

What are the Brain Specializations underlying Human Vocal Control?

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Declaration

All data and interpretations presented in this thesis are my own work with contributions from my two supervisors Profs Rogier Mars and Kate Watkins and from others as outlined below. Funding for this project was provided by a PhD studentship from the Wellcome Trust. The functional magnetic resonance imaging study (chapter 2) was performed at the Oxford Centre for Human Brain Activity, University of Oxford. The study on sulcal anatomy (chapter 3) was performed in collaboration with Prof Michael Petrides at the Montreal Neurological Institute, McGill University. Funding for this research visit to Montreal was provided by the Oxford-Zürich-McGill Partnership in Neurosciences. Scanning of post-mortem macaque samples underlying the data analysed for the comparative study (chapter 4) were conducted by Prof Jerome Sallet and Prof Rogier Mars at the University of Oxford. *In vivo* macaque scans were provided by Prof Kristine Krug from the University of Oxford. Chimpanzee data was obtained from the National Chimpanzee Brain Resource collected at the Yerkes National Primate Research Centre. This thesis is approximately 45.000 words and has only been submitted to the University of Oxford for the degree of Doctor of Philosophy in Neurosciences and no part has been submitted for any other degree at this or any other University.

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Abstract

One uniquely human experience is our ability to communicate via speech and language. Specialized laryngeal motor control has been suggested to be a critical adaptation in the human brain that enabled speech. The aim of this thesis was to study the human brain specializations underlying laryngeal motor control and to study evolutionary principles of brain organization within the primate lineage. I investigated these questions using magnetic resonance imaging and comparative primate neuroanatomy.

To characterize the representations of the larynx in the human brain, I used anatomical and functional studies. First, I mapped the motor representations of the larynx and other effectors using fMRI. I designed a novel functional paradigm to isolate brain activity related to laryngeal activity during vocalization whilst controlling for breathing. Then I characterized the cortical microstructure underlying the activated regions using structural and quantitative neuroimaging. The results revealed the existence of a dorsal larynx representation in primary motor cortex and a ventral larynx representation presumed to be in premotor cortex as indexed by lower cortical thickness and lower myelin content.

Next, I examined whether variability in cortical folding explains inter-individual variability in the location of the larynx representations. I provided a systematic description of the sulcal patterns in the central and subcentral region. I showed that the anterior subcentral sulcus typically consists of two distinct segments, as opposed to being one continuous sulcus as previously described in a brain atlas. I demonstrated a tight link between morphological landmarks in the central and subcentral region and functional activation maxima for several movement types. This result indicates that inter-individual variability in brain morphology

might have contributed to controversies and inconsistent reports in the case of the ventral larynx representation.

Furthermore, I set out to dissociate different scenarios of cortical brain reorganization during primate evolution. Using a surface-based cross-species registration, I studied if changes to the relative location of brain areas and cortical expansions went hand in hand with changes to connectivity profiles. The registration was based on homologous maps of cortical myelin in humans, chimpanzees and macaques to model local cortical expansions and relocations of brain areas. Then we applied this registration to surface tract maps of a set of homologous white matter tracts. Our results demonstrated that several tracts can be predicted well by cortical expansion and relocation, but that the arcuate fasciculus underwent additional evolutionary modifications affecting the temporal lobe connectivity pattern.

