic system in humans as well as (2) improve monitoring of early cholinergic dysfunction in disease. This in vivo multimodal imaging strategy constitutes the core of my Ph.D. thesis.

1.2 Basal forebrain function and anatomical organization

1.2.1 Function: importance in cognition

The basal forebrain is a source of acetylcholine release and is involved in various functions. The molecule acetylcholine activates muscarinic receptors, allowing for a parasympathetic reaction in any organs and tissues where the receptor is expressed. Muscarinic receptors are a type of ligand-gated G-protein coupled receptor, functioning as either simulative regulative G-proteins or inhibitory regulative G-proteins. There are five subtypes of muscarinic receptors (Table 1.1); and among the five subtypes, M1, M3, M5 are stimulatory receptors, whereas M2 and M4 receptors are inhibitory receptors. In addition, ionotropic ligand-gated nicotinic receptors, mostly in the central nervous system, are also responsive to acetylcholine. The subtypes of these receptors and their functions are summarized in table 1.1 (Hillmer et al., 2016; Kudlak & Tadi, 2022; Naganawa et al., 2021).

<table>
<thead>
<tr>
<th>Muscarinic receptor subtypes</th>
<th>Function(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>attention, sleep (including REM), affective response</td>
</tr>
<tr>
<td>M2</td>
<td>cardiac inhibition</td>
</tr>
<tr>
<td>M3</td>
<td>lacrimal, salivary, stimulatory effect</td>
</tr>
<tr>
<td>M4</td>
<td>action on potassium and calcium channels</td>
</tr>
<tr>
<td>M5</td>
<td>regulates dopamine release at stratum terminals</td>
</tr>
<tr>
<td><strong>Nicotinic receptor subtypes</strong></td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>mediation of neuromuscular action potentials</td>
</tr>
<tr>
<td>N2</td>
<td>autonomic functions</td>
</tr>
</tbody>
</table>

*Table 1.1: Acetylcholine receptor subtypes and their functions.*
The diffuse projections of the cholinergic basal forebrain are considered to be critical for controlling sleep and wakefulness. Cholinergic neurons are more active during wakefulness and rapid eye movement (REM) sleep than non-REM (NREM) sleep; and activation of each cell type rapidly induces wakefulness (Xu et al., 2015). However, emerging evidence shows that acetylcholine operates at faster time scales and with fine-grained spatial precision (Sarter & Lustig, 2020) at the level of moment-to-moment cognitive events. Evidence for rapid temporal dynamics includes optogenetics work that showed spatial specificity of cholinergic signaling using photostimulation of the basal forebrain in combination with multielectrode measurement from the visual cortex (Pinto et al., 2013). A more recent study using biosensors for acetylcholine release showed both a temporal and spatial specificity of cholinergic release (Jing et al., 2020).

This topographic organization of BF cholinergic circuits is also evident in cognitive functions where cholinergic signaling plays a crucial role (Ballinger et al., 2016; Muñoz & Rudy, 2014). Failures of cholinergic circuitry are associated with cognitive impairments in neurodegenerative diseases and alternations of the signaling have also been shown to be associated with disorders of attention, memory and cognitive control. The prefrontal cortex (PFC) receives cholinergic inputs from the nucleus basalis of Meynert (NbM) and the diagonal band of Broca’s (DB) which mediates cue detection and cue-triggered changes in goal-oriented behaviors (Howe et al., 2013). Task-oriented information from the PFC is transmitted to the basal forebrain, which signals to the sensory cortex where cholinergic signaling causes decorrelation and enhances response reliability (Kim et al., 2016). Cholinergic denervation in the nucleus subputaminalis (NSP) of BF is linked to primary progressive aphasia which is characterized by profound and localized degeneration of the cortical language area (S. Teipel et al., 2016).

Cholinergic signaling also plays a role in memory encoding. The hippocampus receives the cholinergic signals from the medial septal (MS) and DB of the basal forebrain during spatial memory formation and elevated acetylcholine in the hippocampus has been observed in various memory tasks (Ballinger et al., 2016). Hasselmo (2006) suggests that cholinergic modulation is particularly important for the encoding and retrieval of episodic memories, which involve the integration of sensory information into a coherent representation of an event. In this context, ACh is thought to enhance the encoding and consolidation of memories by promoting the formation of associations between different
sensory features and by facilitating the transfer of information from short-term to long-term memory (Hasselmo, 2006). Emerging evidence shows the volumetric reduction in BF occurs earlier than that of the hippocampus and entorhinal cortex and can predict the cortical spread of Alzheimer’s pathology (Schmitz et al., 2016).

1.2.2 Organization: cholinergic cell bodies in the basal forebrain

The basal forebrain is a heterogeneous nucleus located anterior to the corpus callosum close to the medial and ventral surfaces of the cerebral hemispheres. BF includes substantia innominata (SI), medial septal (MS), nucleus basalis of Meynert (NbM) and nucleus of the diagonal band of Broca (DB). Earlier work on the delineation of the basal forebrain based on their cell groups has been studied by Mesulam on rodents (M. M. Mesulam, Mufson, Wainer, et al., 1983). Mesulam has identified four different cell groups based on their cholinergic connectivity patterns (Figure 1.1) in rodent models (M. M. Mesulam, Mufson, Levey, et al., 1983) summarized in Table 1.2 and Figure 1.1.

<table>
<thead>
<tr>
<th>Cholinergic Cell Groups</th>
<th>Region</th>
<th>Projection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ch1</td>
<td>Medial Septal nucleus</td>
<td>Hippocampus</td>
</tr>
<tr>
<td>Ch2</td>
<td>Vertical limb of the diagonal band nucleus</td>
<td>Hippocampus</td>
</tr>
<tr>
<td>Ch3</td>
<td>Horizontal limb of the diagonal band nucleus</td>
<td>Olfactory bulb</td>
</tr>
<tr>
<td>Ch4</td>
<td>Nucleus basalis of Meynert</td>
<td>Neocortex and Amygdala</td>
</tr>
</tbody>
</table>

Table 1.2: BF cholinergic cell groups and their projections based on rodent and monkey brain (Liu et al., 2015; M. M. Mesulam, Mufson, Wainer, et al., 1983).
Figure 1.1: Cortical and subcortical projection map of basal forebrain in mice brain.  
The figure is modified from the original creation by Kate Onuska using BioRender.com.

The Ch4 cell groups, projecting to the neocortex, are the largest cell groups within the BF and in further studies based on the monkey model. Mesulam has subdivided this into five cell groups based on their cortical projection (M.-M. Mesulam et al., 1984; M. M. Mesulam, Mufson, Levey, et al., 1983; M. -M Mesulam & Geula, 1988) and has suggested the possibility of an additional sixth subsector (* in Table 1.3) in humans as the transition between the anterior and intermediate part (Figure 1.2 and Table 1.3).
Figure 1.2: Cortical projection map of NbM (Ch 4) in the human brain as suggested by Mesulam.

The figure is based on (Liu et al., 2015), created with BioRender.com.

<table>
<thead>
<tr>
<th>NbM Region</th>
<th>Corresponding Ch4 regions</th>
<th>Cortical Projection Regions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior NbM</td>
<td>Ch4am</td>
<td>Medial cortical region, frontal, cingulate</td>
</tr>
<tr>
<td></td>
<td>Ch4al</td>
<td>Frontoparietal region, amygdala</td>
</tr>
<tr>
<td>Intermediate NbM</td>
<td>Ch4ai*</td>
<td>*(not known)</td>
</tr>
<tr>
<td></td>
<td>Ch4i</td>
<td>Laterodorsal frontoparietal, midtemporal region</td>
</tr>
<tr>
<td>Posterior NbM</td>
<td>Ch4i</td>
<td>Superior temporal and temporal pole</td>
</tr>
<tr>
<td></td>
<td>Ch4P</td>
<td></td>
</tr>
</tbody>
</table>

Table 1.3: NbM (Ch4) cholinergic cell groups and their predicted cortical projections in the human brain as suggested by Mesulam (Liu et al., 2015).
However, the delineation of the BF nuclei and its organization in humans is difficult to study. Axonal tracing from postmortem brain tissues can identify the different cholinergic cell groups, but sampling is often limited to incomplete and variable subsets of 2D coronal slices. Current atlases are based on postmortem brains (Kilimann et al., 2014; S. J. Teipel et al., 2005; Zaborszky et al., 2008) which is summarized in Table 1.4.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Teipel et al.</th>
<th>Zaborszky et al.</th>
<th>Killimann et al.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>2005</td>
<td>2008</td>
<td>2014</td>
</tr>
<tr>
<td>Image</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>Space</td>
<td>MNI152 template (1mm)</td>
<td>MNI colin27</td>
<td>IXI-MNI template</td>
</tr>
<tr>
<td>Sub-regions</td>
<td>Ch1-2; Ch3; Ch4; Ch4p</td>
<td>Ch2; Ch3; Ch4am; Ch4p</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td>MRI and histology</td>
<td>Cytoarchitectonic mapping of silver stained histology</td>
<td>In cranio MRI and histology</td>
</tr>
<tr>
<td>Sample #</td>
<td>N = 1</td>
<td>N = 10</td>
<td>N = 1</td>
</tr>
</tbody>
</table>

Table 1.4: Currently available human BF atlases based on postmortem brain(s).

Zaborszky et al. (underlined) atlas is used in Chapter 2 and Chapter 3 of this thesis.

1.2.3 Organization: cholinergic cortical projections

The cholinergic neurons in the BF are known to have very large projections, targeting distal areas of the cortex and amygdala via multiple pathways. Traditionally this cholinergic BF was thought to have diffuse projections (as mentioned in 1.2.1) that were thought to globally modulate the levels of acetylcholine in the cortex through volume transmission. However, new evidence in rodent models suggests that the cholinergic BF projection are more topographically organized and may modulate individual networks (Do et al., 2016; Gielow & Zaborszky, 2017; Li et al., 2017; Wu et al., 2014; Zaborszky et al., 2015). Precise estimation of this projection was difficult to study due to the complexity of axonal
branching. However, the complete morphology of individual cholinergic neurons was visualized in mice using a novel cell labeling technique (Wu et al., 2014).

Recent work using optogenetics also allowed cell type specific targeting of labeled cholinergic neurons (Li et al., 2017) and revealed the large, monosynaptic projections originating from the basal forebrain cholinergic neurons. Here, different clusters of cholinergic neurons in the basal forebrain project to different parts of the cerebral cortex, showing a topographic organization. Moreover, a given cluster of cholinergic neurons in the basal forebrain that tend to project to parts of the cortex are themselves highly interconnected.

However, in humans, there is only one extant paper on the organization of cholinergic projections (Selden et al., 1998). Due to the technical challenges of tracing the cholinergic fibers, which are enormous and can branch >1000 times before reaching the cortex, it is very difficult to study the BF projection. The above-mentioned study was done in an individual postmortem sample and in only one basal forebrain compartment, Ch4. As such, our understanding of the organization of the basal forebrain in humans is far from complete. In the next section, we discuss the importance of understanding the organization and of the BF for research on neurodegenerative diseases, before moving to the potential of using in vivo multimodal neuroimaging to study the organization of the cholinergic basal forebrain in humans.

1.2.4 Selective vulnerability to aging and Alzheimer’s disease

From the 1980s onwards, the cholinergic cortico-projection neurons received particular attention for their loss in Alzheimer’s disease (AD) (M.-M. Mesulam, 1999; Perry et al., 1984; Zaborszky et al., 2008). The nucleus basalis of Meynert (NbM) of the basal forebrain is the primary source of cholinergic innervation to the neocortex (M. Mesulam, 1976) that undergoes neurodegeneration in AD (Whitehouse et al., 1981). This along with the findings of depletion of presynaptic cholinergic markers in the cerebral cortex (Bowen et al., 1976), memory impairment with the cholinergic antagonists and improvement with agonists (Drachman & Leavitt, 1974) led to the cholinergic hypothesis of AD.

Early research on therapeutic drugs was based on this cholinergic hypothesis of AD – indeed, cholinesterase inhibitor therapies showed significant symptomatic improvement (Hampel et al., 2018). However, these drugs weren’t fully successful in treating the disease.
One problem with the implementation of these early anti-cholinesterase clinical trials was the lack of imaging biomarkers specific for cholinergic dysfunction. Patients were included based on mild to moderate cognitive impairment, a stage of prodromal AD in which widespread cholinergic damage has already occurred. Hence, one possibility for the limited efficacy of these trials is that they targeted patients with little remaining cholinergic function to rescue with cholinesterase inhibitors. It is possible that the true efficacy of these drugs will only be realized when patients can be stratified at the early stages of the disease, preceding cognitive impairment. However, this will require biomarkers which are sensitive and specific to early cholinergic dysfunction.

Currently, there are no clinically validated imaging-based biomarkers of cholinergic dysfunction and denervation. As with the need for imaging-based strategies to better understand the basic organization of the cholinergic BF projections in humans, there is a need to develop these imaging strategies for biomarkers of cholinergic dysfunction in early stage AD. Pathological changes occur years before symptoms like cognitive declines in AD (Long & Holtzman, 2019). Unlike toxic protein accumulation that can be detected with recently developing blood tests (Shea et al., 2022), dysfunction in cholinergic projection may not change the amount of acetylcholine in the brain to be detected by such tests. Understanding the organization and function of the BF in humans using multimodal imaging techniques will not only help us understand the basis of cholinergic input in humans but also yields an understanding and development of in-vivo imaging-based biomarkers for early stages of neurodegenerative diseases such as AD. In the remaining sections of this chapter, I will discuss multimodal imaging strategies for studying the basal forebrain cholinergic system in health and disease.

1.3 Neuroimaging of basal forebrain

1.3.1 MRI of the human basal forebrain

As discussed in the preceding sections, much of the knowledge on the organization of the basal forebrain cholinergic cell bodies and their cortical projections is from studies with rodent models, monkey and human postmortem samples. Current atlases of the human basal forebrain are based on postmortem histology (Table 1.4) (Kilimann et al., 2014; S. J. Teipel et al., 2005; Zaborszky et al., 2008). Magnetic Resonance Imaging (MRI) can provide in-vivo connectivity profile of the basal forebrain in 3D context. In this section, we
will briefly review the basic principles of MRI as well as the imaging techniques (diffusion-weighted imaging and resting state-functional MRI) used to study the human basal forebrain organization and projection in-vivo for this thesis.

1.3.2 Principles of MRI

Magnetic Resonance Imaging (MRI) is the dominant in-vivo method for collecting brain structural images. It is minimally invasive and reasonably high in spatial resolution (down to the submillimetre scale). MRI scanners use a powerful external magnetic field, magnetic field gradients and radiofrequency currents to reconstruct 3D images of the tissue of interest, hereby leveraging the high water content of the human body. The strong magnetic field (B0) of the MRI scanner forces the protons (H+) to align with the field which are then spinned out of their equilibrium stage by applying a pulsed radiofrequency (RF) current. Finally, when the RF pulse is turned off and the protons realign with the magnetic field (Figure 1.3), the sensors in the scanner (i.e., receive coils) are used to measure the energy released (FID signal)(Ella et al., 2019). The amount of energy released as the protons realign and the time it takes to realign depends on the nature of the molecules – hence, different tissue types characterized by different molecular compositions have unique magnetic properties that can be imaged using MRI. These images are then reconstructed to obtain the 3D MR images.

![Figure 1.3: Schematic representation of magnetic resonance imaging principles.](image)

Figure recreated based on (Ella et al., 2019). (A) Protons (or hydrogen (H) atoms) are aligned to the strong magnetic field (B0) in the MRI scanner. (B) The application of RF pulse cause the protons to spin out of equilibrium (alignment). (C) Once the RF pulse stops, the protons spin back to their equilibrium state (relaxation) to align with B0. (D) During the previous process
of relaxation, protons lose energy emitting the RF signal called Free Induction Decay (FID). This FID signal is measured by the coil placed above the brain or region of the body being taken and reconstructed to produce the MR images.

With the advancement of MRI technology, various acquisition methods are developed to obtain different images. Two common MRI acquisitions, both of which are used throughout this thesis, are introduced here: diffusion-weighted imaging (DWI) and functional MRI (fMRI). The former, DWI, measures the movement of water molecules (more on this in the next section)(Smith et al., 2020) and the latter measures changes related to deoxygenated iron in the blood (the basic principle of functional MRI – more on section 1.3.5)(Dale et al., 2015).

1.3.3 Diffusion-weighted MRI and Diffusion model

DWI is based on the movement of water molecules (Kroenke, 2020). All particles in a fluid have a random movement, known as the Brownian motion. This Brownian motion is restricted due to the microstructural properties of the tissues in the brain. In the neuron soma (gray matter) the motion is roughly isotropic – water molecules freely move around. However, in the myelinated axon (white matter tracts) water diffusion is more anisotropic and occurs maximally in the same direction as the white matter tracts as demonstrated in Figure 1.4.

![Figure 1.4: Schematic illustration of water diffusion in a neuron.](image-url)

*Created with BioRender.com*
The difference in how water diffuses within a voxel of an acquired image can provide microstructural properties of the tissues within that voxel. A typical DWI acquisition scheme typically acquires multiple diffusion-weighted volumes sensitive to specific directions of water diffusivity, as well as several non-diffusion-weighted volumes. DWI typically has low signal-to-noise ratio (SNR) compared to anatomical images and requires optimization of acquisition parameters and preprocessing techniques to obtain a high-quality image with a reasonable SNR. First of all, water molecule movement produces much lower signal strength compared to protons in the anatomical images, resulting in lower signal strength. The diffusion signal can also be affected by magnetic field inhomogeneities or susceptibility artifacts causing signal dropout. Moreover, diffusion images are susceptible to motion as acquisitions can take a long time which makes it difficult for the subject to remain still for the entire scan. Finally, the large changes in magnetic gradients and direction during the scan cause electromagnetic currents known as eddy currents which can distort the images. These noise and distortions are corrected for each volume and aligned to the anatomical images. The cleaned and aligned images are then used to fit various diffusion models.

The most well-known and widely used model is the Diffusion Tensor (DTI) model (Christiaens & Tournier, 2020). In this model, the local diffusion in a voxel is characterized by a diffusion tensor, $D$, that is related to the attenuation of the diffusion signal along a particular direction. This diffusion tensor is given by:

$$D = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{bmatrix}$$

and by taking the determinant of the matrix one can obtain the three eigenvalues and eigenvectors:

$$D = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{bmatrix} = \begin{bmatrix} V_{1x} & V_{1y} & V_{1z} \\ V_{2x} & V_{2y} & V_{2z} \\ V_{3x} & V_{3y} & V_{3z} \end{bmatrix} \cdot \begin{bmatrix} \lambda_1 \\ \lambda_2 \\ \lambda_3 \end{bmatrix} \cdot \begin{bmatrix} V_{1x} & V_{2x} & V_{3x} \\ V_{1y} & V_{2y} & V_{3y} \\ V_{1z} & V_{2z} & V_{3z} \end{bmatrix}$$

Eigenvalues ($\lambda_1, \lambda_2, \lambda_3$) are important in determining the principal direction of water diffusion and are used to quantify specific aspects of the diffusion as well. For example, in an isotropic diffusion, the three eigenvalues of the diffusion tensor are equal and the ellipsoid takes on a...
spherical shape. However, in an anisotropic diffusion, the ellipsoid’s long axis is oriented in the main direction of diffusion (Figure 1.5).

\[ \lambda_1 = \lambda_2 = \lambda_3 \quad \text{Isotropic Diffusion} \]

\[ \lambda_1 > \lambda_2 \geq \lambda_3 \quad \text{Anisotropic Diffusion} \]

**Figure 1.5:** Diffusion ellipsoid example of isotropic (left) and anisotropic (right) diffusion.

The DTI model also provides scalar metrics of diffusivity, such as mean diffusivity (MD) and fractional anisotropy (FA) which are respectively the mean of the eigenvalues and the normalized variance of the eigenvalues:

\[ MD = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3} \]

\[ FA = \frac{3}{\sqrt{2}} \frac{\sqrt{\text{Var}(\lambda)}}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2} \]

These scalar, tissue microstructure-sensitive metrics are often used to investigate potential biomarkers for diseases. Although the DTI model has limitations in capturing complex fiber orientations, its simplicity is favored to be adopted for many studies.