Abbreviations

ACC	Anterior cingulate cortex
AF	Arcuate fasciculus
ascs	Anterior subcentral sulcus
ascs_lat	Lateral segment of the anterior subcentral sulcus
ascs_op	Opercular segment of the anterior subcentral sulcus
BA	Brodmann area
BOLD	Blood-oxygen-level-dependent
cris	Circular insular sulcus
cs_1 (2, etc.)	Segment of the central sulcus 1 (2, etc.)
CST	Cortico-spinal tract
CUREC	Central University Research Ethics Committee
DCES	Direct current electrical stimulation
dMRI	Diffusion-weighted magnetic resonance imaging
ECoG	Electrocorticography
EPI	Echo-planar imaging
fMRI	Functional magnetic resonance imaging
FSL	FMRIB software library
FWHM	Full width at half maximum
GLM	General linear model
HCP	Human connectome project
IFO	Inferior fronto-occipital fasciculus
ILF	Inferior longitudinal fasciculus
iprs	Inferior precentral sulcus
iprs-i	Inferior ramus of the inferior precentral sulcus
LCA	Lateral cricoarytenoid muscle
LDA	Linear discriminant analysis
LH	Left hemisphere
MDLF	Middle longitudinal fasciculus
MNI	Montreal Neurological Institute
MPM	Multi-parameter mapping
MRI	Magnetic resonance imaging
MSM	Multi-modal surface matching
MTw	Magnetization-transfer-weighted
PCA	Posterior cricoarytenoid muscle
PDw	Proton-density-weighted
PET	Positron emission tomography
pscs	Posterior subcentral sulcus
qMRI	Quantitative magnetic resonance imaging
RH	Right hemisphere
ROI	Region of interest
SD	Standard deviation
SLF	Superior longitudinal fasciculus
SMA	Supplementary motor area
T1w	T1-weighted
T2w	T2-weighted
TMS	Transcranial magnetic stimulation
UF	Uncinate fasciculus
VOF	Vertical occipital fasciculus

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1

Introduction

One uniquely human experience is our ability to communicate via speech and language. However, we see the roots of these behaviours in our closest relatives in the animal kingdom (Petkov and Jarvis, 2012; Fitch, 2017). Given that all species-specific behaviours arise from evolutionary adaptations of the brain, the speech and language system therefore provides a prime example to study brain evolution. Comparing the human to the non-human primate brain can reveal shared and distinct aspects of brain organization to help us understand the neural basis of speech and language. Ultimately this will provide a window into understanding the nature of human uniqueness.

1.1. Speech and Language: An Evolutionary Perspective

It is generally agreed that human speech and language are unparalleled in the animal kingdom. Different theoretical frameworks have been proposed to characterize the differences in vocal behaviour across species. Although the vocal behaviour of humans and that of other animals are strikingly different, the precise definition of a 'uniquely human' aspect has been subject of a long-standing and ongoing debate (Hauser et al., 2002, 2014). The conceptual models describing the behavioural differences shape the way we study the neural architecture underlying these differences. Linguists and cognitive scientists proposed a 'language module' that describes an innate and uniquely human element of our cognitive system (Chomsky, 1980; Fodor, 1983; Pinker, 1994). The notion of a domain-specific language centre that hosts such a module in the brain, however, has been challenged by empirical evidence from functional neuroanatomy in primates. Various research disciplines, in fact, demonstrated that many aspects of our vocal behaviour are shared with other animals. An alternative framework thus describes the faculty of language in terms of a 'broad' and a 'narrow' sense (Hauser et al., 2002). In the 'broad' sense we include behaviours that are shared with other animals, such as vocal sound production and intentional imitation. In the 'narrow' sense, however, the model

describes uniquely human cognitive operations that relate to recursion and combinatorial manipulation. In a more recent account, vocal behaviour has been described as a continuum across species, where humans are considered most advanced in a range of component traits and in particular in the vocal learning component (Jarvis, 2019).

In light of these evolutionary theories of vocal behaviour, neuroanatomical specializations need to be studied in a comparative framework that can account for both shared and distinct features across species (Krubitzer and Kaas, 2005). Functional and anatomical studies across species, particularly in members of the primate lineage, allow us to assess the neural foundations of vocal behaviour. But, similar to the behavioural domain, comparative neuroanatomy faces the challenge of defining homologies. It is not trivial to determine which parts of the brain can be considered homologous, given that evolution affected all aspects of the brain's organization in different ways.

One way to deal with this problem is to transform brains of different species to a common reference space based on a certain modality and then to assess how other aspects changed relative to this transformation (Mars et al., 2018a). Several modalities have been proposed as suitable bases to derive a shared coordinate system across primate species. Comparable neural structures have been defined based on gross anatomy (Van Essen and Dierker, 2007; Hill et al., 2010; Eichert et al., 2019b; Heuer et al., 2019), cytoarchitecture (Petrides, 1999; Zilles and Amunts, 2010), cortical microstructure (Glasser et al., 2014) and brain function (Petkov et al., 2008; Sallet et al., 2011; Neubert et al., 2014) or based on brain connectivity (Mars et al., 2016c, 2018a; Eichert et al., 2019b; Roumazeilles et al., 2020).

Advances in neuroimaging allow us to study both the living human brain and also post-mortem non-human brain specimens (Zilles et al., 2011; Thiebaut de Schotten et al., 2019; Friedrich et al., 2020). Using the same method to acquire structural and functional data from human and non-human brains offers great potential to define homologous structures and systems. Equipped with these tools, the field of comparative neuroscience is at a point where we can formulate and test different evolutionary theories on the origins of speech and language (Mars et al., 2018a). In this thesis, we developed such a comparative approach further to study species differences in primate brain organization. The comparative work was combined with a neuroanatomical characterization of the human speech motor system with a focus on the laryngeal motor cortex given that this is a highly controversial topic in evolutionary neuroscience.

1.2. The Neural Basis of Human Vocal Motor Control

Vocal motor control relies on a distributed system of cortical and subcortical brain areas and this section of the thesis will give a brief overview of some critical components and how they are thought to interact (see Figure 1.1 for an overview). Overall, it is important to note that different elements of this network do not necessarily function in a sequential order, but that the system involves multiple feedback loops that signal in parallel and at different time scales (Friederici and Singer, 2015; Hagoort, 2016; Hickok et al., 2016).

The neural basis of human vocalization and speech production is the topic of ongoing debates (Jürgens, 2002; Price, 2012; Ackermann et al., 2014; Fitch, 2017; Jarvis, 2019). One central aspect of speech production is motor control of the larynx, which serves as primary sound source for all vocalizations. Neural control of the larynx, however, is highly controversial. The anatomy and cortical control of the larynx are therefore introduced in two dedicated sections

below (1.3 and 1.4). Some of the primary evidence discussed here stems from observations in non-human primates, but an exhaustive review of the literature on the neurobiology of primate vocalization is out of scope here.