### 1.3.4 Diffusion Tractography

Using the previously described diffusion model, one can estimate the local diffusion orientations within a voxel and reconstruct the long fibers connecting brain regions - commonly referred as tractography. Keep in mind though, a typical voxel is in the range of cubic millimeters resolution whereas a single axon has a diameter of about one micrometer,
hence a single voxel is a representation of thousands of axons and the diffusion orientation measured in that voxel is a statistical average of multiple axons within that voxel. The basic principle of tractography is based on this phenomenon and the assumption that the dominant fiber tracts can be assumed to be parallel to one of the eigenvectors of the diffusion tensor. If the fiber orientation at one voxel is known, one can take a small “step” in the direction of the underlying fibers, re-assess the fiber orientation at this new voxel, and take another small step in this new direction (Figure 1.6). By repeating this process many times, one can construct a continuous path through space, which is consistent with the underlying fiber orientations, and is, therefore, a reasonable estimate of the trajectory of the underlying long neuronal fibers from which those local orientations were observed (Smith et al., 2020).

![Figure 1.6: Simplified demonstration of basic tractography process.](image)

Constructing this path from a seed (starting point) to a target (termination point) can provide the structural connectivity between these two regions in the brain. Additionally, one can add constraints in reconstructing this path, such as “waypoint” or “include” ROI(s) and/or “exclude” ROI(s). There are two groups of commonly used algorithms in tractography: deterministic and probabilistic. The main difference between these two algorithms is how fiber orientation in a voxel is determined for the streamline propagation of the path construction. In deterministic tractography, streamline propagation is carried out in a “specific” way; the path direction is predictable and is usually similar to the one in the previous step. On the other hand, probabilistic tractography chooses the propagation direction more randomly based on the distribution of fiber orientations in the voxels.
1.3.5 The blood-oxygen-level-dependent (BOLD) signal and functional MRI

Another powerful method for MRI is functional MRI (fMRI), based on the blood-oxygen-level-dependent (BOLD) signal. Functional MRI utilizes the distortion of the magnetic field caused by deoxygenated iron in the blood. Neural activity in the brain requires a large amount of energy that is supported by a constant supply of oxygen. This relationship between neuronal activity and blood flow in the brain is referred to as the neurovascular coupling. The oxygen supply is provided by hemoglobin, an iron-rich protein in the blood. Oxygen-saturated hemoglobin, or oxyhemoglobin, provides the necessary oxygen supply to the region of neural activity through a cascade of metabolic events. Once the oxygen molecules are released from the oxyhemoglobin, it becomes deoxyhemoglobin or oxygen-depleted hemoglobin (Figure 1.7). This hemoglobin state changes from oxyhemoglobin to deoxyhemoglobin underlies BOLD signal fluctuations in the capillaries near the active neuronal site (Ogawa et al., 1990).

![fMRI paradigm](image)

**Figure 1.7: Schematic representation of BOLD response measured in fMRI paradigms.**

created with BioRender.com

The use of functional MRI started with the task-based fMRI paradigm. Depending upon a hypothesis, participants performed various tasks inside the scanner. The timing and modeling of such tasks varied considerably, but many followed a simple method in which
participants alternate between the task and a rest period (i.e., ‘block design’), and the
analysis was based on the subtraction of this task from the rest state. The brain regions with
changes in BOLD signal during the repeated task period vs. rest were considered “active”
during that certain task and involved in the cognitive function required for the task.

1.3.6 Resting-state functional MRI

A significant change occurred following the discovery of the spontaneous BOLD
fluctuations measured at rest that were not related to random noise or a result of
physiological or scanner artifacts but are consistent during rest and could be used to map
the human brain at rest (Biswal et al., 1995). These findings caused a new area of network
research based on functional connectivity using resting-state fMRI. Functional connectivity is
defined by the BOLD signal correlation of two brain regions. Although functional
connectivity is not a direct measure of anatomical connections, it seems to provide insights
into how cortical regions communicate with each other (Honey et al., 2007). Cortical
communication is complex; while tractography (discussed in the previous section) can
provide us with an estimated measure of structural connectivity, it fails to provide how distal
regions or regions without direct connection communicate. Functional connectivity
measured by resting-state fMRI can supplement this information. So far, all previous studies
on data-driven segmentation of the human BF have been focusing on a single modality of
brain connectivity based on resting-state functional MRI and connectivity-based parcellation
(discussed in the next section). None have leveraged the complementary information of
both modalities. As such, in this thesis project, we employ both structural and functional
connectivity measures to get comprehensive insights into how BF is connected to the other
brain regions.

1.4 Connectivity-based parcellation

Methods of connectivity-based parcellation have become increasingly popular for
the in-vivo mapping of the human brain into regional specialization to study the topography
of the brain (Eickhoff et al., 2018). Parcellation of the brain (or structure of the brain) into
distinct regions is typically done in two ways: (1) by extracting discrete clusters, or (2) by
characterizing patterns of smooth transitions in brain connectivity. The former assumes that
the brain is patched up by regions characterized by uniform intra-regional structurally and
functionally connectivity. The letter is referred to as gradients and, contrary to delineating discrete subregions, assumes the brain regions vary gradually along certain spatial axes.

1.4.1 Discrete parcellation: clustering

Discrete parcellation aims to subdivide the brain or a region of the brain into sub-regions with similar connectivity patterns. Using tractography and/or resting-state fMRI, described in the previous sections, structural and/or functional connectivity of the brain region with other areas in the brain can be obtained. These connectivity data are considered to have unique patterns depending on the structural and functional properties. As such, voxels with similar connectivity patterns can be clustered to form a sub-region. Methodologically, the majority of the studies apply unsupervised clustering algorithms (summarized in Table 1.5) to the connectivity data to achieve this (Yeo et al., 2011).
<table>
<thead>
<tr>
<th>Algorithm</th>
<th>K-means</th>
<th>Hierarchical</th>
<th>Spectral</th>
<th>DBSCAN/ HDBSCAN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
<td>Partitional clustering algorithm. Partitions data to minimize intra-partition distance</td>
<td>Find successive clusters using previously established clusters. These algorithms can be either agglomerative (bottom-up) or divisive (top-down)</td>
<td>Graph clustering. A kind of manifold learning, finding a transformation of the original space so as to better represent manifold distances for the data. Once the data is transformed, a standard clustering algorithm is run (such as k-means)</td>
<td>Density based algorithm. DBSCAN transforms the space according to the density of the data: points in dense regions are left alone, while points in sparse regions are moved further away. Applying single linkage clustering to the transformed space results in a dendrogram, which we cut according to a distance parameter</td>
</tr>
<tr>
<td><strong>Use case</strong></td>
<td>General-purpose, even cluster size, flat geometry, not too many clusters</td>
<td>Many clusters, possibly connectivity constraints, non-Euclidean distances</td>
<td>Few clusters, even cluster size, non-flat geometry</td>
<td>Non-flat geometry, uneven cluster sizes</td>
</tr>
<tr>
<td><strong>Geometry</strong></td>
<td>Distances between points</td>
<td>Any pairwise distance</td>
<td>Graph distance (e.g. nearest-neighbor graph)</td>
<td>Distances between nearest points</td>
</tr>
</tbody>
</table>

**Table 1.5: Major clustering algorithms.**

Although there are differences in how each algorithm subdivides the clusters, they all aim to optimize inter-cluster homogeneity and intra-cluster dissimilarity (Eickhoff et al., 2015). Historically, parcellation analyses were preferred by many researchers due to their simplistic view of brain connectivity and efficacy in interpretation (Eickhoff et al., 2018).
However, there are limitations to this method. First, the absence of ground truth makes it very challenging to validate the results. Whether cortical or subcortical, it is rare to have a ground truth segmentation of the brain that can be used to validate this data-driven parcellation. In the absence of such a standard metric, other indirect measures are used to validate the discrete parcellation, including internal indices that measure the inter-cluster homogeneity and intra-cluster dissimilarities (more in Chapter 2 methods), consistency with other (human and non-human) studies, test-retest reliability, and accuracy in prediction of clinical or behavioral outcome. Second, it fails to capture voxel-level differences in connectivity patterns. Although clustering analyses can differentiate broad differences in connectivity patterns, it fails to capture subtle differences at voxel-level. This can be problematic for parcellating small subcortical regions in the brain. Finally, while the general assumption is that a brain region is functionally specialized and has a unique and homogeneous connectivity profile, this is almost never the case (Van Essen & Glasser, 2018).

1.4.2 Gradient representation

In contrast to parcellation, the goal of gradient representation is to characterize the spatial variation of the connectivity profile of the region of interest. This overcomes the problem of capturing subtle connectivity differences allowing us to examine changes in voxel-level connectivity patterns. Gradient approach enables keeping the voxel resolution of connectivity profiles, providing better insights into the organization of the brain region of interest. However, when looking at the connectivity profiles at the voxel-level resolution, the large size of the data became problematic. In theory, one could visualize and examine connectivity patterns of each and individual voxel of their interest, but brain regions (especially at higher resolution MRI data) usually consist of hundreds and thousands of voxels (in our study, Chapter 2 and 3, seed BF consists of 599 voxels and cortical targets were 360) resulting in a huge matrix (599 x 360) of connectivity patterns. Qualitative analysis of these individual connectivity metrics is not only tedious and impractical but lacks quantitative measures to systematically evaluate the heterogeneity in the connectivity patterns across the brain region of interest. This challenge calls for the development of methodological tools that preserve the high voxel-level resolution of connectivity patterns but condense the high dimensional data into selective features. This is what the gradient approach does.
The gradient maps basically represent the data in a reduced dimensional space in a way that preserves the underlying structure of the data. The first step in estimating the gradients is to construct a similarity matrix based on pairwise similarities between the data points i.e. any two voxels. Various similarity measures can be used to construct this similarity matrix, such as cosine similarity (Margulies et al., 2016), eta^2 coefficient (Haak et al., 2018) and normalized angle similarity (Vos de Wael et al., 2020). Next, a low-dimensional subspace for the connectivity profiles that preserves the underlying data structure has to be estimated. This process is modeled using a random walk on the graph defined by the similarity matrix created, where each voxel represents a node and the similarity between them is represented by the edges in the graph. The process works in a way such that data points that are more similar to each other are more likely to be visited by the random walk and by applying mathematical techniques, such as principal component analysis (PCA), Laplacian Eigenmaps, or Diffusion Mapping (used in Chapter 3) a set of low-dimensional embeddings, or subspace, for the data points can be obtained. In this subspace, pairs of voxels with highly similar connectivity patterns are situated close to one another, whereas dissimilar voxels are far apart.

Projecting these low-dimensional subspace coordinates back to the brain can be effective in visualizing the spatial changes in connectivity patterns over that brain region. Furthermore, unlike discrete parcellation, gradient estimation provides quantitative values that can be used to assess fine-grained connectivity differences in multi-modal imaging. In other words, gradient maps can be used to systematically compare structural and functional BF-cortical connectivity differences.

1.5 Goals of this thesis and overview of projects

The current thesis aims to explore the 3D anatomy of the basal forebrain in an in-vivo, data-driven, and multi-modal framework. There are multiple prior studies examining subregional connectivity of the BF using either structural or functional MRI. For instance, connectivity based parcellation using functional connectivity profiles has been used in previous studies (Fritz et al., 2019; Markello et al., 2018; Yuan et al., 2019) to segregate the subregions of the BF. Moreover, diffusion work has examined the white matter connectivity of the NbM subregion of the BF (Nemy et al., 2020; Selden et al., 1998)). However, no study has systematically used these strategies together in an integrated analytical framework.
The projects described in this thesis will be the first to explore the whole brain connectivity of the entire BF using high-resolution 7 Tesla (7T) MRI data spanning structural (DWI) and functional (rsfMRI) measures in healthy cognitively normal younger adults. Chapter 2 explores these MRI connectivity metrics employing discrete parcellation using spectral clustering on the basal forebrain. Chapter 3 explores the same connectivity metrics utilizing the gradient representation of connectivity. Furthermore, we integrate these MRI-based connectivity data with in-vivo cell type-specific molecular imaging of the presynaptic cholinergic nerve terminals, assayed using positron emission tomography (PET), as well as several other cortical features relevant to basal forebrain connectivity including geodesic distance between BF and cortex and cortical myelin content. We will use these multimodal data to generate a novel human atlas of cortical cholinergic projections. The analyses and findings of Chapter 3 are currently under peer review at PNAS. In Chapter 4, we will conduct a meta-analysis of placebo controlled pharmacological fMRI studies evaluating cholinergic agonists in combination with executive function tasks. We will compare how our atlas of cortical cholinergic connectivity relates to the spatial map produced by pharmacological meta-analysis.
1.6 References


Hampel, H., Mesulam, M.-M., Cuello, A. C., Farlow, M. R., Giacobini, E., Grossberg, G. T.,


Chapter 2

Connectivity based discrete parcellation of the human basal forebrain

2.1 Goals of this chapter

The aims of this chapter are to demonstrate connectivity-based clustering analysis in the basal forebrain for both structural and functional MRI data. We anticipate that (1) connectivity data derived from diffusion-weighted imaging (DWI) can parcellate the basal forebrain, recapitulating the known nuclear subdivisions of this structure based on cell labeling and tractography studies (Selden et al.), and (2) connectivity data derived from resting state fMRI (rsMRI) can also parcellate the basal forebrain, recapitulating previous studies which have shown rsfMRI-based parcellation differentiating the anteromedial and posterolateral basal forebrain subregions (Fritz et al., 2019; Markello et al., 2018; Yuan et al., 2019). To date, however, these two modalities of structural and functional MRI data have yet to be systematically integrated within the same sample to evaluate multimodal basal forebrain connectivities. In this chapter, I will explore the possibility of structural connectivity-based parcellation, whether it can parcellate the anteromedial to posterolateral subregions similar to the functional connectivity-based parcellation of the human basal forebrain.

2.2 Introduction

The magnocellular cholinergic neurons of the BF were originally thought to send diffuse projections to the cortex and amygdala, with relatively little sub-regional spatial organization at the level of individual BF nuclei (Saper, 1984; Woolf, 1991). These diffuse projections were thought to globally modulate the levels of acetylcholine in the cortex through volume transmission. However, new evidence from axonal tracer studies in rodent and non-human primate models suggests that the cholinergic BF projections are topographically organized according to the distinct BF nuclei and their cortico-amygdalar projections (Chapter 1, section 1.2.2 and 1.2.3) (Muñoz & Rudy, 2014; Turchi et al., 2018). Rodent optogenetics study has shown different clusters of cholinergic neurons in the basal forebrain project to different parts of the cerebral cortex (Li et al., 2017).

However, our understanding of the organization and functional relevance of basal forebrain projections in humans is limited. Axonal tracing from postmortem brain tissues can identify the different cholinergic cell groups, but sampling is often limited to incomplete and
variable subsets of 2D coronal slices. Selden and Mesulam (1998) conducted the first, and as of yet, the only paper to explicitly examine basal forebrain axonal projections in an ex-vivo human brain. This paper focused exclusively on tracts emanating from the NbM (Ch4) nucleus of the basal forebrain. Three tracts were found in this study: one medial pathway and two lateral pathways. The medial pathway originates in the anterior NbM (Ch4a) and goes around the corpus callosum, mostly within the cingulum. The lateral pathways originate from the posterior part of the NbM (Ch4p) and are divided into two divisions based on the white matter fibers they travel through. One of the two lateral pathways, the capsular division travels in a densely packed and highly organized bundle of fibers immediately next to the putamen in the external capsule. The other lateral pathway, perisylvian division, is located within the claustrum, curving laterally into the white matter of the inferior frontal and superior temporal gyri.

In-vivo diffusion-weighted MRI overcomes many of the challenges of postmortem tissue analysis such as its liability to grasp the 3D structure. Drawing on the Ch4 tracts observed ex-vivo by Selden and Mesulam (1998), Nemy et al (2020) conducted an in-vivo diffusion-weighted (DWI) tractography study focusing a priori on the Ch4 projections. In contrast to ex-vivo slices, in-vivo DWI can provide the structural connectivity profile in a 3D volume to map the white matter projections emanating from the cholinergic BF. As discussed in the previous chapter, diffusion tractography can be used to visualize white matter tracts and measure the microstructural properties of white matter, such as fractional anisotropy (FA) and mean diffusivity (MD). FA reflects the degree of coherence or organization of white matter fibers, while MD reflects the degree of freedom or hindrance of water diffusion within the tissue.

In the study performed by Nemy et al (2020), the authors used tractography to examine the integrity of cholinergic white matter pathways in the brain. They focused on two specific pathways - the uncinate fasciculus (UF) and the cingulum bundle (CB) - which connect brain regions involved in attention and memory and receive cholinergic projections from the nucleus basalis of Meynert. FA and MD values were calculated for the UF and CB tracts in each participant. The authors then examined the association between these measures and cognitive performance on tests of attention and memory. The results showed that FA values in the UF and CB tracts were positively associated with cognitive performance, indicating that greater white matter integrity was related to better cognitive
performance. In contrast, MD values in the UF and CB tracts were negatively associated with cognitive performance, indicating that greater water diffusion within white matter (less integrity) was related to worse cognitive performance. Followup work examining diffusion tractography of the basal forebrain suggests these findings are reliable across independent cohorts (Lin et al., 2022; Nemy et al., 2022; Schumacher et al., 2022).

Diffusion tractography based structural connectivity data has also been used to demonstrate the ability to parcellate subcortical regions (other than BF) by distinct white matter projections. For example, Behrens et al (2003) used probabilistic tractography to map the white matter pathways connecting the thalamus and cortex in human subjects. The results showed that the method could successfully identify several well-established thalamocortical pathways, including the connections between the thalamus and primary motor cortex, primary somatosensory cortex, and visual cortex. Additionally, the authors identified several novel pathways connecting the thalamus to other cortical regions, such as the prefrontal cortex and posterior parietal cortex. Overall, the study demonstrated the potential of diffusion imaging and probabilistic tractography to non-invasively map subcortical-cortical connectivity in humans and parcellate the subcortex into subregions based on these connectivity features.

While structural connections are inferred from DWI data using diffusion tractography based on the hindered or restricted motion of water molecules within the axonal fiber bundles, temporal correlation of spontaneous blood-oxygenation-level-dependent (BOLD) fluctuations can be used to assess functional connectivity between brain regions from functional MRI data. Inherent to this, diffusion tractography is restricted to regions that are anatomically connected, while the BOLD signal can also highlight complementary, indirect connections between regions. Previous studies have explored the data-driven discrete parcellation of the human basal forebrain with rsfMRI (Fritz et al., 2019; Markello et al., 2018; Yuan et al., 2019). All three studies found anteromedial to posterolateral subdivisions within the basal forebrain and patterns of BF-cortical functional connectivity in distinct BF subregions. Critically, these connectivity profiles, derived from subcortical-cortical connectivity overlapped with cortico-cortical networks derived from independent resting-state functional data (Yeo et al., 2011). The results of these studies are summarized in Table 2.1.
<table>
<thead>
<tr>
<th>Authors</th>
<th>Markello et al.,</th>
<th>Fritz et al.,</th>
<th>Yuan, Biswal and Zaborszky</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>2018</td>
<td>2019</td>
<td>2019</td>
</tr>
<tr>
<td>Resolution</td>
<td>3mm isotropic</td>
<td>3mm isotropic</td>
<td>1.5mm isotropic</td>
</tr>
<tr>
<td>Sub-regions found (final cluster #)</td>
<td>2 anterior, posterior</td>
<td>2 anterior, posterior</td>
<td>3 ch123, ch4a, ch4p</td>
</tr>
<tr>
<td>Clustering Method</td>
<td>hierarchical clustering using Euclidean distance and Ward's criterion</td>
<td>k-means clustering (maximum 1,000 iterations)</td>
<td>Spectral clustering</td>
</tr>
<tr>
<td>Intrinsic Validation</td>
<td>maximum silhouette score</td>
<td>mean silhouette value for all voxels</td>
<td>variation of information and Hartigan metric</td>
</tr>
<tr>
<td>number of subjects(N)</td>
<td>N = 100</td>
<td>N = 85</td>
<td>N = 21</td>
</tr>
<tr>
<td>BF ROI (seed)</td>
<td>BF probabilistic maps in the SPM Anatomy toolbox, resampling to functional space, and thresholding at &gt;40% probability. masks were 95 voxels in size for the entire BF (Ch1-4, BF), and 37 voxels for MS/DB (Ch1-3), and 51 voxels for NBM (Ch4).</td>
<td>Based on combined information from existing stereotactic atlases of basal forebrain cholinergic nuclei in MNI space (Kilimann et al., 2014; Teipel et al., 2005; Zaborszky et al., 2008). resampled to a final voxel size of 3 mm isotropic with 189 voxels.</td>
<td>Original Zaborszky maximum probability maps down-sampled into 1.5mm per voxel.</td>
</tr>
</tbody>
</table>

Table 2.1: Summary of functional connectivity-based parcellation of the basal forebrain.

There are a few methodological obstacles that one should consider when employing DWI and rsfMRI to parcellate small brain regions according to their structural and functional connectivity profiles:

1) Spatial resolution: The spatial resolution of DWI and rsfMRI is limited by factors such as voxel size and signal-to-noise ratio. In small brain structures, the limited spatial
resolution can make it difficult to accurately delineate white matter tracts and their connections.

2) Parcellation method and validation: As discussed in Chapter 1 (section 1.4.1) there are various clustering algorithms that can be used to parcellate the brain regions. Employing the “wrong” algorithm can lead to faulty parcellation. Another challenge is to validate the parcellation results. There’s no gold standard for the delineation of the human BF to compare with the resultant parcellation.

The first challenge can make it difficult to accurately reconstruct white matter pathways and estimate functional time series in small brain structures. To overcome the spatial resolution problem, high spatial-resolution MRI data can be used. Additional strategies to improve the quality of DWI are to use high angular resolution diffusion imaging (HARDI) acquisition, which can provide higher spatial resolution and hence better tractography results (Berman et al., 2013). It should be noted that all but one of the aforementioned connectivity studies examining the basal forebrain were conducted at field strengths of 3T and lower, typically with voxel sizes >2mm³. Moreover, none of these studies systematically examined DWI and rs-fMRI estimates of basal forebrain connectivity within the same individuals. Hence, there has yet to be a comprehensive DWI- and rsfMRI-based survey of the complete set of basal forebrain nuclei, including the anteromedial nuclei, Ch12, Ch3, in addition to Ch4a and Ch4p.

To address this obstacle we used DWI and rsfMRI data from the Human Connectome Project (HCP). The HCP is a large-scale initiative aimed at mapping the brain's structural and functional connectivity patterns in healthy adults. The HCP 7T MRI data is freely available to researchers through their online platform and provides high-resolution MRI data, including 7 Tesla (7T) data. The second obstacle was addressed by using spectral clustering that has shown promising result in previous study using functional connectivity data (Yuan et al., 2019) and employing multiple intrinsic validation metrics as suggested by (Eickhoff et al., 2015) as well as examining the cortical correspondence of the parcellation results in comparison to previous rodent and human studies.

2.3 Materials and Methods

We used high-resolution minimally pre-processed 7T MRI HCP data (n=173) (Glasser et al., 2013) and the existing stereotactic atlas of the BF (Zaborszky et al., 2008) to build
structural and functional connectomes. Workflows were built using Snakemake (Mölder et al., 2021) with the full workflow available on GitHub (see data and code available for specifics). Individual connectomes were reduced to a 2-dimensional m-by-n matrix describing the pairwise connectivity strength between m BF ROI voxels and n cortical regions (Glasser, Coalson, et al., 2016). The scikit-learn toolbox (Pedregosa et al., 2012) was used to run spectral clustering on the connectivity data which, as well as any further analysis, was done using Jupyter Notebook (Kluyver et al., 2016).

2.3.1 Data

High-resolution 7T dMRI and rsfMRI data were downloaded from the HCP data repository (Van Essen et al., 2013). We used the minimally pre-processed data described in Glasser et al., (2013) consisting of 173 healthy subjects (69 male, 104 female) aged 22 to 35 years. The dMRI images were collected with 1.05 mm3 isotropic voxels, TR=7000 ms, TE=71.2 ms, b-values=1000, 2000 s/mm2, FOV=210 x 210 mm2. Resting-state fMRI images were collected with 1.6 mm3 isotropic voxel size, TR=1000 ms, TE=22.2 ms, FOV=208 mm2, spanning 4 runs of 16-minute duration each, per subject. For anatomical imaging, two T1-weighted (T1w) scans were obtained using a three-dimension (3D) magnetization-prepared rapid gradient-echo (MPRAGE) (Mugler & Brookeman, 1990) sequence and two T2-weighted (T2w) images using a 3D T2-SPACE sequence, all with identical geometries and a 0.7 mm3 isotropic voxel size. Full details of the acquisition parameters can be found in the HCP S1200 release reference manual (https://www.humanconnectome.org/storage/app/media/documentation/s1200/HCP_S1200_Release_Reference_Manual.pdf).

2.3.2 Basal Forebrain ROI

The basal forebrain (BF) (Figure 2.1) region-of-interest (ROI) was created using the existing stereotactic atlas of the BF (Zaborszky et al., 2008). This stereotactic BF atlas is based on histological sections obtained from 10 postmortem brains, the magnocellular cell groups were delineated in each slice, 3D reconstructed and warped into the MNI single-subject reference space (Collins et al., 1994). The atlas consists of 4 subregions of the BF defined in the nomenclature: Ch1-2, Ch3, Ch4, and Ch4p. For each subregion, a stereotactic probabilistic map has a range of 0 to 10 indicating the number of brains containing the specific magnocellular cell groups in the given voxel. Our BF ROI is created by thresholding these subregion masks to 50% first and then combining all to get a mask.
covering full BF. This BF ROI mask was then warped into MNI152 non-linear 6th generation atlas (MNI152Nlin6Asym).

**Figure 2.1:** A 3D view of labeled BF subdivisions defined by (Zaborszky et al., 2008) projected on glass brain (left) and scatter plot of the same labeled BF (right; A: anterior, P: posterior).

### 2.3.3 Structural Connectivity Reconstruction

Diffusion tractography was performed to get a connectivity matrix for diffusion data. As part of the minimal preprocessing pipeline data release, all subjects underwent FreeSurfer processing (v5.3.0-HCP; [https://github.com/Washington-University/HCPpipelines](https://github.com/Washington-University/HCPpipelines)). The BF ROI mask was then first resampled and transformed to the individual subjects’ minimally preprocessed volume space (0.7mm³) using the warp fields provided by HCP. Volumetric cortical labels were built by mapping the HCP-MMP 1.0 surface parcellation (Glasser, Coalson, et al., 2016) using Connectome Workbench’s ribbon-constrained label-to-volume-mapping function and FreeSurfer-derived surfaces. The BF ROI voxels were used as seeds, and the 180 cortical regions in each hemisphere were combined and used as targets to perform probabilistic tractography using FSL’s probtrackx (Behrens et al., 2007) with 5000 streamlines per BF ROI voxel. The resulting probability maps in the BF quantified the number of streamlines that reached each target. The maps were resampled to MNI space (Grabner et al., 2006) in 1.6mm³ resolution to match the functional connectivity matrix and reduced to a 2-dimensional m-by-n matrix, where m represents the voxels in the BF ROI (599 voxels) and n is the cortical targets (180 each hemisphere) with their corresponding number of streamlines. This m-by-n connectivity feature matrix for all 173 subjects was concatenated horizontally to run spectral clustering.

### 2.3.4 Functional Connectivity Reconstruction

First, the BF ROI mask in the minimally preprocessed volume space was resampled to 1.6mm³ isotropic voxel size to match the resolution of the rsfMRI data and added to the
subject’s subcortical parcellation. A functional connectivity matrix was then created for each subject by calculating the temporal correlation between BF voxels and cortical ROIs. All four runs (i.e., two sets of 16 min. runs with posterior-to-anterior and anterior-to-posterior phase-encoding) of the minimally preprocessed and ICA-FIX denoised rsfMRI data (Griffanti et al., 2014) were used. Since the BF ROI is not included in the dense timeseries provided by HCP, these were regenerated using the updated subcortical parcellation to include the BF ROI voxels for further processing. Subsequent processing included ROI-constrained subcortical smoothing to match the cortical sampling density using the scripts provided by HCP, as well as additional signal filtering (i) based on the average WM and CSF timeseries using scikit (Dickie et al., 2019) and (ii) by applying a Wishart filter as proposed previously (Glasser, Smith, et al., 2016; Tian et al., 2020) to selectively smooth unstructured noise more than the structured blood oxygen level-dependent signal. Average cortical ROI timeseries (concatenated across runs) were then extracted using the HCP-MMP 1.0 surface parcellation (Glasser, Coalson, et al., 2016). Functional correlation maps were calculated by calculating the Pearson’s correlation coefficient for each voxel within the BF to each of the cortical parcels. The resulting correlation maps were reduced to a 2-dimensional m-by-n matrix, where m represents the voxels in the BF ROI (599 voxels) and n are the cortical targets (180 in each hemisphere) with their corresponding functional correlation. This m-by-n connectivity matrix for all 173 subjects was concatenated to calculate group-wise clustering results.

2.3.5 Spectral clustering

Spectral clustering was run using the scikit-learn toolbox (Pedregosa et al., 2012). Group concatenated connectivity matrices were used as input to the SpectralClustering function, using the cosine affinity matrix, zero random states, discrete label assignment and a maximum of 15 clusters. Spectral clustering showed promising result with functional connectivity data (Yuan et al., 2019) and is also more suitable for non-uniform cluster sizes across the data, unlike k-means clustering which is more suitable for even clusters and flat geometry (Pedregosa et al., 2012). Cholinergic BF neuron clusters are not of equal size or continuous geometry. The cosine distance metric was used to calculate the affinity matrix since it is useful in determining the dissimilarity of two objects irrespective of their size - our connectivity matrices are not symmetrical, i.e. cortical target (360) vs. BF voxels (599). BF
voxels that are characterized by similar connectivity patterns will be clustered together and these clusters were then mapped back onto the BF space to visualize.

In addition, to determine the cortical correspondence of these clusters, we extracted the voxel rows specific to each cluster label from the BF in the original connectivity matrix and calculated their average to generate a single cortical map. The distribution of this cortical projection was then decomposed into seven functional networks (Yeo et al., 2011) using the HCP-MMP 1.0 parcellation-based Yeo networks as defined in Byre & Kennedy (2019). These networks include visual, somatomotor, dorsal attention, ventral attention, limbic, frontoparietal, and default mode.

2.3.6 Cluster Validation

One of the most challenging aspects of discrete parcellation is choosing the right cluster number. In addition to comparison with the ground truth (if available; here, we compared with the labeled probabilistic atlas (Zaborszky et al., 2008)) evaluation of the clustering model itself without the reference to external information is an important step in validating the cluster analysis fit. Here, we used three internal indices to evaluate how well the results of our cluster analysis fit the data without reference to external information. These three indices were Calinski-Harabasz, Davies-Bouldin, and Silhouette Coefficient (Caliński & Harabasz, 1974; Davies & Bouldin, 1979; Rousseeuw, 1987). All of these indices measure how similar data points are within a cluster and dissimilar with those from another cluster.

More specifically, Calinski-Harabasz index (CHI) (Caliński & Harabasz, 1974) is based on the inter-cluster (between clusters) and intra-cluster (within cluster) variance. Maximized inter-cluster and minimized intra-cluster variance suggest a good clustering fit. The CHI is calculated by the ratio of the sum of between-cluster dispersion and of within-cluster dispersion with higher index values suggesting clusters are homogeneous within a cluster and well separated from another.

Davies-Bouldin index (DBI) (Davies & Bouldin, 1979) is also based on variance but the dissimilarity between two clusters is computed based on the distance difference between centroids. The index provides the mean of the sum of intra-cluster variances divided by the difference between centroids. An index value closer to zero means a better partition of cluster solutions. DBI is defined as:
\[ DBI = \frac{1}{N} \sum_{i=1}^{N} \max_{i \neq j} R_{ij} \]

where, for \( N \) number of clusters it indicates the average of the similarity measures of each cluster with a cluster most similar to it. The best choice is where the average similarity is minimized, hence value closer to zero indicates a better cluster solution.

\( R_{ij} \) is calculated as:

\[ R_{ij} = \frac{S_i + S_j}{M_{ij}} \]

where, \( S_i \) is the intra-cluster dispersion of cluster \( i \) and \( S_j \) is the intra-cluster dispersion of cluster \( j \), and \( M_{ij} \) is the distance between centroids of clusters \( i \) and \( j \).

Finally, the Silhouette coefficient (Rousseeuw, 1987) is based on distance within and between clusters and measures the cohesion. In other words, how closely related data points are within a cluster by averaging the distance in the cluster and separation, as well as how distinct a cluster is from another cluster by average distance to other clusters. A coefficient close to one implies that a large portion of voxels is labeled to the right cluster solution. Silhouette coefficient for a data point \( x \) is given as:

\[ s(x) = \frac{b(x) - a(x)}{\max\{a(x), b(x)\}} \]

where, \( a(x) \) is the average distance in the cluster, and \( b(x) \) is the average distance to other clusters. All of the internal indices for our data were calculated using the formula implemented in the scikit-learn toolbox (Pedregosa et al., 2012).

### 2.3.7 Data and code Availability

The Human Connectome (HCP) project dataset is available at [http://www.humanconnectomeproject.org/](http://www.humanconnectomeproject.org/). The workflow for reconstructing the structural connectivity matrix from the HCP data and a subcortical region of interest is available at [https://github.com/sudesnac/diffparc-smk](https://github.com/sudesnac/diffparc-smk) (Khan & Chakraborty, 2023); and the functional connectivity workflow at [https://github.com/khanlab/subcorticalparc-smk](https://github.com/khanlab/subcorticalparc-smk) (Kai et al., n.d.). All other code used to conduct the reported analyses and create the figures are available at [https://github.com/sudesnac/BF_clustering](https://github.com/sudesnac/BF_clustering).

### 2.4 Results

Both structural and functional connectivity data based parcellation suggest an anteromedial to posterolateral differentiation similar to previous studies. Cortical projection
and correspondence with the Yeo 7 Network suggest the most posterolateral subdivision overlap with the ventral attention network while the anterior cluster(s) overlap more broadly with limbic and default mode networks.

2.4.1 Choosing the right cluster solution

The first step in discrete parcellation analysis is to find the right k, i.e. cluster solution for the data. Three internal indices for the data were calculated separately for diffusion and functional data. In addition to the subject concatenated data, group averaged data were also used to calculate the indices which is less noisy and may provide better representation of the connectivity between the seed and targets. The results are plotted in Figure 2.2.

![Graphs showing Calinski-Harabasz Index, Davies-Bouldin Index, and Silhouette Scores for Structural (DWI) and Functional (rsfMRI) data.](image)

**Figure 2.2: Intrinsic validation of diffusion and functional clustering solutions.**

*Three internal indices for Structural (diffusion: top) and functional (rsfMRI: bottom) data. Blue straight line for all plots indicates concatenated data, while the dashed blue line indicates the group averaged data. The Red dashed line on structural plots indicates the k=5 cluster solution.*

The index results for diffusion data suggest k=5 cluster solution to be the best choice. Both concatenated and average data show a similar pattern, the dashed line (average data) yield better results for all indices. All three indices exhibit a stiff jump at k=5 compared to the neighboring cluster solution. CHI and Silhouette show a bump up at k=5, and slowly decrease to plateau afterwards. DBI indicates a sharp drop at k=5 (note that lower DBI values indicate better partitioning) as well.
In comparison to the diffusion data, the internal index results for the functional data is not very informative. The results for concatenated and average data do not coincide very much with one another and indicate \( k=2 \) as the best solution. However, qualitative comparison with an earlier study (Yuan et al., 2019) suggests \( k=3 \) overlap well with their results, hence this cluster solution for the functional data is chosen to further analyze against the probabilistic atlas as well as for sub-division specific cortical projection.

Based on the connectivity profiles, the data-driven parcellation of both the diffusion and functional data divided the basal forebrain along the rostral–caudal axis. Roughly, both subdivided the Ch123 and Ch4a/Ch4p subregion (Figure 2.1) at \( k=2 \) and further subdivided the Ch4a/Ch4p subregion at higher \( k \) solutions. However, the subdivision was not identical to each other, suggesting a dissimilarity between the two modalities. This dissimilarity in clustering solution grew stronger at higher cluster subdivisions as seen in Figure 2.3. The similarity between left and right of BF for each modality and between modalities decreases with increasing partitions (\( k \)) as well.

**Figure 2.3:** BF spectral clustering results up to \( k=5 \), chosen cluster solution for each modality is highlighted with a red rectangle (A: anterior, P: posterior).
2.4.2 Histological validation

The chosen cluster solution based on internal indices (k=5 for structural and k=3 for functional) was further analyzed for spatial overlap with the probabilistic atlas (Zaborszky et al., 2008) using the Dice Similarity Index (DSI); a statistical measure for calculating the similarity between two samples. The results are plotted in Figure 2.4.

**Figure 2.4: Spatial overlap between the probabilistic atlas (Zaborszky et al., 2008) divided in Ch123 and Ch4a/Ch4p subregions.**

Each of the subdivisions (color coded similarly to Figure 2.3) in the chosen cluster solutions using the Dice Similarity index (DSI).

Among the five subdivisions of structural parcellation, cluster-5 (purple) overlaps entirely with the anterior Ch123 subregion of the probabilistic atlas, while, cluster-3 (blue) and cluster-4 (yellow) overlap mostly with posterior Ch4a/Ch4p subregion. The remaining cluster-1 (red) covers more of the Ch4a/Ch4p subregion compared to Ch123 and cluster-2 (green) has equal overlap with both subregions of the BF atlas. The three subdivisions of the functional parcellation is clearly divided between the Ch123 and Ch4a/Ch4p with cluster-3 (blue) overlapping with Ch123 and cluster-1 (red) and cluster-2 (green) overlapping with Ch4a/Ch4p subregions.
2.4.4 **Cortical Projection of cluster subdivisions**

We then analyzed the cortical association of each subdivisions of the chosen cluster solutions for structural and functional parcellations. As shown in Figure 2.5, the cortical projection for structural parcellation (left) shows high overlap between subdivisions. Cluster-1 and cluster-2 show a similar pattern of strong visual, somatomotor, temporal, parahippocampal region correspondence, as well as frontal and anterior cingulate cortex association. Cluster-3 and cluster-4 also show strong visual, somatomotor, temporal, parahippocampal region but a reduced association with the anterior cingulate cortex. Cluster-5 exhibits a different pattern of cortical correspondence with higher frontal and anterior cingulate cortex association. For the functional parcellation (Figure 2.5 right), posterior cluster-1 is associated with several cortical regions, the anterior cingulate cortex, the parahippocampal region and the middle temporal gyrus, and the frontal gyrus. The most posterior cluster-2 shows similar but stronger anterior cingulate cortex association and less temporal and frontal gyrus, but additional somatomotor area projection. Finally, the anterior cluster-3 shows strong frontal, temporal gyrus, and parahippocampal region correspondence.

**Figure 2.5:** Cortical projection of each cluster subdivisions in structural (left) and functional (right) parcellation.
2.4.5 **Brain Network Correspondence**

Finally, the correspondence between each cluster subdivision and well-defined resting-state fMRI based 7 brain networks (Yeo et al., 2011) was measured. As shown in Figure 2.6, both structural and functional parcellation show a preferable connection with different networks among the subdivisions. For the chosen cluster solution (indicated with red circle) based on structural connectivity, cluster-1,2 and 5 show a strong connection with default mode and limbic networks. Cluster-4 shows a preferable connection with dorsal attention and visual networks along with cluster-3, but cluster-3 also shows strong connection with limbic network. For functional parcellation, cluster-1 shows almost equal connection strength to all seven networks. But cluster-2 subdivision clearly shows strong correspondence with the ventral attention network, while cluster-3 indicates a preferable connection with default mode, limbic and somatomotor networks. This pattern of preferable correspondence among the subdivisions of the cluster solution (limbic, default vs. visual and dorsal attention for structural and ventral attention vs. default, limbic and somatomotor networks in functional parcellation) is preserved in other k-cluster solutions. The results for k=2 to 5 are shown in Figure 2.6.
Figure 2.6: Spider plots showing the Yeo network (Byrge & Kennedy, 2019; Yeo et al., 2011) correspondence of each cluster subdivision in structural (top) and functional (bottom) parcellation (chosen cluster solution for each modality is highlighted with a red rectangle).
2.5 Discussion

Using high-resolution DWI and rsfMRI data, this study demonstrated that both BF structural and functional connectivity with the cortex can subdivide the BF into compartments along the anteromedial to posterolateral axis. Each subdivision of the cluster had distinct and overlapping cortical correspondence. The posterior subdivisions appeared more associated with the dorsal and ventral attention networks whereas the anterior subdivisions corresponded more with limbic and default mode networks.