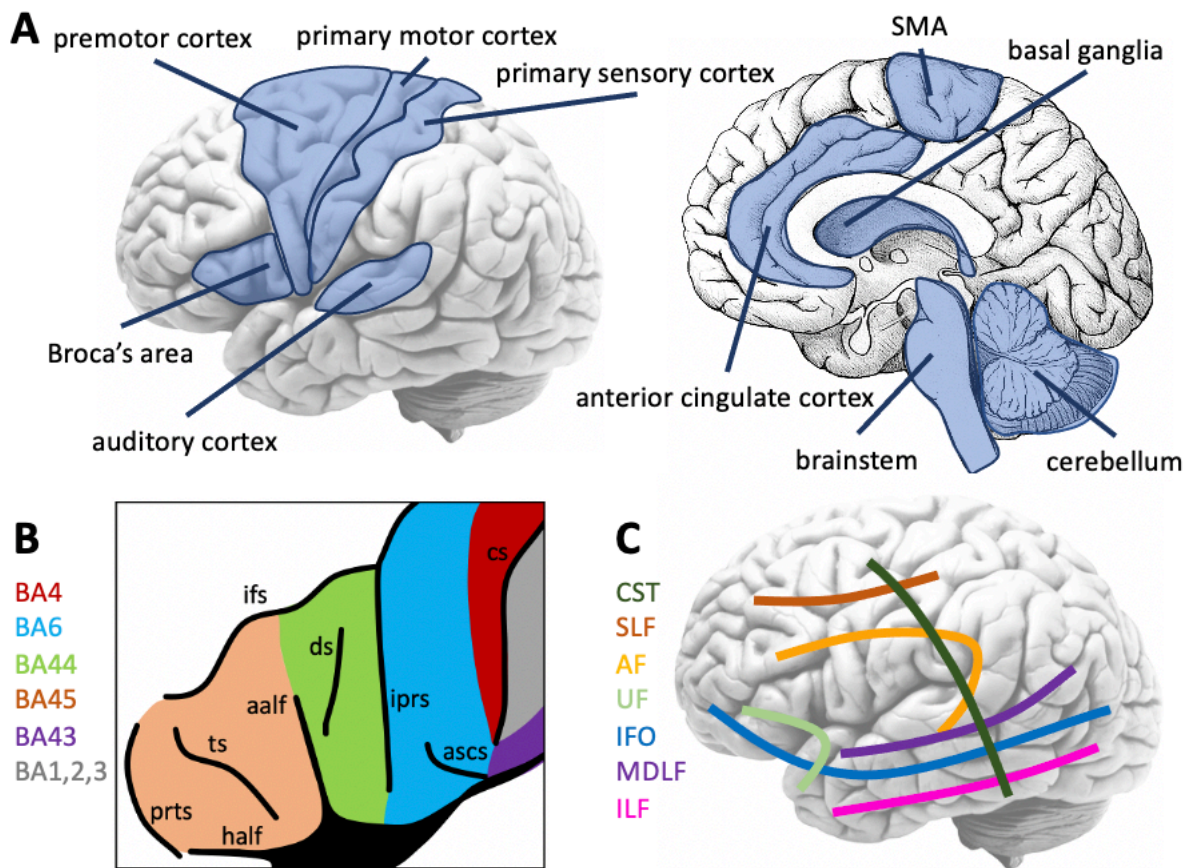


Figure 1.1 Brain regions and pathways critical for human speech and language.

A: Approximate locations of brain regions, which have a central role in human speech and language. **B:** The patterning of Brodmann areas in left inferior frontal cortex and proposed relations to sulci (adapted from Brodmann, 1909 and Petrides and Pandya, 2009). *cs*: central sulcus, *iprs*: inferior precentral sulcus, *ascs*: ascending subcentral sulcus, *ds*: diagonal sulcus, *ifs*: inferior frontal sulcus, *aalf*: anterior ascending ramus of the lateral fissure, *half*: horizontal anterior ramus of the lateral fissure, *prts*: pretriangular sulcus, *ts*: triangular sulcus. **C:** The approximate course of the major long-range fiber bundles involved in speech and language. CST: cortico-spinal tract, SLF: superior longitudinal fasciculus, AF: arcuate fasciculus, UF: uncinate fasciculus, IFO: inferior fronto-occipital fasciculus, MDLF: middle longitudinal fasciculus, ILF: inferior longitudinal fasciculus.

1.2.1. Brain areas in the vocal motor system

The primary motor cortex, cytoarchitectonic brain area Brodmann Area (BA) 4 (Brodmann, 1909), is located on the anterior wall of the central sulcus and the precentral gyrus. Primary motor cortex plays a crucial role in voluntary movements of the body (Vogt and Vogt, 1919; Penfield and Boldrey, 1937). Different effectors are represented in a somatotopic fashion and those involved in speech production and vocalization are located in the ventral portion of the central sulcus (Penfield and Boldrey, 1937; Grabski et al., 2012; Bouchard et al., 2013; Breshears et al., 2015). Posterior to the central sulcus, somatosensory cortex (BA1,2,3) and the subcentral area BA43 are located. Dense connections between motor and sensory cortex provide the basis for continuous somatosensory feedback, which is critical for rapid adjustments during speech production (Tremblay et al., 2003; Lametti et al., 2012). The precise nature of the signal that the motor cortex encodes, however, is currently still debated (Graziano et al., 2002; Conant et al., 2018). The primary output of the motor system is conveyed via connections from upper motoneurons in the primary motor cortex to motoneurons in the brainstem and the spinal cord.

The brainstem contains all afferent and efferent projections from and to the brain and it contains the bodies of lower motoneurons, which are arranged in nuclei. The muscles controlling articulators such as lip and tongue, are controlled by a specific set of brainstem nuclei including the nucleus hypoglossus and the trigeminal motor nucleus. Motoneurons that signal to the intrinsic laryngeal muscles, however, are located in nucleus ambiguus. Note that the innervation of the larynx is described in more detail in a dedicated section below (1.3.3). The connections between primary motor cortex and brainstem nuclei are typically mediated via local interneurons (Iwatsubo et al., 1990). In addition to indirect connections also direct, that

is monosynaptic, connections exist between primary motor cortex and brainstem nuclei (Kuypers, 1958a). It has been suggested that the indirect connections are phylogenetically older (Porter and Lemon, 1993) and that the development of direct brainstem connections played a critical role in the evolution of fine motor control in humans (Porter and Lemon, 1993; Simonyan and Jürgens, 2003a; Simonyan, 2014).