The ascending BF cholinergic projections are highly branched and may reflect complex topographical organization in connectivity with the cortex (Gielow & Zaborszky, 2017; Muñoz & Rudy, 2014). Cell-type specific labeling of cholinergic BF neurons in rodents has indicated that neurons which are clustered in close proximity to one another tend to send their projections to functionally related areas in the cortex (Zaborszky et al., 2015). Extending this finding to humans, previous functional parcellation studies have also shown functional connectivity patterns in distinct BF subregions overlapping with specific cortico-cortical networks (Yuan et al., 2019). Our results further expand on these findings by showing that a structural parcellation of BF using DWI data reveals an anteromedial to posterolateral subdivisions, similar to the functional parcellation, that divides the histologically defined Ch123 from Ch4a/Ch4p. These findings suggest that mesoscopic differences in structural and functional connectivity patterns along the BF region coincide with each other.

We also observed that the structural parcellation further divided the Ch4a/Ch4p region into subcompartments. However, it is difficult to confirm the reliability of these subdivisions. Unlike for example the hippocampus, there is no clear benchmark or anatomical delineation visible on MRI images to manually segment the BF to use as ground truth reference (DeKraker et al., 2018). As an alternative, we used the existing stereotactic atlas of the BF (Zaborszky et al., 2008) as a proxy to examine the broad overlap between Ch123 vs. Ch4a/Ch4p.

We then examined how the spatial profiles of BF structural and functional connectivity relates to the known cortico-cortical networks derived from rsfMRI data. The anteromedial cluster exhibited preferential correspondence to limbic and default mode networks. The posterolateral cluster exhibited preferential connectivity to visual and dorsal...
attention networks. These results are consistent with a prior unimodal imaging study examining the BF’s functional parcellation (Yuan et al., 2019).

This study has few limitations. First, the clustering-based approach to connectivity makes it difficult to directly compare the rsfMRI and DWI with quantitative analysis. Although there were coarse-scale qualitative similarities in the structural and functional clusters, these patterns may mask subtle but meaningful intermodal divergences. Recent work suggests that estimates of structural and functional connectivity exhibit tethering in MRI data. Structure-function tethering in connectivity refers to the idea that structural and functional connectivity between brain regions are closely linked and may influence each other. In other words, the structure of the brain, such as the white matter connections between regions, can influence the functional connectivity between those regions, and vice versa. Structure-function tethering can be observed in several ways. For example, regions that are structurally connected tend to also have higher levels of functional connectivity, suggesting that the strength of structural connectivity influences functional connectivity. Conversely, regions that are functionally connected tend to be more likely to have direct structural connections, suggesting that functional connectivity may influence the development or strengthening of structural connections. We address this limitation with gradient-based analyses in Chapter 3.

Another limitation of our study is that MRI based estimates of basal forebrain connectivity are not specific to cholinergic neurons. The basal forebrain is a complex region of the brain that contains various cell types, including large projection GABAergic interneurons and glutamatergic neurons (Do et al., 2016). The GABAergic neurons produce the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) and regulate the activity of other neurons in the basal forebrain. The glutamatergic neurons produce the excitatory neurotransmitter glutamate and are involved in various functions, including the regulation of sleep and wakefulness. MRI-based estimates of basal forebrain functional and structural connectivity cannot distinguish the relative contributions of these different cell types to the observed clustering solutions. We also address this limitation in Chapter 3 by integrating DWI and rsfMRI measures with cell type specific cortical maps of the presynaptic cholinergic nerve terminals, as estimated by $^{[18F]}$ FEOBV PET.

Altogether our initial study found evidence for functional subdivisions within the magnocellular cell groups in the basal forebrain, which are known to be involved in
attention, learning, and memory processes (Ballinger et al., 2016; Sarter et al., 2006). Specifically, we found that the anteromedial magnocellular group was functionally connected with the anterior cingulate cortex, ventromedial prefrontal cortex, and hippocampus, which are regions associated with attention, emotion regulation, and memory processes. In contrast, the posterolateral magnocellular group was functionally connected with the superior parietal lobule, intraparietal sulcus, and precuneus, which are regions involved in visuospatial attention and working memory (Obermayer et al., 2019; Raghanti et al., 2008). These findings are consistent with a coarse-scale mesoscopic topographical organization of subregional BF projections according to their distinct cortical projection zones.
2.7 Reference


system and mesoscopic projectome analysis of basal forebrain cholinergic neurons. 


Raghanti, M. A., Stimpson, C. D., Marcinkiewicz, J. L., Erwin, J. M., Hof, P. R., & Sherwood, C.


Chapter 3

3 Multimodal gradients of human basal forebrain connectivity

3.1 Goals of this chapter

In the previous chapter, using clustering analyses on high-resolution diffusion-weighted (DWI) and resting-state functional (rsfMRI) data, we have demonstrated that both BF cortical structural and functional projections can subdivide the BF into compartments along the anteromedial to posterolateral axis. However, we also observed that the structural and functional parcellation results tended to become dissimilar with increasing partitions (k).

The gradient approach (Haak et al., 2018) is often considered to be more sensitive than parcellation in fMRI research because it is based on a continuous measure of functional connectivity between brain regions along a gradient of spatial organization in the brain, whereas parcellation is based on identifying discrete regions or "clusters" of the brain that exhibit synchronized activity over time. One reason why gradients may be more sensitive is that it can reveal more nuanced and subtle relationships between brain regions that may not be apparent using cluster analysis alone and could more closely resemble the actual biological situation in the regions. Cluster analysis can be useful for identifying discrete networks of brain regions that are involved in specific functions or tasks, but it might miss more subtle connections between regions that do not fit neatly into these discrete networks.

Another reason why gradients may be more sensitive is that it can help to address issues with spatial smoothing in fMRI data. Spatial smoothing is a common preprocessing step in fMRI data analysis that is used to improve the signal-to-noise ratio, but it can also blur the boundaries between functional regions in the brain. This is of particular concern for analyzing connectivity in anatomically small regions such as the subcortical nuclei of the basal forebrain. Analyses of connectivity gradients can help to mitigate this issue by examining functional connectivity along all voxels, rather than relying on discrete regions defined by spatial smoothing (Jbabdi et al., 2013). Finally, gradient based approaches enable direct quantitative comparisons of gradient maps derived from different imaging modalities such as DWI and rsfMRI. These types of analyses are critical for evaluating structure-function tethering. A

1 A version of this chapter is submitted for consideration in PNAS
number of recent studies (Vázquez-Rodríguez et al., 2019) have used gradient maps from DWI and rsfMRI to examine structure-function tethering in cortico-cortical connectivity (reviewed in greater depth below).

In this chapter, we therefore systematically examined the relationship between the structural and functional connectivities of BF cortical projections using connectivity gradients and explored the reason behind the observed structure-function dissimilarity. A version of this chapter is currently under review at Proceedings of the National Academy of Sciences (PNAS).

3.2 Introduction

The basal forebrain (BF) (Figure 3.1A) is a collection of subcortical cholinergic cell groups which provide the major sources of acetylcholine to the neocortex and hippocampus (Mesulam et al., 1983). Structurally, the ascending cholinergic projections are highly branched, with individual cells often targeting multiple different cortical areas (Do et al., 2016; Li et al., 2017; Wu et al., 2014). The total arborization of a single human cholinergic BF neuron is estimated to have a length in excess of 100 meters (Wu et al., 2014).

The organization of ascending BF cholinergic projections may reflect complex spatial topographies of connectivity with the cortex (Gielow & Zaborszky, 2017; Muñoz & Rudy, 2014). Within the BF, subregional structural changes in gray matter and white matter integrity are associated with distinct patterns of cortical degeneration and cognitive dysfunction (Kilimann et al., 2014; Nemy et al., 2020; Scheef et al., 2019; Schmitz et al., 2018; Teipel et al., 2014). In neurodegenerative diseases such as Alzheimer’s (AD), early dysfunction or loss of specific BF cholinergic fibers may alter local neuronal functions in cholinoreceptive cortical areas (Fernández-Cabello et al., 2020). Consistent with a topographical organization, axonal tracing studies suggest that BF cholinergic neurons are grouped into ensembles which target functionally interrelated cortical areas (Zaborszky et al., 2015). Moreover, patterns of functional connectivity in distinct BF subregions have been found to overlap with distinct cortico-cortical networks (Fritz et al., 2019; Markello et al., 2018; Yuan et al., 2019). Although these separate lines of evidence suggest the
cortex expresses topographies of BF structural and functional connectivity, the intermodal relationship of these topographies to one another is unknown.

How does the structural organization of cholinergic BF projections relate to their functional integration in the cortex? One possibility is that BF structural and functional connectivity is closely tethered. In tethered connections, spatially varying profiles of white matter projections and hemodynamic co-fluctuations within the BF would overlap to one another and share common cortical targets. Studies examining profiles of cortico-cortical white matter and resting state connectivity consistently observe strong intermodal tethering in unimodal cortex, which is thought to reflect a preponderance of highly myelinated short range connections among neuronal populations with similar functional repertoires (Paquola et al., 2019; Vázquez-Rodríguez et al., 2019). Alternatively, BF structural and functional connectivity may diverge from one another, exhibiting little overlap within the BF and distinct cortical fingerprints. In cortico-cortical connectivity, this profile of structure-function detethering is observed in the association cortex, where weakly myelinated longer range connections provide neuronal populations with greater integration and a more diverse functional repertoire (Paquola et al., 2019; Vázquez-Rodríguez et al., 2019).

Here we addressed the relationship between structural and functional connectivity in the ascending basal forebrain projections. We used multimodal imaging combining high resolution 7 Tesla (7T) diffusion (dMRI) and resting-state functional MRI (rsfMRI) in a cohort of 173 individuals from the Human Connectome project (Van Essen et al., 2013). We derived gradients of the BF in each modality (Vos de Wael et al., 2020) employing diffusion map embedding to elucidate fine-grained continuous maps of its connectivity. To quantify intermodal tethering, we computed the residual variance between (a) the gradients of structural and functional connectivity within the BF and (b) the expression of these gradients on the cortical surface. This method allowed us to ask if spatial topographies of BF structure and function overlap one another and whether the degree of this spatial overlap is homogeneous or inhomogeneous across different BF subregions. We found greater inhomogeneity in structure-function tethering moving from anteromedial to
posterolateral BF, with the strongest inhomogeneities localized in the nucleus basalis of Meynert (NbM).

Next, we explored the spatial overlap of the structural and functional gradients with known cortico-cortical networks, focusing on the most dominant BF gradient observed in each imaging modality and computing their gradient-weighted cortical maps. Similar to the BF gradients, we quantified the similarity between the structural and functional gradient-weighted cortical maps by calculating their residuals. The resulting residual cortical map exhibited pronounced dissimilarity in the transmodal cortex associated with the ventral attention network. Finally, we examined what may account for this structure-function detethering by examining its spatial relationships to: (a) the cortical geodesic distances from BF, (b) cortical myelination estimated from the T1w/T2w ratio (Glasser & Van Essen, 2011), and (c) the cortical concentrations of cholinergic nerve terminals estimated from cell type specific molecular imaging of the presynaptic vesicular acetylcholine transporter (VACHT). We found that the higher BF structure-function detethering corresponds to shorter distance from the BF, lower myelination and relatively higher concentrations of cholinergic innervation.

### 3.3 Materials and Methods

We used high-resolution minimally pre-processed 7T MRI HCP data (n=173) (Glasser et al., 2013) and the existing stereotactic atlas of the BF (Zaborszky et al., 2008) to build structural and functional connectomes. Any further pre-processing and connectivity matrix construction was done on the compute cluster. Workflows were built using Snakemake (Mölder et al., 2021) with the full workflow available on GitHub (see data and code availability for specifics). Individual connectomes were averaged and reduced to a 2-dimensional $m$-by-$n$ matrix describing the pairwise connectivity strength between $m$ BF ROI voxels and $n$ cortical regions (Glasser, Coalson, et al., 2016). The BrainSpace toolbox (Vos de Wael et al., 2020) was used to capture the gradients which, as well as any further analysis, was done using Jupyter Notebook (Kluyver et al., 2016).
3.3.1 Data Acquisition

High-resolution 7T dMRI and rsfMRI data were downloaded from the HCP data repository (Van Essen et al., 2013). We used the minimally pre-processed data described in ref (Glasser et al., 2013) consisting of 173 healthy subjects (69 male, 104 female) aged 22 to 35 years. The dMRI images were collected with 1.05 mm³ isotropic voxels, TR=7000 ms, TE=71.2 ms, b-values=1000, 2000 s/mm², FOV=210 x 210 mm². Resting-state fMRI images were collected with 1.6 mm³ isotropic voxel size, TR=1000 ms, TE=22.2 ms, FOV=208 mm², spanning 4 runs of 16-minute duration each, per subject. For anatomical imaging, two T₁-weighted (T₁w) scans were obtained using a three-dimension (3D) magnetization-prepared rapid gradient-echo (MPRAGE) (Mugler & Brookeman, 1990) sequence and two T₂-weighted (T₂w) images using a 3D T₂-SPACE sequence, all with identical geometries and a 0.7 mm³ isotropic voxel size. Full details of the acquisition parameters can be found in the HCP S1200 release reference manual (March, 2018).

3.3.2 Basal Forebrain Mask

The basal forebrain (BF) region-of-interest (ROI) was created using the existing stereotactic atlas of the BF (Zaborszky et al., 2008). This stereotactic BF atlas is based on histological sections obtained from 10 postmortem brains, the magnocellular cell groups were delineated in each slice, 3D reconstructed and warped into the MNI single-subject reference space (Collins et al., 1994). The atlas consists of 4 subregions of the BF defined in the nomenclature: Ch1-2, Ch3, Ch4, and Ch4p. For each subregion, a stereotactic probabilistic map has a range of 0 to 10 indicating the number of brains containing the specific magnocellular cell groups in the given voxel. Our BF ROI is created by thresholding these subregion masks to 50% first and then combining all to get a mask covering full BF. This BF ROI mask was then warped into MNI152 non-linear 6th generation atlas (MNI152NLin6Asym) (Collins et al., 1994).

3.3.3 Structural Connectivity Reconstruction

Diffusion tractography was performed to get a connectivity matrix for diffusion data. As part of the minimal preprocessing pipeline data release, all subjects underwent FreeSurfer processing (v5.3.0-HCP) (HCPpipelines: Processing Pipelines for the HCP, n.d.). The BF ROI mask was then first resampled and
transformed to the individual subjects’ minimally preprocessed volume space (0.7mm$^3$). Volumetric cortical labels were built by mapping the HCP-MMP 1.0 surface parcellation (Glasser, Coalson, et al., 2016) using Connectome Workbench’s ribbon-constrained *label-to-volume-mapping* function and FreeSurfer-derived surfaces. The BF ROI voxels were used as seeds, and the 180 cortical regions in each hemisphere were combined and used as targets to perform probabilistic tractography using FSL’s *probtrackx* (Behrens et al., 2007) with 5000 streamlines per BF ROI voxel. The resulting probability maps in the BF quantified the number of streamlines that reached each target. The maps were resampled to MNI space (Grabner et al., 2006) in 1.6mm$^3$ resolution to match the functional connectivity matrix and reduced to a 2-dimensional $m$-by-$n$ matrix, where $m$ represents the voxels in the BF ROI (599 voxels) and $n$ is the cortical targets (180 each hemisphere) with their corresponding number of streamlines. This $m$-by-$n$ connectivity feature matrix for all 173 subjects was averaged to calculate the gradients.

### 3.3.4 Functional Connectivity Reconstruction

First, the BF ROI mask in the minimally preprocessed volume space was resampled to 1.6mm$^3$ isotropic voxel size to match resolution of the rsfMRI data and added to the subject’s subcortical parcellation. A functional connectivity matrix was then created for each subject by calculating the temporal correlation between BF voxels and cortical ROIs. All four runs (i.e., two sets of 16 min. runs with posterior-to-anterior and anterior-to-posterior phase-encoding) of the minimally preprocessed and ICA-FIX denoised rsfMRI data (Griffanti et al., 2014) were used. Since the BF ROI is not included in the dense timeseries provided by HCP, these were regenerated using the updated subcortical parcellation to include the BF ROI voxels for further processing. Subsequent processing included ROI-constrained subcortical smoothing to match the cortical sampling density using the scripts provided by HCP (*HCPpipelines: Processing Pipelines for the HCP*, n.d.), as well as additional signal filtering (i) based on the average WM and CSF timeseries using *ciftify* (Dickie et al., 2019) and (ii) by applying a Wishart filter as proposed previously (Glasser, Smith, et al., 2016; Tian et al., 2020) to selectively smooth unstructured noise more than the structured blood oxygen level-dependent signal. Average cortical ROI timeseries (concatenated across runs) were then extracted using the HCP-MMP 1.0 surface...
parcellation (Glasser, Coalson, et al., 2016). Functional correlation maps were calculated by calculating the Pearson’s correlation coefficient for each voxel within the BF to each of the cortical parcels. The resulting correlation maps were reduced to a 2-dimensional $m$-by-$n$ matrix, where $m$ represent the voxels in the BF ROI (599 voxels) and $n$ are the cortical targets (180 each hemisphere) with their corresponding functional correlation. This $m$-by-$n$ connectivity matrix for all 173 subjects were averaged over subjects to calculate group-wise gradients.

3.3.5 Gradient Calculation

Connectivity gradients were calculated using the BrainSpace toolbox (Vos de Wael et al., 2020). Group averaged connectivity matrices were used as input to the GradientMaps function, using the normalized angle kernel and diffusion map embedding approach. This nonlinear dimension reduction method transforms the connectivity matrix into a low-dimensional representation to construct connectivity gradients (Coifman et al., 2005). BF voxels that are characterized by similar connectivity patterns will have a gradient value closer together, whereas voxels with litter or no similarity are farther apart. These gradients were then mapped back onto the BF voxel space to visualize transitions in functional and structural connectivity patterns.

In addition, gradient-weighted cortical maps were created by multiplying each row of the BF-cortical connectivity matrix with the corresponding gradient value of that BF voxel (Guell et al., 2020; Katsumi et al., 2023) (Supplemental Figure 3.1). The distribution of cortical gradient-weighted values was then decomposed into seven functional networks (Yeo et al., 2011) using the HCP-MMP 1.0 parcellation-based Yeo networks as defined in previous works (Byrge & Kennedy, 2019; Yeo et al., 2011). These networks include visual, somatomotor, dorsal attention, ventral attention, limbic, frontoparietal, and default mode.

3.3.6 Weighted Residual map of BF

Weighted residual values for each of the BF voxels results were reconstructed by regressing the structural gradients against the functional gradients of BF and computing the residuals. First, residuals of all the pairs of selected gradients were calculated. For each pair of structure-function correlation, a combined weight was computed by adding the variance of corresponding structural and functional gradient
components. This weighting was multiplied by the squared residual values and all 24 pairs were summed to produce the average weighted residual map of BF:

$$\bar{x} = \sum_{k=1}^{n} (w_i, k + w_j, k)x_k^2$$

where $k$ is the structure-function pair, $w_i$ is the explained variance of structural gradient and $w_j$ is the explained variance of functional gradient, $x_k$ is the residual values of $k$ structure-function pair, and $n$ is the number of structure-function pairs (i.e. 24).

### 3.3.7 Geodesic Distance

Geodesic distance along the cortical surface was calculated using the geodesic library ([https://github.com/the-virtual-brain/tvb-gdist](https://github.com/the-virtual-brain/tvb-gdist)) based on the algorithm that approximates the exact distance along the shortest path between two nodes (or vertices) on a triangulated surface mesh (Mitchell et al., 1987). An average BF seed node was created for the left and right hemispheres separately by (i) projecting the BF mask onto the 59k_fs_LR white matter surface of the individual subjects using Connectome Workbench’s `volume-to-surface-mapping` function, (ii) averaging across all subjects to get a probability map, (iii) resampling to the 10k_fsavg surface-space as suggested by the HCP study ([https://wiki.humanconnectome.org](https://wiki.humanconnectome.org)) and (iv) then by thresholding at 0.5 to obtain a final binary BF seed on the cortical surface. A distance value was then assigned to each cortical vertex based on the minimum geodesic distance along the 10k_fsavg pial surface to the BF seed node, hereby avoiding the medial wall. To match with the resolution of the cortical connectivity results, the geodesic distance map was parcellated using the HCP-MMP 1.0 atlas as implemented in the neuromaps toolbox (Markello, Hansen, Liu, Bazinet, Shafiei, Suárez, Blostein, Seidlitz, Baillet, Satterthwaite, Mallar Chakravarty, et al., 2022), and rescaled to values between 0 and 1 (Pedregosa et al., 2011).

### 3.3.8 PET $[^{18}\text{F}]$FEOBV maps

Positron emission tomography (PET) data were chosen to compare with our geodesic distance map (Figure 3.3A left) and gradient-weighted cortical residual map (Figure 3.2C top). These are $[^{18}\text{F}]$fluoroethoxybenzovesamicol ($[^{18}\text{F}]$FEOBV) imaging
data targeting the vesicular acetylcholine transporter (VACht) protein (Albin et al., 2018; Kanel, van der Zee, Sanchez-Catasus, et al., 2022). Each individual $[^{18}\text{F}]$FEOBV PET image was intensity normalized to the subject’s supratentorial white matter uptake to create a parametric $[^{18}\text{F}]$FEOBV PET image (Kanel, van der Zee, & Sanchez-Catasus, 2022). The original PET atlases were transformed to 10k_fsavg surface-space and parcellated to HCP-MMP 1.0 surface (Glasser, Coalson, et al., 2016). The values of each cortical parcel encoding the relative concentration of cholinergic nerve terminals were rescaled (Pedregosa et al., 2011) and visualized on an inflated surface (Figure 3.4A). Spatial spin tests (Alexander-Bloch et al., 2018; Markello, Hansen, Liu, Bazinet, Shafiei, Suárez, Blostein, Seidlitz, Baillet, Satterthwaite, Mallar Chakravarty, et al., 2022) were used to statistically quantify the relationship between the geodesic distance map and cortical residual map. Additional FEOBV maps (Aghourian et al., 2017; Bedard et al., 2019) were obtained from the Neuromap toolbox (Markello, Hansen, Liu, Bazinet, Shafiei, Suárez, Blostein, Seidlitz, Baillet, Satterthwaite, Mallar Chakravarty, et al., 2022) to examine the reproducibility of our results.

### 3.3.9 Myelin Map

Individual T$_1$-weighted divided by T$_2$-weighted ($T_1w/T_2w$) as a proxy measure for intracortical myelin maps made available in the HCP minimally-preprocessed data (Glasser & Van Essen, 2011), were averaged across subjects to create a group myelin map. This group myelin map was then transformed to the 10k_fsavg surface space and parcellated using the HCP-MMP 1.0 atlas and values were rescaled for quantification of its relationship with cortical residual map.

### 3.3.10 Statistical analyses

Permutation tests with surrogate maps (Burt et al., 2020) were used to compute statistical significance for the BF gradients and the distribution of residuals. BF gradient values for structural and functional connectivities were first rescaled between 0 and 1 and Euclidean distance was used to calculate the distance matrix among all voxels within the original BF ROI (Pedregosa et al., 2011). Variograms were then permuted (N=1000) using the SurrogateMaps function implemented in the BrainSpace toolbox (Vos de Wael et al., 2020). Parameters were adjusted in the case of a suboptimal fit compared to the empirical data (pv=60, random_state=1234). The
final variograms were used to build and compare mean and variation null probabilities between BF subregions.

Spin tests (Alexander-Bloch et al., 2018), as implemented in the neuromaps toolbox (Markello, Hansen, Liu, Bazinet, Shafiei, Suárez, Blostein, Seidlitz, Baillet, Satterthwaite, Chakravarty, et al., 2022), were used to compare cortical maps based on N=10k permuted maps. All cortical maps were parcellated using the HCP-MMP 1.0 atlas (Vos de Wael et al., 2020) and values were rescaled between 0 and 1 (Pedregosa et al., 2011). In addition to the spin tests, a bootstrapping analysis was performed to quantify the difference between structural and functional connectivity and their correlation with geodesic distance. Here, bootstrapping was applied 10k times (by randomly selecting sets of regions during each iteration) to build a null probability of correlation coefficients for statistical inference based on the empirical difference between the two modalities.

3.3.11 Data and code Availability

The Human Connectome (HCP) project dataset is available at http://www.humanconnectomeproject.org/. The workflow for reconstructing structural connectivity matrix from the HCP data and a subcortical region of interest is available at https://github.com/sudesnac/diffparc-smk (Khan & Chakraborty, 2023); and the functional connectivity workflow at https://github.com/khanlab/subcorticalparc-smk (Kai et al., n.d.). All other code used to conduct the reported analyses and create the figures are available at https://github.com/sudesnac/HumanBF-Connectivity (Chakraborty, n.d.).

3.4 Results

We used high-resolution 7T MRI HCP data (n=173) (Glasser et al., 2013) and a widely used stereotactic atlas of the BF (Zaborszky et al., 2008) to build structural and functional connectomes. Individual connectomes were averaged and reduced to a 2-dimensional $m$-by-$n$ matrix, where $m$ represent the voxels in the BF ROI and $n$ are the cortical targets (Glasser, Coalson, et al., 2016) with their corresponding connectivity strengths. To capture the gradients, we used diffusion embedding - a nonlinear dimension reduction approach that identifies multiple axes of variation in
connectivity along the BF voxels - separately for structural (dMRI) and functional (rsfMRI) connectivity matrices (Vos de Wael et al., 2020).

3.4.1 Primary structural and functional BF gradients

The first gradient for both structural and functional connectivity data explained the most (30%) variance (Figure 3.1B) followed by a reduction in explanatory power by 50% (~15%) for the second gradient. Given this dominance of the first gradients, we therefore focused on the first, principal gradient in each modality. In both the structural and functional gradient, a smooth gradient transition from anteromedial to posterolateral BF was observed (Figure 3.1C). We then examined if this anteromedial-to-posterolateral gradient patterns differentiated known histologically defined subregions of the BF (Figure 3.1A), namely the Ch123 subregion containing the septal nucleus and diagonal band of Broca (in red), versus the Ch4a/Ch4p subregion containing the NbM (in green).

We then examined if the distributions of gradient values within Ch123 and Ch4a/Ch4p differed from one another using permutation tests with fitted surrogate maps (see Methods). We performed tests to compute the difference in means and difference in coefficients of variation (CoV) between Ch123 and Ch4a/Ch4p. The mean gradient values were significantly different between these two subregions for structural (mean difference=-0.336, \( p_{\text{perm}}=0.05 \)) and functional (mean difference=-0.469, \( p_{\text{perm}}=0.003 \)) connectivities. The Ch123 region exhibited higher overlap with the gradient lower bound (blue) whereas the Ch4a/Ch4p exhibited higher overlap with the gradient upper bound (red) (Figure 3.1D). There was no significant difference in variance for both structural (\( p_{\text{perm}}=0.28 \)) and functional (\( p_{\text{perm}}=0.36 \)) primary gradients (sG1 and fG1, respectively). Difference in coefficients of variation suggested Ch123 has significantly higher CoV than Ch4a/Ch4p for both sG1 and fG1 (\( p_{\text{perm}}<0.001 \)).
Figure 3.1: Structural and functional gradients across BF.

(A) A 3D view of histologically defined BF subdivisions defined in (Zaborszky et al., 2008) projected on glass brain. (B) Scree plots showing the variance explained by each component of the gradients in structural (left) and functional connectivity (right). (C) The first principal gradient of the BF based on structural (sG1; left) and functional (fG1; right) connectivity both revealed an anteromedial to posterolateral axis. Lower bound of gradient values are represented by blue (and –) while the upper bound is represented by red (and +). (D) Strip plots showing the distribution of BF structural (sG1; left) and functional (fG1; right) gradient distribution within the C123

62
and C4a/Ch4p stereotactic BF subregions (Zaborszky et al., 2008). (E) Pairwise $R^2$ heatmap of the structural (6 components) and functional (4 components) BF gradients, significant pairs are bolded ($p<0.002$). (F) Weighted residual values for each of the BF voxels were reconstructed from calculating all the pairwise correlation between structural and functional BF gradients (see Methods). Maximum values (red) indicate divergence between structural and functional BF connectivity. (G) Strip plot showing the distribution of the weighted residuals within the C123 and C4a/Ch4p stereotactic BF subregions (Zaborszky et al., 2008).

3.4.2 Multimodal gradients of BF structure-function relationship

We next examined the magnitude of shared variance, or tethering, between BF structural and functional gradients to determine their spatial similarity to one another. Although sG1 and fG1 represent the most explanatory intra-modal gradients, they are not necessarily the most explanatory pair of gradients in terms of shared intermodal variance. It could be the case, for example, that sG1 and fG2 share more spatial similarity than sG1 and fG1. To explore this possibility, we computed the voxelwise intermodal associations ($R^2$ value) for all structural and functional gradients whose initial components fell above the variance plateau (Figure 3.1B). This yielded a matrix of 24 structure-function gradient pairs (sG1-6 and fG1-4). Figure 2E shows the heatmap matrix of $R^2$ values for each pairwise structure-function gradient (significant ones are bolded $p<0.002$). The shared variance across all 24 pairs was low (mean $R^2=0.05$). However, the range of $R^2$ varied from $1.4 \times 10^{-4}$ to 0.23.

We then computed voxelwise regressions for each of the 24 structure-function gradient pairs in this matrix and extracted their corresponding residuals, which quantify the magnitude of unexplained variance for that pair. All 24 pairs of residuals were weighted according to the initial variance explained (Figure 3.1B) for that pair and then summed to produce a weighted average residual map encoding BF structure-function detethering (see Methods). This weighted average residual map was projected back to BF voxel space, which revealed an anteromedial to posterolateral topography, with the highest structure-function detethering localized in posterolateral subregions (Figure 3.1F). Examining this detethering pattern according to the histologically defined boundaries of Ch123 and Ch4a/4p...
(Zaborszky et al., 2008), the mean weighted residual values were not different between the two subregions (mean difference=-0.008, p<sub>perm</sub>=0.40) (Figure 3.1G). However, the residual values in Ch4a/Ch4p exhibited significantly greater variability in comparison to Ch123 (CoV difference=-34.78, p<sub>perm</sub>=0.01). This latter finding suggests that structure-function detethering within Ch4a/Ch4p was more inhomogeneous than Ch123.

3.4.3 The cortical expression of BF structural and functional gradients

We next computed gradient-weighted cortical maps (Guell et al., 2020; Katsumi et al., 2023) to determine how BF gradients were expressed by the cortex. The gradient-weighted cortical maps were created by multiplying each row of the initial connectivity matrix (<sup>BF</sup> voxels x <sup>cortical</sup> parcels) with the corresponding sG1 or fG1 value to create a gradient-weighted connectivity matrix (<sup>BF</sup> voxels x <sup>cortical</sup> parcels). Finally, all rows of this gradient-weighted matrix (i.e. <sup>BF</sup> voxels) were averaged to produce a single cortical representation of the particular gradient (see Supplemental Figure 3.1 and Methods).

For the gradient-weighted cortical map corresponding to sG1 (sG1ctx; Figure 3.2A top), we observed a smooth macroscale transition from the anteromedial to posterolateral cortical surface. By contrast, the gradient-weighted cortical map corresponding to fG1 (fG1ctx; Figure 3.2B top) exhibited a more patch-like pattern. We then examined if the spatial topographies of sG1ctx and fG1ctx exhibited any relationship to the spatial topographies of intrinsic cortico-cortical resting state networks (Yeo et al., 2011). To do so, we examined the distributions of gradient values captured by each of 7 macroscale resting state networks covering the entire human cerebral cortex (Figure 3.2AB bottom).

A pattern in which the distributions of these gradient values are well delineated from one another across different networks would be consistent with a high level of topographic mapping between specific networks and specific spatial locations along the anteromedial to posterolateral axis of the BF. A pattern in which the distributions of weights are more spread out and overlapped across different networks would be consistent with low topographic mapping. For both sG1ctx and fG1ctx, we observed an intermediate pattern of delineation, which was stronger for some networks than others. For example, we observed greater spread across the
default mode, limbic, ventral attention and frontoparietal networks. A common feature of these networks is that they have hubs located exclusively in the transmodal cortex (see supplemental Figure 3.2 for the distribution of the 7 networks on the cortical surface).

As done for the BF gradients, we quantified the similarity between the structural and functional gradient-weighted cortical maps by calculating their pairwise unexplained variance (i.e., residuals) across cortical parcels. The resulting residual cortical map (Figure 3.2C top) exhibited increasing dissimilarity moving from unimodal to transmodal cortex, with highest dissimilarity in the anterior cingulate cortex. These cortical parcels with greater structure-function dissimilarity tended to overlap primarily with the ventral attention network (Figure 3.2C bottom).

**Figure 3.2:** Structural and functional gradient-weighted cortical maps and their relationship.

(A) Structural G1-weighted map projected to the cortical surface (top), the black dot represent the BF seed, lower bound of G1-weighted gradient values are represented by blue (and –) while the upper bound is represented by red (and +); and histogram plot (bottom) showing the distribution of G1-weighted gradient values separately for
each of the 7 networks color-coded based on the ref (Yeo et al., 2011) (Supplemental Figure 3.2). The networks are ordered by the mean values. (B) Functional G1-weighted cortical map (top) and histogram plot (bottom) of network distribution ordered by the mean values. (C) Weighted cortical residual map calculated similarly to weighted residual map of BF (Figure 3.1F; see Methods) and the corresponding distribution of the residual values for each of the 7 networks ordered by their mean values.

3.4.4 BF structure-function tethering is shaped by cortical geodesic distance and myelination

What could be the reason behind the observed structure-function detethering in the transmodal cortex? Structure-function tethering accounts of cortico-cortical connectivity propose that the divergence between a cortical area’s functional and structural connectivity increases as a function of its geodesic distance from unimodal sensory cortex (Buckner & Krienen, 2013; Margulies et al., 2016; Vázquez-Rodríguez et al., 2019). This is due to cortical expansion, which disproportionately affects the more recently evolved association cortices. Under this account, phylogenetically newer cortical areas are less constrained by the short-range wiring of sensory cortex, yielding higher levels of functional integration and divergence from structural connectivity, particularly among areas in the frontoparietal cortex. One possibility is that, like increasing geodesic distance from the unimodal cortex, increasing geodesic distance from BF would be associated with increased detethering of structure and function. However, our residualized BF gradient weighted cortical maps suggest a striking inversion of this pattern (Figure 3.2C top). At closer rather than farther distances from the BF, structure and function were more detethered (more unexplained variance between modalities), with the most unexplained variance in proximal hubs of the ventral attention network. By contrast, the least unexplained variance was concentrated in distal visual and somatomotor networks.

To more directly explore constraints of distance on BF-cortical connectivity, we examined our original structural (number of white matter streamlines) and functional (Pearson r, encoding hemodynamic correlations) BF seed-based
connectivity maps in relation to the intrinsic geometry of the cortex measured by geodesic distance from BF to each cortical parcel. We started with creating an approximate BF seed label on the cortical surface. This is done by sampling the original volumetric BF mask onto the subject’s white matter (WM) surface across all subjects, average them to get a probability map and then thresholded for a final binary BF label on the cortical surface (see Methods). This BF seed was then used to calculate the minimum geodesic distance between all points on the cortical surface and the seed. Finally, since our seed-based connectivity maps are parcellated based on the HCP-MMP 1.0 (Glasser, Coalson, et al., 2016) this geodesic distance map was parcellated using the same surface atlas which is visualized on the inflated cortical surface (Figure 3.3A left). We then quantified the spatial relationships of the geodesic distance map with each modality-specific connectivity map using spin tests against spatial null models (Alexander-Bloch et al., 2018; Markello, Hansen, Liu, Bazinet, Shafiei, Suárez, Blostein, Seidlitz, Bailet, Satterthwaite, Mallar Chakravarty, et al., 2022). We found that the relationship of BF-cortical structural connectivity with BF-cortical geodesic distances was negligible (R=-0.02, p_{spin}=0.96). By contrast, a significant negative correlation was detected between BF-cortical functional connectivity and BF-cortical geodesic distances (R=-0.67, p_{spin}=0.01). The magnitude of this association was significantly higher than that observed for structural connectivity (average difference of -0.624 between correlation coefficients with 95% CI [-0.51,-0.73], p_{boot}<0.001, as revealed by bootstrap analysis). The strength of BF connectivity diverged more strongly between modalities in transmodal cortical areas at smaller geodesic distances from BF (Figure 3.3A right). Hence, these findings provide quantitative support for our observation that structure-function detethering tends to increase in cortical areas at decreasing geodesic distances from BF.

A second and related explanation given for structure-function detethering comes from work examining the relationships of rsfMRI measures of cortico-cortical connectivity with cortical myelin content (Huntenburg et al., 2017; Vázquez-Rodríguez et al., 2019). From this work, close tethering between structural and functional connectivity is consistently observed among areas in unimodal cortex, where temporal co-fluctuations in hemodynamic responses coincide with highly myelinated short-range white matter connections. Transitioning to association...
cortices, function detethers from structure as hemodynamic co-fluctuations among different cortical areas increasingly reflect weakly myelinated long-range connections. We therefore examined if structure-function detethering of BF gradient weighted cortical maps also reflected cortical myelin content. To do so, we examined the spatial relationship between our gradient-weighted cortical residuals map (Figure 3.2C top) and a group averaged map of cortical myelin content (Glasser, Coalson, et al., 2016) using spin tests (Alexander-Bloch et al., 2018; Markello, Hansen, Liu, Bazinet, Shafiei, Suárez, Blostein, Seidlitz, Baillet, Satterthwaite, Mallar Chakravarty, et al., 2022). Individual myelin maps provided by the HCP (Glasser & Van Essen, 2011) were averaged across subjects to create a group myelin map. This group myelin map was then transformed to the 10k_fsavg surface space and parcellated using the Glasser atlas (Glasser, Coalson, et al., 2016) and projected on the inflated surface (Figure 3.3B left). Consistent with patterns observed for cortico-cortical connectivity, we found that the highest magnitudes of BF detethering were localized to the most weakly myelinated areas of transmodal cortex (R=-0.355, p_{spin}=0.001; Figure 3.3B right).

Figure 3.3: Multimodal connectivity in relation to cortical geodesic distance and myelination.
(A) Parcellated (Glasser, Coalson, et al., 2016) geodesic distance (left) from the cortical BF label (black spot) to each point on the cortical surface, darker red indicating farther geodesic distance from the BF seed and scatter plot (right) of the structural (blue) and functional (orange) seed-based connectivity against the geodesic distance demonstrating significant negative correlation for the functional connectivity but no relationship with structural connectivity. Each point in the scatter plot represents cortical parcels based on Glasser parcellation (Glasser, Coalson, et al., 2016) and spin test using spatial null model (Alexander-Bloch et al., 2018) results are reported in the box corresponding to the scatter plots. (B) Parcellated T1w/T2w ratio (myelin) map provided by the HCP (Glasser & Van Essen, 2011) and averaged across subjects with the cortical BF label indicated by black dot, red color indicating stronger myelination while blue indicates weaker (left); and scatter plot of weighted cortical residual map against the myelin map showing significant negative relationship (right). Each point in the scatter plot represents cortical parcels and is color-coded by the 7 network (Yeo et al., 2011) identical to Supplemental Figure 3.2.

3.4.5 BF structure-function tethering reflects the density of cortical cholinergic innervation

Why might structure and function diverge in weakly myelinated cortical regions situated at closer distances to BF? Due to its anteromedial location in the brain, the BF is closer to many transmodal cortical areas than the unimodal cortex. However, this proximity to transmodal cortical areas does not explain why BF structure and function would exhibit closer tethering in cortical areas at greater geodesic distances. One possibility is that the number of axon terminals (branches) per cholinergic neuron varies from cell to cell in the BF. Under this account, cortical areas expressing divergent structure-function tethering with BF may receive more inputs per cholinergic neuron (cells with more branches), while cortical areas expressing closer structure-function tethering with BF may receive fewer inputs per cholinergic neuron (cells with fewer branches).

We tested this hypothesis using in vivo positron emission tomography in combination with the $[^{18}\text{F}]$ FEOBV (Albin et al., 2018; Kanel, van der Zee, Sanchez-Catasus, et al., 2022), a radiotracer which binds to the vesicular
acetylcholine transporter (VACHT). The VACHT is a glycoprotein expressed solely by cholinergic neurons, with the highest density of binding sites on the presynaptic terminals. We acquired intensity normalized distribution maps of \[^{18}F\]FEOBV binding from a group of healthy cognitively normal young adults (N=13; mean age=24.54, 3 females) (Kanel, van der Zee, & Sanchez-Catasus, 2022), and produced an average map representing the BF cortical cholinergic projectome (Figure 3.4A; see Methods).

Using spin tests against spatial null models (Alexander-Bloch et al., 2018; Markello, Hansen, Liu, Bazinet, Shafiei, Suárez, Blostein, Seidlitz, Baillet, Satterthwaite, Mallar Chakravarty, et al., 2022), we extracted the cortical expression of BF structure-function detethering (cortical residualized map from Figure 3.2C top) and cholinergic innervation estimated from VACHT concentrations using the common cortical parcellation (Glasser, Coalson, et al., 2016). Consistent with our hypothesis that BF neurons are diverse in terms of branch complexity, we found that cortical areas exhibiting greater divergence in BF structure-function tethering also exhibit greater density of BF cholinergic input, i.e. higher VACHT concentration (R=0.28, \(p_{\text{spin}}=0.02\); Figure 3.4B). Geodesic distances from the BF to cortex also reflected the spatial distribution of the cortical cholinergic innervation: cortical areas closer to the BF tended to express higher VACHT concentrations (R=−0.592, \(p_{\text{spin}}=0.0003\); Figure 3.4C). We replicated these associations with three other atlases of FEOBV PET publicly available (Markello, Hansen, Liu, Bazinet, Shafiei, Suárez, Blostein, Seidlitz, Baillet, Satterthwaite, Mallar Chakravarty, et al., 2022) and found similar results of showing significant (\(p_{\text{spin}}<0.05\)) positive spatial correlation with cortical residual maps for all three VACHT maps and significant negative spatial correlation with geodesic distance in two of the maps (Supplemental Figure 3.3).
Figure 3.4: Cortical cholinergic innervation in relation to cortical residual map and geodesic distance.

(A) Parcellated and rescaled FEOBV PET map, pink indicating higher values while sky blue color indicating lower with the cortical BF label indicated by black spot. (B) Scatter plot against the weighted cortical residual map indicating positive correlation. Each point in the scatter plot represents cortical parcels based on HCP-MMP 1.0 parcellation (Glasser, Coalson, et al., 2016) and is color-coded by the 7 network (Yeo et al., 2011) identical to Supplemental Figure 3.2. Spin test using spatial null model (Alexander-Bloch et al., 2018) results are reported in the box corresponding to the scatter plots. (C) Scatter plot of the FEOBV PET map against the geodesic distance showing significant negative relationship.

Altogether our findings reveal a multimodal gradient in the human cerebral cortex which expresses both BF connectivity and cholinergic innervation. Along this gradient, cortical areas which express divergent BF structure-function coupling, shorter distances from the BF and weaker myelination receive dense cholinergic innervation from highly branched neurons (Figure 3.5). These areas closely resemble hubs of the ventral attention network. Hubs of the dorsal attention and default mode networks express an intermediate profile in this gradient. By contrast, cortical areas which express convergent BF structure-function coupling, larger distances from the BF and stronger myelination receive sparse cholinergic innervation from neurons with fewer branches. These areas tend to overlap the primary visual and sensorimotor cortex, suggestive of a hierarchical organization in BF cholinergic innervation.
3.5 Discussion

Using high resolution dMRI and rsfMRI, we observed gradients of BF structural and functional connectivity with the cortex. The BF white matter
projections and temporal co-fluctuations with neuronal populations in the cortex both change along a mesoscopic axis from anteromedial to posterolateral BF. Although the axes of these BF structural and functional gradients are qualitatively similar to one another, quantitative comparison of their spatial organization revealed localized structure-function divergences, with the strongest detethering concentrated in the posterolateral NbM. Examination of where structure-function divergences were most strongly expressed in the cortex revealed a set of regions overlapping hubs of the ventral attention network. Further analyses of cortical properties thought to shape structure-function tethering, including interregional geodesic distances and myelin content, revealed novel insights into how BF-cortical connectivity differs from cortico-cortical connectivity. We found that structural and functional connectivity is more divergent in cortical areas at closer, as opposed to farther, geodesic distances from the BF. These proximal cortical areas tend to be weakly myelinated. To determine what features of BF connectivity may account for this unexpected pattern, we combined our multimodal MRI analyses of BF connectivity with in vivo PET imaging of VACHT, a cell type specific marker of the presynaptic BF cholinergic cortical projectome. We found that cortical areas with higher VACHT tend to express greater BF structure-function detethering, consistent with a highly branched innervation whereby individual BF cholinergic neurons target diverse cortical areas with numerous axonal collaterals.

Cell type specific labeling work in non-human animal models indicates that the axonal projections of BF cholinergic neurons vary in terms of their branch complexity, both in terms of total number of branches per cell and diversity of cortical targets (Bloem et al., 2014; Chandler et al., 2013; Do et al., 2016; Li et al., 2017; Wu et al., 2014). Our findings in living humans indicate that this diversity in branch complexity is reflected by BF structure-function tethering, geodesic distance from the BF, myelin content and VACHT concentration (Muñoz & Rudy, 2014). Cortical areas receiving cholinergic input from highly branched neurons exhibit divergent BF structure-function coupling, closer proximity to BF, lower myelin content and higher VACHT concentration. These areas were located exclusively in the transmodal cortex. The populations of BF cholinergic neurons providing these highly branched projections tend to be located in the posterolateral NbM, where structure-function
gradient divergences were higher and more variable than the medial septum and diagonal band of Broca (Figure 3.1FG).

In rodents, the medial septum and diagonal band are as large as the NbM. In macaques and humans, by contrast, the NbM is considerably larger than medial septum and diagonal band (Semba, 2004). The human NbM is also more densely populated with choline-o-acetyltransferase (ChAT) expressing cholinergic neurons (~90% of cells) compared to medial septum (10%) and diagonal band (max ~70%) (Kasashima et al., 1998; Mesulam et al., 1983; Mesulam & Geula, 1988). The phylogenetic structural progression of the NbM’s size and complexity may reflect the evolutionary expansion of the transmodal cortical areas it projects to (Galvin et al., 2018; Semba, 2004). Nevertheless, the human NbM is estimated to contain only about 400,000 cholinergic neurons (Arendt et al., 1985), a small proportion of the ~16 billion neurons estimated for the human cerebral cortex (Azevedo et al., 2009). If every neuron in the cerebral cortex directly synapsed with an NbM cholinergic fiber, this would require ~40,000 branches per axon. A more likely scenario is that individual NbM cholinergic fibers provide input at the level of cortical ensembles, i.e., groups of neurons with similar feature tuning. Although the size of these ensembles varies across cortical areas, in vivo (Herculano-Houzel et al., 2008) and in silico (Onesto et al., 2020) evidence suggests that the upper bound for ensemble size is ~200 neurons. Assuming a uniform average ensemble size of 100 neurons, the ratio of cerebral cortical ensembles to NbM cholinergic neurons is 160,000,000:400,000. Only 400 branches per cholinergic neuron are required in this latter architecture, which is well within the range of empirically verified axonal branch counts for individual BF cholinergic neurons (Wu et al., 2014).

How might the observed gradient of cortical cholinergic innervation translate to the role of acetylcholine signaling in attention? When ensembles of neurons receive driving input from their preferred stimulus features, the responses of individual neurons are suppressed, or normalized, by the total activity of its ensemble and neighboring ensembles (Carandini & Heeger, 2011). This divisive normalization moderates noisy responses from individual neurons and prevents runaway excitation. Directed attention is thought to bias these mutually suppressive competitive interactions among ensembles, enabling some stimulus representations
to dominate over others (Desimone & Duncan, 1995; Reynolds & Heeger, 2009). Spatially localized acetylcholine release at the level of cortical ensembles may represent a key neurochemical basis of these biasing signals (Schmitz & Duncan, 2018). However, it remains poorly understood whether and how acetylcholine signaling changes from unimodal to transmodal stages of the cortical hierarchy. Our findings imply that the branch complexity of cholinergic projections may reflect properties of the cortical ensembles they target. Moving up the cortical hierarchy, neuronal ensembles with increasingly diverse repertoires or cortical-cortical connectivity may similarly receive input from increasingly branched BF cholinergic neurons. In terms of hierarchical integration, the ventral and dorsal attention networks may represent the apex of the BF cortical cholinergic innervation. This proposal is in line with research indicating that these networks play supervisory roles in both the voluntary and involuntary direction of attentional biasing signals throughout the brain (Alves et al., 2022; Corbetta et al., 2008).

A diversity of branch complexity in BF cholinergic neurons may also account for differences in their vulnerability to aging and disease. Cell type specific labeling and transcriptomic analyses examining morphological and functional properties which increase a neuron’s vulnerability to age-related neurodegenerative disease such as AD have consistently demonstrated large axonal projections as a key risk factor (Mattson & Magnus, 2006; Roussarie et al., 2020; Saxena & Caroni, 2011; Wu et al., 2014). The observed structure-function detethering in the BF, and in particular the NbM, is consistent with neurons exhibiting large arborizations. This morphofunctional property of NbM cholinergic neurons may increase their vulnerability to dysfunction in the aging brain. In parallel, our observation that ventral attention network may receive input from the most highly branched NbM cholinergic neurons implies that these cortical areas might exhibit higher vulnerability to dysfunctional cholinergic signaling in the aging brain. It is also notable that the BF cholinergic neurons with fewer arborizations, which our findings suggest primarily target the primary and somatosensory cortices, constitute projection zones which are relatively spared by pathology in early stages of AD.

Our findings are subject to several important methodological considerations. First, the basal forebrain is a small subcortical structure with poorly defined
anatomical boundaries. We therefore used a probabilistic atlas to localize its constituent nuclei. However, when using probabilistic BF atlases in combination with data collected at spatial resolutions typical of 3T structural (1.5 mm$^3$) and functional MRI (3 mm$^3$), aliasing of adjacent structures has been shown to systematically overestimate the BF gray matter (Maier-Hein et al., 2019). To mitigate this issue, we used high spatial resolution dMRI (1.05 mm$^3$) and rsfMRI (1.6 mm$^3$) data acquired at 7T. Second, our measures of structural connectivity were estimated using streamline tractography on diffusion-weighted imaging, which can be susceptible to false positives and negatives in certain brain areas (Maier-Hein et al., 2019). It is therefore possible that the regional variation in BF structure–function correspondence is partly explained by regional variation in tractography performance. Another concern is the susceptibility-related spatial distortions near the BF region for fast readout scans, such as used for the rsfMRI acquisitions by the HCP. Although corrected for using a separately acquired field map, these spatial distortions might lead to suboptimal probing of BF voxels in such data with possible contamination from white matter (WM) tissue and cerebro-spinal fluids (CSF). To limit the impact of the latter on the functional timeseries analysis, additional denoising using the average WM and CSF timeseries was performed.
3.6 Acknowledgments

RH was supported by a BrainsCAN postdoctoral fellowship. This research was enabled in part by the support provided by the Digital Research Alliance (https://alliancenan.ca), and the Natural Sciences and Engineering Research Council (TWS). Data was provided in part by the Human Connectome Project, WU-Minn Consortium (Principal Investigators: David Van Essen and Kamil Ugurbil; 1U54MH091657) funded by the 16 NIH Institutes and Centers that support the NIH Blueprint for Neuroscience Research; and by the McDonnell Center for Systems Neuroscience at Washington University. The PET data used in the analysis was supported by the National Institutes of Health [P01 NS015655, RO1 NS070856, P50 NS091856, P50 NS123067].
3.7 References


Yuan, R., Biswal, B. B., & Zaborszky, L. (2019). Functional Subdivisions of


3.8 Supplemental Figures

Supplemental Figure 3.1: Gradient-weighted cortical mapping method. Gradient-weighted cortical maps were created by first, multiplying each row of the initial connectivity matrix $\left( M_{BF\text{ voxels} \times N_{cortical\ \text{parcels}}} \right)$ in left with the corresponding gradient value of that BF voxel from gradient result (e.g. $G_1$ here). This produce a $G_1$-weighted connectivity matrix $\left( G_{BF\ \text{voxels} \times N_{cortical\ \text{parcels}}} \right)$, where all rows of the matrix (i.e. $G_{BF\ \text{voxels}}$) were averaged to produce a single cortical representation of the particular gradient.

Supplemental Figure 3.2: A seven–network parcellation based on resting-state fMRI of the human cerebral cortex by Byrge & Kennedy (2019), and Yeo et al., (2011).
Supplemental Figure 3.3: Cortical cholinergic innervation in relation to cortical maps of weighted residuals and geodesic distance.

Three publicly available FEOBV PET maps (Aghourian et al., 2017; Bedard et al., 2019) were obtained from the Neuromap toolbox (Markello, Hansen, Liu, Bazinet, Shafiei, Suárez, Blostein, Seidlitz, Baillet, Satterthwaite, Chakravarty, et al., 2022); they were parcellated, rescaled and projected on the cortical surface (A), pink indicating higher values while sky blue color indicating lower with the cortical BF label indicated by black spot. (B) Scatter plot against the weighted cortical residual map indicating positive correlation. Each point in the scatter plot represents cortical parcels based on the Glasser parcellation (Glasser, Coalson, et al., 2016) and is color-coded by the 7 networks (Yeo et al., 2011) identical to Figure 3.2. Spin test using spatial null model (Alexander-Bloch et al., 2018) results are reported in the box corresponding to the scatter plots. (C) Scatter plot of the FEOBV PET maps against the geodesic distance showing negative relationship.
Chapter 4

4 Pharmacological meta-analysis of cholinergic modulation on attention

4.1 Goals of this chapter

Thus far, our analyses of the cholinergic BF projections in humans spanning chapters 2 and 3 have focused principally on connectomic and anatomical features derived from diffusion-weighted imaging (DWI), resting-state fMRI (rsfMRI), [18F] FEOBV PET, cortical myelin and cortical geodesic distance metrics. In Chapter 4, we focus on how acetylcholine release from the cholinergic basal forebrain affects cortical function and cognition. Since direct stimulation of the basal forebrain cholinergic neurons is not feasible in humans, researchers have leveraged pharmacological strategies for examining the influence of increased or decreased cholinergic signaling on brain activity and cognitive performance.

To increase cholinergic signaling, a drug classified as a cholinergic agonist is delivered via injection, dermal patch or oral medication. A cholinergic agonist is a drug or chemical compound that activates or enhances the activity of the cholinergic system in the brain and body. Cholinergic agonists can be further classified based on their mechanism of action. For example, some cholinergic agonists, such as nicotine, directly bind to and activate nicotinic acetylcholine receptors (nAChRs). Other compounds, such as choline and acetylcholine precursors, increase the availability of acetylcholine (ACh) in the brain by providing the building blocks for its synthesis. There are also drugs that block the action of acetylcholinesterase, the enzyme that rapidly breaks down ACh after its release from cholinergic neurons. Donepezil and galantamine are examples of cholinesterase inhibitors that increase the concentration of ACh in the brain by inhibiting its breakdown. Cholinergic agonists have a variety of medical and therapeutic uses, such as treating disorders associated with cholinergic dysfunction, including Alzheimer’s disease, Parkinson’s disease, and myasthenia gravis. They can also be used to enhance cognitive function and improve memory and attention, although the use of cholinergic agonists for cognitive enhancement is still controversial and requires further research.

To decrease cholinergic signaling, a drug classified as a cholinergic antagonist is delivered via injection, dermal patch or oral medication. Like cholinergic agonists,
cholinergic antagonists can be further classified based on their mechanism of action. For example, atropine is a natural cholinergic antagonist found in the belladonna plant that blocks muscarinic acetylcholine receptors (mAChRs). Scopolamine is a synthetic cholinergic antagonist used to treat motion sickness and as a pre-anaesthetics to reduce secretions. Both atropine and scopolamine directly bind to and block cholinergic receptors. Cholinergic antagonists have a variety of medical and therapeutic uses, such as treating disorders associated with overactive cholinergic function, including bradycardia, asthma, and gastrointestinal disorders. They can also be used as anesthetics or pre-anesthetics to reduce salivary and bronchial secretions during surgery.

Pharmacologic neuroimaging with either fMRI or PET has been employed to characterize the impact of an acute drug effect on human brain function (Bentley et al., 2011; Salmeron & Stein, 2002; Sutherland et al., 2015; Wise & Tracey, 2006). Multiple studies have examined drug-induced changes in brain activity and cognition after administration of a cholinergic agonist (Bentley et al., 2004, 2008; Hahn et al., 2007; Iglesias et al., 2021; Kumari et al., 2003; Thiel & Fink, 2008) or antagonist (Furey et al., 2000; Schon et al., 2005; Thienel et al., 2009; Voss et al., 2012). Collectively, these studies provide an opportunity to study the impact of cholinergic modulation of brain activity and cognitive performance at the meta-analytic level. The purpose of fMRI meta-analysis is to combine the results from multiple fMRI studies in order to identify consistent patterns of brain activation across different experiments. Meta-analysis can help overcome the limitations of single studies, such as small sample sizes, heterogeneity of methods, or inconsistent findings, by pooling data and increasing statistical power and generalizability.

In this chapter, we perform quantitative meta-analysis of placebo controlled pharmacological fMRI studies in which cholinergic agonists were administered to cognitively normal younger adults engaged in cognitively demanding tasks. The aims of this chapter are threefold: (1) we will derive spatial maps where task-related brain activity is consistently modulated by cholinergic agonists; (2) we will use spin tests to quantitatively relate these spatial maps to anatomical and connectomic maps expressing the basal forebrain cholinergic projections (derived from Chapter 3); (3) we will perform meta-analyses on response latency and response accuracy measures
from the fMRI studies. A version of this chapter is submitted for consideration in the *Journal of Neurochemistry*.

### 4.2 Introduction

Acetylcholine (ACh) is a neurotransmitter that plays a critical role in modulating neural activity and information processing in the brain. The primary source of neocortical, hippocampal and amygdalar ACh originates from the cholinergic neurons in the basal forebrain (BF), which consists of several subcortical nuclei situated adjacent to the hypothalamus (M.-M. Mesulam & Geula, 1988; Woolf, 1991; Zaborszky et al., 2015). Individual BF cholinergic neurons have immense axonal projections, which can branch >1000 times before reaching their synaptic targets in the cerebrum (Wu et al., 2014).

Most of what we understand about the influence of ACh on cortical function comes from research in non-human animal models. Optogenetic and electrophysiological studies demonstrate that cholinergic BF neurons emit precisely timed ACh signals in response to the detection of novel or salient sensory stimuli, as well as task-relevant outcomes such as rewards and errors, at the millisecond timescale (Bennett et al., 2012; Guo et al., 2019; Hangya et al., 2015; Harrison et al., 2016; Laszlovszky et al., 2020; Letzkus et al., 2011; Pinto et al., 2013; Tu et al., 2022). Advances in biosensor strategies indicate that ACh signaling can exhibit spatial specificities ranging from the macroscopic scale of different brain regions (Teles-Grilo Ruivo et al., 2017) down to the micron scale of cortical ensembles and receptive fields (Jing et al., 2020; Sethuramanujam et al., 2021). These discoveries have revealed ‘wired’ transmission modes of ACh signaling, which in turn have necessitated revisions to our understanding of its role in cortical function (Sarter et al., 2009; Sarter & Lustig, 2020; Schmitz & Duncan, 2018; Záborszky et al., 2018). At the network level, wired ACh neurotransmission may hierarchically integrate cortical areas to a “read-in” mode, while suppressing cortical consolidation, or “read-out”, of memory (Hasselmo & McGaughy, 2004). High levels of ACh release thus appear to set local and global neural dynamics that are optimal for attention, encoding and learning.
We recently demonstrated with multimodal MRI and PET data acquired from humans that cortical regions including the dorsal anterior cingulate, anterior insula and frontal operculum express molecular, structural and functional markers of BF cholinergic innervation which are disproportionately higher than other cortical regions (Chapter 3; Chakraborty et al., 2023) (Figure 4.1A). Further analysis revealed that these regions closely overlap with hubs of the ventral attention network (Alves et al., 2022; Corbetta et al., 2008; Vossel et al., 2014; Yeo et al., 2011) and salience network (Seeley, 2019; Seeley et al., 2007). The anatomical core of the salience and ventral attention networks comprises a set of midcingulo-insular cortical hubs, according to recent taxonomies of cortical networks (Uddin et al., 2019). The midcingulo-insular network mediates switching between the default mode and central executive networks (Sridharan et al., 2008; Uddin, 2015), consistent with a domain-general role in coordinating attentional resources throughout the brain (Downar et al., 2000; Menon & Uddin, 2010; Vossel et al., 2014). The phylogenetic cortical homologues of a midcingulo-insular network are also observed in the intrinsic cortical connectome of the mouse (Mandino et al., 2022; Sforazzini et al., 2014), suggesting evolutionary conservation of a core attention system for saliency, switching, and control (Figure 4.1B).

**Figure 4.1:** Homologous midcingulo-insular hubs of the ventral attention/salience networks in the human and mouse brain.

(A) Surface rendering displaying the human ventral attention network (cortical hubs delineated by white borders) overlaid on a multimodal map of the BF connectome.
(Chapter 3; Chakraborty et al., 2023), estimated from cortical VACHT concentration ([18F] FEOBV PET), BF structural (diffusion-weighted MRI) and functional connectivity (resting-state fMRI), geodesic distance from BF, and cortical myelin (T1w/T2w MRI). Areas with the darkest color exhibit higher VACHT, higher structure-function detethering in measures of BF connectivity, shorter geodesic distances to BF, and lower cortical myelin. The BF seed label is indicated by the black dot visible on the medial wall of the cortical surfaces just posterior to the subgenual cingulate cortex.

(B) The ventral attention/salience networks exhibit homologous midcingulo-insular cortical architecture and function in mouse (left) and human (right); this figure is adapted with permission from Xu et al (2022).

However, the direct link between ACh signaling and attention has proven challenging to study experimentally in humans. There are no non-invasive biomarkers available that can reliably measure the time-varying functional activity of the cholinergic system in humans with cell type specificity. One strategy for overcoming this obstacle is to measure task-related brain activity with neuroimaging techniques such as fMRI or PET while participants are administered pharmacological interventions targeting cholinergic function. Drugs which alter cholinergic function fall into one of two general categories: ACh agonists and ACh antagonists. For reviews, see Bentley et al (2011) and Sutherland et al (2015). From this work, a complex and sometimes contradictory picture of the relationship of ACh with task-related activations and task performance has emerged. For instance, pharmacological activation of ACh alters task-related brain activation in areas associated with attentional orienting, including cortical hubs of the ventral attention/salience network. However, across studies, many other patterns are reported, including increased task-related activity in parietal and sensory areas, and decreased activity in cortical midline areas. Similarly, while the impact of ACh on behavioral markers of attention, such as response latency and accuracy, is generally thought to reflect facilitation, there is inconsistency in whether and how these data are reported from study to study.

Meta-analysis is a powerful tool for improving consensus across empirical research studies. Well-curated meta-analytic inclusion criteria can provide focused
integration of research examining a target population with a common set of experimental factors. Moreover, meta-analysis provides quantitative integration of observations across studies. The resulting increase in sample size leads to increased statistical power to detect smaller effects, such as a common pattern of task-evoked brain activation or behavior, that may have been missed in individual studies. The pharmacological neuroimaging literature on ACh is broad. Studies have examined populations ranging from cognitively normal to those with conditions thought to affect central cholinergic integrity, including schizophrenia, substance abuse disorders, autism, and Alzheimer’s disease. Moreover, many studies use tasks which are not designed to engage directed attention, such as passive viewing, or use task-free resting-state fMRI paradigms. Heterogeneity in these experimental factors poses a major challenge to synthesis of coherent patterns across studies.

We therefore performed several meta-analyses to examine the influence of ACh and directed attention on human brain function and behavior. Our strategy combined (1) focused meta-analysis on a well curated sample of pharmacological neuroimaging studies with (2) discovery and validation meta-analyses on larger independent samples of non-pharmacological neuroimaging studies (See Figure 4.2). To address heterogeneity in experimental factors, the pharmacological imaging meta-analysis focused on experiments that met the following three criteria: (1) employed ACh agonists with placebo control; (2) report explicit task comparisons of high versus low attentional demand conditions; (3) assessed cognitively normal younger adults (<50 years). Our meta-analyses of non-pharmacological imaging studies provided data-driven discovery of cortical areas that co-activate with the BF during task engagement, and validation that cortical areas modulated by ACh and attention (identified in the meta-analysis of pharmacological neuroimaging) also co-activate with one another during task engagement. Sample sizes in both the discovery and validation meta-analyses exceeded 80 unique neuroimaging studies, comprising >1300 individuals. Lastly, we performed to our knowledge the first behavioral meta-analysis of ACh pharmacological neuroimaging studies to determine if cholinergic modulation impacts response latency and accuracy measures of attentional performance.
Altogether, we demonstrate that pharmacological activation of ACh alters distributed patterns of midcingulo-insular brain activity during directed attention. These regions exhibit strong co-activation with the BF and with one another during task engagement, close spatial alignment with a multimodal map quantifying the gradients of cortical cholinergic innervation originating from the BF, and are associated behavioral markers of facilitated attentional performance.

**Figure 4.2: Neuroimaging meta-analysis strategies.**

(A) A probabilistic atlas of the Ch123,Ch4a/4p BF nuclei (Zaborszky et al., 2008) was used as a seed region for meta-analytic connectivity mapping (MACM). Additional search filters restricted our sample to neuroimaging studies reporting task activation in cognitively normal adults. (B) The MACM localized coordinates for brain areas that co-activate with the BF under attentional engagement (goldenrod dots in semi-transparent render). These coordinates were submitted to a discovery activation likelihood estimation (ALE) analysis (Figure 4.3). (C) We identified 24 placebo (Pla) controlled pharmacological neuroimaging studies that: (1) employed cholinergic agonists (ACh); (2) report explicit task comparisons of high versus low (H > L) attentional demand condition; (3) assessed cognitively normal younger adults (<50 years). ALE was performed separately on Drug x Task interactions where ACh either increased activation under ACh and attention (red dots: [ACh (H > L)] > [Pla (H > L)]), or decreased activation under ACh and attention (blue dots: [ACh (H > L)] < [Pla (H > L)])
Suprathreshold ALE clusters for activation increases and decreases were merged into a seed region (magenta) for MACM. The MACM localized coordinates for brain areas that co-activate with the ACh modulated cortical regions under attentional engagement (magenta dots in semi-transparent render). These coordinates were submitted to validation ALE analysis (Figure 5).

4.3 Materials and Methods

4.3.1 Literature Search and Selection Criteria for Pharmacological neuroimaging studies

We conducted a literature search on the PubMed database (https://pubmed.ncbi.nlm.nih.gov) to identify pharmacological functional imaging studies of potential relevance to the current hypothesis up to April 2023. The search terms used were adopted from Bentley et al. (2011) and contained the following combinations of keywords: [cholinergic OR acetylcholine OR nicotine OR scopolamine OR cholinesterase OR smoking OR varenicline] AND [functional imaging OR fMRI OR PET]. Meta-analytic review papers (Sutherland et al., 2015) and reference lists were also examined to retrieve additional relevant studies. Following the initial screening of titles and abstracts, the selected articles underwent further screening based on predefined eligibility criteria. Studies were included in the meta-analysis if they (1) used fMRI or PET (2) reported activation data obtained from healthy, neurologically intact, non-elderly (i.e. mean age < 50 years) participants; (3) involved cholinergic manipulation of participants by either pharmacological administration or cigarette smoking in a placebo-(or abstinence) controlled within-subjects or between-subjects design; (4) employed tasks with at least two levels of cognitive load/demand; (5) reported activation coordinates for brain regions that demonstrated interaction between drug and task; and (6) reported peak activation coordinates in standardized stereotaxic space (i.e. either Montreal Neurological Institute (MNI) or Talairach space). Studies investigating functional connectivity were excluded from this meta-analysis.

4.3.2 Activation Likelihood Estimation (ALE)

Relevant coordinates from the selected studies were extracted and converted into MNI coordinates, where necessary, using the Lancaster transform included in
GingerALE v.3.0.2 (Lancaster et al., 2007). For ACh agonists, activation foci were separated according to their pattern of task-evoked activity under ACh modulation and attention. We then ran two meta-analyses: (1) Activation increases under ACh modulation and attention \([\text{ACh-Ag (H} > \text{L)} > \text{Pla (H} > \text{L)}]\) and (2) Activation decreases under ACh modulation and attention \([\text{ACh-Ag (H} > \text{L)} < \text{Pla (H} > \text{L)}]\). We also conducted an exploratory meta-analysis of pharmacological neuroimaging papers using ACh antagonists, such as atropine and scopolamine, in combination with attentionally engaging tasks. As with the ACh agonists, activations for antagonists were separated according to their pattern of task evoked activity: (1) activation increases under ACh blockade and attention \([\text{ACh-Ant (H} > \text{L)} > \text{Pla (H} > \text{L)}]\) and (2) activation decreases under ACh blockade and attention \([\text{ACh-Ant (H} > \text{L)} < \text{Pla (H} > \text{L)}]\).

ALE analyses were performed using GingerALE v.3.0.2 (http://www.brainmap.org/ale/). ALE is a widely used coordinate-based meta-analysis technique which identifies brain regions showing significant convergence in activation across studies. Detailed information regarding ALE and its algorithm has been previously described (Eickhoff et al., 2009). Briefly, this approach treats each reported foci as the center of a 3D Gaussian probability distribution rather than as single points in order to account for spatial uncertainty due to between-subject and between-template variance. The width of these distributions is inversely related to the sample size of the corresponding experiment. The probability distribution of all activation foci in each individual contrast is then combined to obtain the modelled activation (MA) maps. ALE maps are subsequently generated by taking the voxel-wise union of these MA maps across all studies. Finally, the computed ALE maps are compared against a null distribution reflecting a random spatial association of experiments to differentiate true convergence of activation foci from random clustering. Following the thresholding guidelines recommended in Eickhoff et al. (2012, 2017), we applied a cluster-level family-wise error (FWE) threshold of \(p > 0.05\) with a cluster forming threshold of \(p < 0.001\) and 5,000 permutations for each pharmacological imaging ALE analysis.
4.3.3 Meta-Analytic Connectivity Mapping (MACM)

We performed MACM analyses using Sleuth (https://brainmap.org/sleuth/) separately on two seed regions of interest (ROIs): (1) the a priori anatomically defined nuclei of the BF; (2) the suprathreshold clusters identified in the ALE analysis of ACh agonist imaging studies (increases and decreases combined). The binarized MNI space masks for each ROI were entered into the BrainMap database, along with two other search criteria specifying (1) normal mapping and (2) activations. Using the search criteria, MACM queries the database for imaging studies which report coordinates for brain areas that are co-activated with the seed ROI during a particular task or under specific conditions. The coordinates are then entered into an ALE to identify brain regions that are consistently activated across studies.

4.3.4 Spin tests assessing spatial correspondences among brain maps

Brain maps encoding the ALE Z values were transformed from MNI volume-space to 10k_fsavg surface-space using neuromaps toolbox (Markello et al., 2022) and parcellated using the HCP-MMP 1.0 atlas (Glasser et al., 2016). We used a multimodal surface map of the BF connectome derived from (Chapter 3; Chakraborty et al., 2023); Figure 4.1A). In neuroimaging studies, spin tests evaluate the statistical significance of spatial relationships between different cortical surface features (Alexander-Bloch et al., 2018). Specifically, spin tests can be used to assess whether the observed spatial relationships are significant and unlikely to have occurred by chance. To conduct a spin test on neuroimaging maps, the maps can be randomly permuted or "spun" in a way that maintains the spatial structure of the data but disrupts the relationship between different brain regions. By comparing the observed spatial relationship between two neuroimaging maps to the distribution generated by the spin test, the significance of the relationship can be inferred relative to the spatial null. Spin tests were implemented using the algorithm developed by (2018) to compare cortical maps based on 10,000 permutations.

4.3.5 Behavioral meta-analysis

We computed separate behavioral meta-analyses for measures of response latency and response accuracy (where available) using the same set of ACh Agonist pharmacological imaging papers reported in the ALE analyses. We used Cohen's $d$ to quantify the standardized mean difference between Drug and Placebo for each
behavioral measure. In cases where a measure of effect size other than Cohen’s $d$ was reported (e.g. an $F$ or $t$ statistic), we converted these effect sizes to Cohen’s $d$. In cases where no measure of effect size was reported, we computed Cohen’s $d$ from the means and standard deviations of each group as follows:

For between-group comparisons, Cohen’s $d$ was calculated by dividing the difference between the means of two groups by the pooled standard deviation of the two groups. The formula for Cohen’s $d$ in this case is:

$$d = \frac{M_1 - M_2}{SD_{pooled}}$$

Where $M_1$ and $M_2$ are the means of the two groups being compared, $SD_1$ and $SD_2$ are the standard deviations of the two groups being compared, and $SD_{pooled}$ is the pooled standard deviation.

For repeated measures comparisons, Cohen’s $d$ was calculated using the standard deviation of the differences between the two conditions, divided by the mean of the differences. The formula for Cohen’s $d$ in this case is

$$d = \frac{M_1 - M_2}{SD_{diff}}$$

Where $SD_{diff}$ is the standard deviation of the differences between the two conditions, $r$ is the correlation between the two conditions, and $n$ is the sample size. The value of $r$ is rarely reported and was therefore set to 0.1 in all applicable cases, which assumes a weak correlation between repeated measures (higher values may lead to an overestimation of the effect size).

Studies containing $n>1$ statistical comparisons for the effect of ACh on response latency or accuracy, e.g. due to multiple experiments, contributed $n$ Cohen’s $d$ values to the meta-analyses. Using Cohen’s $d$ estimates from each study, we calculated a random-effects weighted average effect size across studies, which took into account both the within- and between-study variance and estimated the precision of this population effect estimate using 95% confidence intervals (Field & Gillett, 2010).
4.3.6 Data and code availability

All MACM and ALE input data, behavioral meta-analysis input data (Cohen’s d), and code used to conduct the reported spin test analyses and create the figures are available at https://github.com/sudesnac/Ach-phfMRI (Chakraborty, n.d.).

4.4 Results

4.4.1 BF co-activates with cortical areas enriched with cholinergic synapses during attention

Extending on our prior work examining the BF cholinergic connectome with multimodal imaging (Chapter 3; Chakraborty et al., 2023), we first examined which brain areas are consistently co-activated with the BF during attentional engagement. To do so, we employed meta-analytic connectivity mapping (MACM) of the BF (see Methods). MACM provides a robust and replicable query of brain imaging studies in the BrainMap database (>4,000 studies representing >100,000 participants), returning a list of brain activation coordinates which have been observed to co-occur with activation in a priori seed region. Our MACM query returned 88 task imaging experiments reporting BF task-related co-activations in 1357 cognitively normal adults (Figure 4.2B). We then performed a discovery ALE analysis on the list of brain activation coordinates matching our search criteria to generate a map of the regions that are most likely to be co-activated with BF across experiments. The resulting suprathreshold foci included bilateral anterior insula and frontal opercular cortex, consistent with midcingulo-insular hubs of the ventral attention/salience networks (Figure 4.3A).

We were interested in whether the continuous whole brain map of these ALE effect sizes, encoded by the Z value at each voxel, was spatially related to the multimodal gradient of cortical cholinergic innervation (Chapter 3; Chakraborty et al., 2023) (Figure 4.1A). To test this relationship, we transformed the volume map encoding the ALE Z values to the same cortical surface space as the BF cholinergic projectome (Figure 4.1A). We then performed spin tests against the spatial null to examine their topographical correspondence. The spin test yielded a significant positive correlation ($r=0.44$, $p_{\text{spin}}=0.0001$), indicating that cortical areas exhibiting the
highest task-related co-activations with BF also receive the densest BF cholinergic innervation.

Figure 4.3: Correlation of BF task co-activation with the multimodal gradient of cortical cholinergic innervation.

(A) The ALE Z values for the BF MACM analysis projected onto the 10k_fsavg cortical surface and parcelled using the HCP-MMP 1.0 atlas; cortical BF label is indicated by black dot. (B) Scatter plot showing the spatial relationship of ALE Z values for the BF MACM (x-axis) with the gradient of cortical cholinergic innervation (y-axis). The r and p-value are derived from a spin test (Alexander-Bloch et al., 2018) between these two surface maps against the spatial null. Each point in the scatter plot represents cortical parcels based on HCP-MMP 1.0 parcellation (Glasser et al., 2016) and is color-coded by the 7 network parcellation from Yeo et al (2011), which is also shown in the upper left inset for reference.

4.4.2 ACh induces both increased and decreased cingulo-opercular activity during attention

Turning to our core question on the relationship of ACh to brain activity and behavioral performance under attention, we performed meta-analyses on a sample of placebo-controlled fMR studies examining ACh agonists (e.g. nicotine or acetylcholinesterase inhibitors) in cognitively normal younger adults (<50 years of age) performing experimental tasks containing a manipulation of attentional demand across high (H) and low (L) conditions (see Methods). We identified 24 experiments fitting these criteria, yielding a meta-analysis consisting of 672 subjects (Figure 4.2C).
We conducted ALE separately on attention-related activation patterns characterized either by increases under ACh and attention \([\text{ACh} (H > L) > \text{Pla} (H > L)](\text{Figure } 4.4A)\), or decreases under ACh and attention \([\text{ACh} (H > L) < \text{Pla} (H > L)](\text{Figure } 4.4B)\).

The meta-analyses revealed that cholinergic modulation by ACh yielded a distributed pattern of both increased and decreased attention-related activity compared to placebo (cluster level FWE corrected \(p<0.05\)). Areas exhibiting increases under ACh and attention were localized to the right anterior cingulate cortex overlapping Brodmann area 32 (Figure 4.4C). By contrast, areas exhibiting decreased activity under attentional demand and ACh were localized to the right opercular and anterior insular cortices overlapping Brodmann area 13 (Figure 4.4D).

The use of ACh antagonists is less common in the neuroimaging literature. From our meta-analytic search criteria above (Methods), we identified a total set of 8 experiments, consisting of 126 subjects. The sample size is considerably less than 17 which has been suggested as a lower bound for well powered ALE meta-analyses (Eickhoff et al., 2017; Yeung et al., 2023). We provide the configured ALE input tables in our GitHub repository for future use.
Figure 4.4: Meta-analyses of task activations under ACh and Attentional demand.

(A and B) Schematic representations of the Drug x Task interactions reported in the literature: (A) ACh increases are characterized by relatively larger increases in activity with attentional demand compared to placebo. (B) ACh decreases are characterized by relatively smaller increases in activity with attentional demand compared to placebo. (C) The ACh increase pattern was observed in the anterior cingulate overlapping Brodmann area 13. (D) The ACh decrease pattern was observed in the insular and opercular cortex overlapping Brodmann area 32 (D). ALE maps in C and D are thresholded at a cluster level FWE corrected p<0.05 and rescaled to facilitate surface rendering.

4.4.3 ACh modulates cortical areas enriched with cholinergic synapses during attention

The meta-analytic observations of activity increases and decreases under ACh agonists (Figure 4.4) imply that BF cholinergic projections exert a distributed
modulatory effect on right anterior cingulate, opercular and insular cortical areas during directed attention. However, the different activation clusters observed in a standard meta-analysis are not necessarily co-active with one another. For instance, it could be the case that different sets of pharmacological imaging studies in our sample contributed to the significance of each of the observed suprathreshold ALE clusters. We separately confirmed that this was indeed the case; only one experiment reported concurrent activation increases in anterior cingulate and decreases in cingulo-opercular cortex (Furey et al., 2008). Are these clusters consistently co-activated with one another under task demand, perhaps reflecting functional embedding within a network, or do they simply reflect coincidental suprathreshold activations loci influenced by different types of experimental tasks? To address this question, we created an ‘ACh’ seed region composed of all suprathreshold clusters exhibiting either increases or decreases under ACh (Figure 4.4CD) and performed a validation MACM and ALE analysis to explicitly test the likelihood of co-activation among these regions across a much larger independent sample of non-pharmacological imaging studies. Using this ACh seed region, along with the same set of additional search criteria used for the BF MACM (constraining to task-related brain activations in normal populations), our query returned 108 experiments, spanning observations from 1530 individuals (Figure 4.2C bottom). From this sample, we then subjected the activation foci from experiments matching the ACh MACM criteria to ALE analysis of spatial consistency in activity patterns.

We found significant co-activation among the ACh modulated cortical regions during task engagement in this independent sample (Figure 4.5A). Moreover, extending on the discovery ALE of BF co-activation (Figure 4.3A), this observed spatial pattern of cortical co-activations provides further validation that these clusters form hubs of the midcingulo-insular network. As with the discovery ALE of BF co-activation, we used spin tests to determine whether the continuous map of ALE Z values encoding the cortical ACh co-activation pattern was also spatially related to the multimodal gradient of cortical cholinergic innervation originating from BF (Chapter 3; Chakraborty et al., 2023) (Figure 4.1A). Consistent with a structure-function link between cortical cholinergic innervation and attention-related functional activations, the spin test yielded a significant positive correlation ($r=0.31$, 103
indicating that cortical areas exhibiting the highest task-related co-activations under ACh also receive the densest BF cholinergic innervation (Figure 4.5B). Altogether, these findings indicate that pharmacological activation of ACh under attentional demand evokes heterogeneous but functionally integrated activation patterns in cortical hubs which overlap the midcingulo-insular hubs of the ventral attention/salience network.

Figure 4.5: Correlation of ACh cortical co-activation with the multimodal gradient of cortical cholinergic innervation.

(A) The ALE Z values for the ACh cortical MACM analysis projected onto the 10k_fsavg cortical surface and parcellated using the HCP-MMP 1.0 atlas. (B) Scatter plot showing the spatial relationship of ALE Z values for the ACh cortical MACM (x-axis) with the multimodal BF connectome (Figure 4.1A; y-axis). The r and p-value are derived from a spin test between these two surface maps against a spatial null and is color-coded by the 7 network parcellation from Yeo et al (2011), which is also shown in the upper left inset for reference.

4.4.4 Modulation of ACh speeds responses with no tradeoff in accuracy

A core inclusion criterion for our meta-analysis of ACh pharmacological fMRI studies (Figure 4.4) was that they employ experimental task manipulations of attentional demand. Of the 24 experiments which met this criterion, some used N-back probes of working memory while others used cue-target selection probes of visuospatial orienting. Although these manipulations probe distinct components of attention, their behavioral correlates are typically reported from common units of
response latency and response accuracy, where faster responses in combination with higher or sustained response accuracy, i.e. negligible speed-accuracy tradeoff (Bogacz et al., 2010), are thought to reflect superior attentional performance. We therefore computed Cohen’s $d$ effect size estimates for comparisons between ACh and placebo (main effect of Drug) on measures of response latency and accuracy. Cohen’s $d$ values for each comparison were then submitted to separate random-effects analyses of the weighted average effect size (see Methods, Figure 4.6).

For behavioral measures of response latency, we found that ACh yielded significant speeding of target selection compared to placebo (weighted Cohen’s $d=0.87$; 95% CI=0.67,1.12; $z=7.95$; $p<0.001$). In a subset of these studies ($n=13$) which also reported Drug x Task interactions, we also detected a significant speeding of responses by ACh under high compared to low attentional demand (weighted Cohen’s $d=1.29$; 95% CI=0.90,1.68; $z=6.42$; $p<0.001$). For behavioral measures of response accuracy, however, differences between ACh and placebo were negligible (weighted Cohen’s $d=0.3$; 95% CI=-0.12,0.72; $z=1.41$; $p=0.16$). The number of Drug x Task interactions ($n=3$) reported for response accuracy was insufficient for random effects meta-analysis. Altogether, these behavioral meta-analytic findings imply that ACh facilitates faster decisions about targets with negligible tradeoff in target selection accuracy.
**Figure 4.6:** Behavioral meta-analyses for main effects of ACh on response latency and accuracy in pharmacological neuroimaging studies.

Cohen’s $d$ values (open circles) estimating the effect of ACh versus placebo for response latencies and response accuracies across experiments. Positive signed Cohen’s $d$ reflect effect sizes from statistical comparisons which favored performance facilitation under ACh (faster responses, higher accuracy). Negative signed Cohen’s $d$ reflect effect sizes from statistical comparisons which favored performance facilitation under Placebo. Horizontal lines (red) are the means and vertical lines are the 95% confidence intervals (CI) derived from a random effects meta-analysis on the weighted Cohen’s $d$.

### 4.5 Discussion

In this study, we explored the relationship between ACh and attention in humans using meta-analytic strategies targeting both activation patterns in pharmacological and non-pharmacological neuroimaging studies, along with pharmacologically induced changes in behavioral response speed and accuracy. Compared to placebo, ACh evoked both increased and decreased activation patterns primarily in right midcingulo-insular cortical areas. We found that these cortical areas exhibit strong task-related co-activation with one another, and with the BF in large independent meta-analyses of non-pharmacological neuroimaging research. Finally, we show that the cortical topographies of BF co-activation and ACh modulation
during directed attention both closely overlap with the multimodal gradient of cortical cholinergic innervation (Chapter 3; Chakraborty et al., 2023). Concurrent to the influence of ACh on brain activity, we found that individuals exhibit faster target selection, without sacrificing selection accuracy, during directed attention.

There is relatively little intersectionality between lines of imaging research on BF connectivity and ACh pharmacology in humans. As such, there are many gaps in our understanding of how the structural organization of the BF cholinergic projections into the cortex shapes cortico-cortical connectivity, and how cortical ACh release within the BF projectome facilitates directed attention to stimuli, task representations and behavioral responses. To measure intrinsic BF connectivity in the human brain in vivo, researchers have typically employed either PET or MRI. For PET studies, researchers have targeted the vesicular acetylcholine transporter (VACHT), a glycoprotein expressed exclusively by cholinergic neurons, with the [18F] FEOBV radiotracer (Albin et al., 2018; Kanel et al., 2022). Because VACHT is expressed most strongly on the presynaptic terminals, these studies have provided insights into the anatomical distribution of the BF cholinergic projections throughout the human cerebrum with cell type specificity. This work is complemented by a growing number of MRI studies examining the white matter and resting state functional connectivity of the BF (Fritz et al., 2019; Grothe et al., 2021; Li et al., 2014; Lin et al., 2022; Markello et al., 2018; Nemy et al., 2020; Oswal et al., 2021; Ray et al., 2015; Teipel et al., 2011; Yuan et al., 2019; Zhang et al., 2017). We recently employed multimodal PET/MR imaging to directly examine the spatial relationships among cortical VACHT concentrations, BF white matter projections, and BF resting-state functional connectivity (Chapter 3; Chakraborty et al., 2023). We demonstrated that cortical areas exhibiting higher VACHT concentrations receive BF projections with greater neuronal branch size and complexity, as estimated by structure-function detethering (Paquola et al., 2019; Suárez et al., 2020; Vázquez-Rodríguez et al., 2019). Moreover, this multimodal map of the BF cortical cholinergic projectome exhibited a striking convergence with midcingulo-insular hubs of the ventral attention network (Corbetta et al., 2008; Menon & Uddin, 2010; Seeley, 2019; Seeley et al., 2007; Sridharan et al., 2008; Uddin, 2015; Uddin et al., 2019; Vossel et al., 2014), consistent with the idea that cortical ACh signaling plays a key role in allocating attentional resources
throughout the brain (see Figure 4.1). However, pharmacological neuroimaging is needed to determine whether and how ACh modulates cortical function, and how this functional modulation translates to attentional performance. In this study, we therefore used meta-analytic techniques to bridge imaging research on ACh connectivity and ACh pharmacology. We hope that these studies motivate further hypothesis-driven research to strengthen our understanding of the critical roles of ACh in human brain function.

Our meta-analytic findings indicate that with increasing attentional demand, ACh induced both increased activity in the right cingulate cortex and decreased activity in right insular-opercular relative to placebo. This pattern is difficult to interpret from the brain imaging modalities used by studies in our meta-analyses, which included fMRI blood oxygenation level dependent (BOLD) signals and $H_2^{15}$O PET regional cerebral blood flow (rCBF). Neither of these imaging modalities can distinguish excitatory and inhibitory neuronal activity. However, multiple lines of electrophysiological, imaging, histological and optogenetic evidence in non-human animal models indicates that ACh release in the cortex may alter both excitation and inhibition in parallel across different spatiotemporal scales (Chen et al., 2012; Do et al., 2016; Galvin et al., 2018, 2020; Khalighinejad et al., 2020; Kuchibhotla et al., 2017; Medalla & Barbas, 2012; M. M. Mesulam, 2004; Saunders et al., 2015; Vijayraghavan et al., 2018). From this work, ACh appears to exert its modulatory effects on cortical activity primarily via synaptic connections with multiple classes of interneurons and astrocytes, as opposed to direct synapses with pyramidal neurons (Chen et al., 2012; Kuchibhotla et al., 2017). These modulatory effects can be inhibitory or disinhibitory, depending on task context and sensory stimuli. The BF is also populated by diverse types of neurons in addition to cholinergic neurons, including long-range projecting glutamatergic neurons and GABAergic interneurons, which regulate cortical states through coordinated activity (Do et al., 2016). Adding yet further complexity to these modulatory effects, BF cholinergic neurons are capable of manufacturing and co-releasing ACh and GABA (Saunders et al., 2015).

The evidence for parallel excitatory/inhibitory drive of cortical ACh release fits well with the normalization model of attention (Bloem & Ling, 2019; Carandini & Heeger, 2011; Reynolds & Heeger, 2009). Under this model, the responses of
individual neurons are scaled or normalized based on the activity of surrounding neurons. This normalization helps ensure that the neural responses are more robust and less affected by unattended stimuli or background activity. Attention operates by adjusting the normalization process. When attention is directed toward a particular stimulus, it enhances the activity of the neurons representing that stimulus, while simultaneously suppressing the activity of surrounding neurons. This selective modulation allows for improved processing and prioritization of attended stimuli. Normalization is thought to reflect a canonical cortical computation (Carandini & Heeger, 2011), potentially implemented via a canonical cortical microcircuit (Fu et al., 2014; Kepecs & Fishell, 2014; Pi et al., 2013; Xue et al., 2014). Rapid and spatially localized ACh release may represent a key neurochemical basis of the biasing signals which adjust normalization (Schmitz & Duncan, 2018). In this way, directed attention and ACh signaling may coordinate to bias competitive interactions among stimuli and task representations throughout the cortical hierarchy, enabling some representations to dominate over others when they become behaviorally relevant (Desimone & Duncan, 1995) or when their behavioral relevance becomes unreliable within an expected probability range (Moran et al., 2013; Yu & Dayan, 2005). The concurrent ACh-mediated activity increases and decreases detected by our meta-analyses may reflect biasing signals adjusting midcingulo-insular normalization of internal states related to task representations, stimuli and responses, though this is highly speculative. In a non-pharmacological fMRI study, Bloem et al (2019) explicitly tested the normalization model of attention by designing orientation stimuli which evoked varying levels of parallel excitation and inhibition in the visual cortex due to competitive interactions among different orientation-tuned neurons. By carefully measuring the orientation-tuned BOLD responses in the visual cortex, they demonstrated parallel attentional modulation and suppression of attended and unattended stimuli, respectively, consistent with biasing of cortical normalization processes. Whether similar effects are detectable with fMRI in the frontal cortex is unknown. Nevertheless, Bloem et al (2019) provide an experimental framework to study the normalization model of attention in humans with fMRI, and, by extension, its potential neurochemical basis with pharmacological fMRI.
This study has limitations related to meta-analysis in general and pharmacological neuroimaging in particular. Generally, meta-analysis is susceptible to publication bias and variability in data quality, study populations and experimental designs. We used several study inclusion criteria to mitigate heterogeneity in the populations examined and in the experimental designs employed across studies. A limitation more specific to our meta-analysis of pharmacological neuroimaging studies concerns the relatively small size of our sample (N=24), which may increase risk for both Type I and Type II error. To mitigate this limitation, we conducted larger discovery and validation meta-analyses using non-pharmacological imaging studies. Finally, it should be noted that the interrelationships among pharmacological manipulation of ACh, fMRI BOLD, rCBF PET and neuronal activity are indirect. Inferences drawn from these studies about the modulatory effects of ACh on neuronal activity merit caution. The cholinergic system is a complex network of neuronal and receptor subtypes. These include striatal and cortical cholinergic interneurons, in addition to the large projection cholinergic neurons of the BF (Ahmed et al., 2019). ACh signaling via nicotinic and muscarinic receptor subtypes is increasingly understood to operate on potentially separate spatiotemporal scales (Obermayer et al., 2017). This physiological complexity is difficult to resolve with imaging techniques currently feasible in humans. The cholinergic system is also involved in many critical physiological functions, such as vasodilation, some of which may directly impact the cerebral blood flow component of BOLD and rCBF responses (Hamner et al., 2012). Nevertheless, we show that the spatial pattern of brain co-activations identified by our meta-analyses closely resembles areas which are densely innervated by the BF cholinergic projections, assayed by cell type specific PET radiotracers and multimodal MRI measures of BF connectivity.

In sum, the present meta-analytic findings provide further evidence that the midcingulo-insular network is a cortical ‘hotspot’ for BF cholinergic innervation and ACh modulatory activity during attentionally demanding tasks.
4.6 Reference


112


Lin, C. P., Frigerio, I., Boon, B. D. C., Zhou, Z., Rozemuller, A. J. M., Bouwman, F. H.,


Internationale Neuropsychopharmacologica, 12(10), 1307–1317.


Yeo, B. T. T., Krienen, F. M., Sepulcre, J., Sabuncu, M. R., Lashkari, D., Hollinshead, M.,

118


Chapter 5

Conclusions and Future Directions

5.1 Summary of Thesis

The basal forebrain (BF) is a collection of subcortical nuclei that provide the major sources of acetylcholine to the neocortex and hippocampus. In the past decade, cell type-specific labeling techniques have led to several major revisions in our understanding of the morphology and function of BF cholinergic neurons. First, the BF cholinergic neurons are enormous, with individual cells having more than 1000 axonal branches observed in mice. Second, the organization of cholinergic neurons, with respect to the position of their cell bodies within the BF and the cortical targets of their projections, appears to reflect a topography. Moving from anteromedial to posterolateral nuclei of the BF, cholinergic neurons differ in terms of their preferred cortical projection targets. Despite the fact that the BF cholinergic system is highly conserved across mammalian species, humans have evolved a much larger cerebral cortex compared to mice. How are the BF cholinergic neurons organized in the human brain?

Much of the knowledge on the BF organization and cortical projections is from non-human animal studies. Current atlases of the human BF are based on postmortem histology, and all previous studies on in-vivo BF connectivity-based parcellation use a single modality of functional data. In Chapter 2, we used HCP 7T diffusion MRI and resting-state fMRI data within the same sample to evaluate multimodal (structural and functional) basal forebrain connectivities. Discrete parcellation using spectral clustering analyses of both functional and structural data showed broad differentiation of anteromedial from posterolateral nuclei of BF, similar to previous functional studies on human BF, as well as to the topography of projections observed in mice. However, we also observed that the structural and functional parcellation results are not identical and tended to become dissimilar with increasing partitions (k). This dissimilarity leads us to ask whether the organization of the human BF reflects its functional and structural integration with the cortex.

We addressed this question in Chapter 3 using the same multimodal imaging data as in Chapter 2 but used a gradients approach to elucidate fine-grained continuous maps of connectivity. Additionally, we employed a PET radiotracer
targeting the vesicular acetylcholine transporter (VACht) to quantify the locations and density of presynaptic BF cholinergic terminals with cell type specificity. Similar to the topography of BF cholinergic projections observed in Chapter 2, we found that the human BF exhibits a gradient broadly differentiating anteromedial from posterolateral nuclei both structurally and functionally. We then examined the interrelationship between gradients of structural and functional connectivity—how closely their spatial profiles are tethered. We found that structure-function tethering varied across the BF, with the greatest detethering concentrated within the posterolateral nucleus basalis of Meynert—a subregion that has undergone disproportionate phylogenetic progression in size and complexity in humans. We also examined where this detethering was most strongly expressed in the cortex and found that the highest detethering cortical regions closely recapitulated the hubs of the ventral attention network.

We then proceeded to explore what might account for this observed detethering. Our first hypothesis was axonal branching; we expected that branching in BF cholinergic neurons would drive detethering between measures of BF structural and functional connectivity. Consistent with this hypothesis, we observed that VACht concentrations were highest in cortical areas exhibiting greater detethering, such as the ventral attention network, as opposed to cortical areas in which BF structural and functional connectivity were more closely tethered, such as the unimodal sensory cortex. Cortical properties that further strengthened this spatial relationship included shorter geodesic distance to the BF and lower cortical myelination (Figure 5.1).

**Figure 5.1:** Summary of our findings on BF cortical connectivities (from Chapter 3).
Darker pink indicates higher detethering and VACHT concentration, shorter geodesic distance and weaker myelination while the faded color indicates lower detethering and VACHT concentration, longer geodesic distance and stronger myelination. The white outline indicates the border of the ventral attention network.

Finally, in Chapter 4, we investigated how acetylcholine release from cholinergic BF neurons affects cortical function and cognition. As direct stimulation of these neurons in humans is not feasible, we performed a quantitative meta-analysis of placebo controlled pharmacological fMRI studies. These studies administered cholinergic agonists to cognitively normal younger adults engaged in cognitively demanding tasks. Our results demonstrate that patterns of cingulo-opercular brain activity evoked by directed attention tasks are altered by pharmacological activation of acetylcholine (ACh) compared to placebo. These patterns spatially overlap with cortical targets that receive dense BF cholinergic projections, which are estimated from the intrinsic BF cholinergic connectivity (Figure 5.1 and Chapter 3) and BF meta-analytic connectivity mapping in a large sample of task fMRI studies. Furthermore, measures of concurrent behavioral performance in these studies revealed that ACh, compared to placebo, yields faster responses while maintaining equivalent accuracy.

5.2 Future Directions

5.2.1 The basal forebrain-hippocampus projection

The cholinergic BF projects to subcortical structures such as hippocampus and amygdala as well as to the neocortex (Liu et al., 2015; M. M. Mesulam, 2004; M. M. Mesulam et al., 1983). This thesis primarily focused on the BF-cortical projection, although the HCP-MMP 1.0 (Glasser et al., 2016) used in our study includes the "hippocampus" region, the BF-hippocampal projection is not completely covered in this thesis. The hippocampus is also known for its important role in learning and memory (Cohn-Sheehy et al., 2021; Tulving & Markowitsch, 1998) and denervation at the early stage of neurodegenerative diseases such as Alzheimer's disease (AD) (DeTure & Dickson, 2019). A careful investigation of BF-hippocampal projection can not only provide comprehensive insight into the cholinergic BF connectivity but can
also help us better understand cognitive processing and its decline in aging and neurodegenerative diseases.

5.2.2 The basal forebrain projectome in aging and Alzheimer's disease

It is well-known that cognition declines with normal aging (Park & Reuter-Lorenz, 2009). During directed attention tasks, older adults are more likely to encode unattended information compared to younger adults, a process which is linked to a loss of executive function (Gazzaley et al., 2005; Gazzaley & Nobre, 2012; Quigley et al., 2010; Schmitz et al., 2010, 2014). Additionally, older adults process sensory information with less selectivity compared to younger adults (Geerligs et al., 2014; Goh, 2011; Koen & Rugg, 2019). Age-related decline in cognition is prominent in attentional tasks and speed of processing, and a significant decline is also observed in memory function. Our BF-connectivity map derived from younger adults in Chapter 3 indicates the highest structure-function divergence in the transmodal cortex, including the anterior cingulate cortex, and areas corresponding to the ventral attention network. How does the BF projectome differ for older adults? Interestingly, studies of individuals immune to normal age-related cognitive impairment (so-called SuperAgers) have demonstrated significantly thicker anterior cingulate cortex (Harrison et al., 2012; Katsumi et al., 2022).

Moreover, as mentioned in Chapter 1 (section 1.2.4), BF is vulnerable in neurodegenerative diseases such as AD. Recent in vivo structural MRI findings from several research groups, primarily using voxel-based morphometry, have indicated that the structural integrity of the basal forebrain provides a sensitive marker of early risk factors for AD (M.-M. Mesulam, 1999; Teipel et al., 2005, 2011). These findings most likely reflect early and selective loss of the magnocellular cholinergic neurons which populate this region. However, it should be noted that degeneration of the cell bodies of cholinergic neurons located in the basal forebrain most likely follows degeneration of the axonal projections throughout the cortex and amygdala. This potentially even earlier biomarker of cholinergic degeneration has received comparatively less research attention due to the technical challenges of quantifying structural and functional measures of connectivity in vivo. Our methodology can be employed to study the role of cholinergic projection in the normal and abnormal...
cognitive decline associated with aging. It may also help in developing potential imaging-based biomarkers for neurodegenerative diseases such as AD.
5.3 Reference