In addition to the brainstem, other subcortical structures, such as the basal ganglia and cerebellum play an important role in the vocal motor system. The relevance of the basal ganglia in motor behaviour and learning of motor sequences is well established. As part of a motor processing loop, primary motor cortex sends signals to and receives signals from the basal ganglia, the latter via the thalamus (Kotz et al., 2003; Postuma and Dagher, 2006). During well-learned motor sequences, such as speaking, it is assumed that the basal ganglia provide internal timing cues about the upcoming movements to the cortex (Ferrandez et al., 2003; Alm, 2004). The cerebellum plays a role in a variety of cognitive and motor functions as it integrates information from several brain areas to control fine-tuning, coordination and precision of movements. During motor learning, the cerebellum helps to adapt motor behaviour based on a mismatch of expected and actual sensory consequences (Tseng et al., 2007; Lametti et al., 2018).

Supplementary motor area (SMA) proper, pre-SMA and cingulate cortex are cortical midline structures that are involved in the initiation and the control of speech movements (Goldberg, 1985; Cunnington et al., 2002). SMA and pre-SMA are critical for self-initiated vocalizations and they contain a somatotopic map of motor representations (Picard and Strick, 1996; Loh et al., 2017, 2020). In non-human primates and in humans, the anterior cingulate cortex (ACC) is active during the initiation of involuntary vocalizations such as laughter and cry (Jürgens and

Pratt, 1979). It has been suggested that ACC signals directly to the brainstem, as part of an ancestral ‘cingulate vocalization system’, which bypasses the primary motor cortex (Jürgens and Pratt, 1979; Loh et al., 2017).

As opposed to the cingulate system of vocal motor control, which is shared with non-human primates, the neocortical vocalization system involves activation of inferior frontal cortex and premotor cortex (BA6) (Ploog, 2002; Hage and Nieder, 2016). In humans, the role of left inferior frontal cortex, or Broca’s area, in linguistic processes is widely accepted (Price, 2000; Tremblay and Dick, 2016). The anterior portion of Broca’s area, BA45 or pars triangularis, is involved in semantic processing, and the posterior part, BA44 or pars opercularis, is involved in phonological processing (Devlin et al., 2003; Gough et al., 2005). While the role of inferior frontal cortex in linguistic and cognitive processes has been well established, it is not critical for production of basic speech movements (Price et al., 2011; Flinker et al., 2015).

Premotor cortex (BA6) is located posterior to Broca’s area and anterior to primary motor cortex (Petrides, 1999). Premotor cortex is extensively connected to other cortical regions of the vocal motor control system (Dum and Strick, 1991) and has been shown to play a modulatory role during conditional action selection and initiation (Cisek and Kalaska, 2005; O’Shea et al., 2007; Hage et al., 2013). It has been suggested that the central somatotopic map in primary motor cortex may extend into premotor cortex (Woolsey et al., 1952; Graziano et al., 2002), but others argue that there are multiple separate motor maps in the frontal lobe, one of them hosted in premotor cortex (Rizzolatti and Luppino, 2001). The functional organization patterning of brain areas the inferior frontal cortex and in the subcentral region is highly complex (Ono et al., 1990; Sprung-Much and Petrides, 2018, 2020). Figure 1.1B shows the proposed relations between Brodmann areas and sulcal morphology (Brodmann, 1909; Petrides

and Pandya, 2009). These relations, however, as well as their implications for the functional organization of cortex are controversial (Zilles et al., 1997).

The auditory cortex in posterior superior temporal cortex processes neural signals during auditory perception. It is therefore active during self-generated vocalizations and mediates auditory feedback, which is a critical aspect of speech production (Liberman et al., 1967; Cogan et al., 2014; Hickok et al., 2016). Furthermore, high-level auditory areas specialized to process conspecific voices have been identified in the temporal lobe throughout the primate lineage (Belin et al., 2000; Petkov et al., 2008). Other temporal brain areas are involved in higher cognitive functions that critically relate to language function, such as semantic processing in the middle temporal gyrus (Dronkers et al., 2004; Whitney et al., 2011) and social cognition in posterior temporal lobe (Sallet et al., 2011; Schurz et al., 2014).

1.2.2. Major brain pathways in the vocal motor system

Given that the brain regions involved in vocal motor control are widely distributed across the entire brain, a broad and complex network of connections is required to mediate communication within this system (Figure 1.1A) (Friederici, 2009; Catani and Bambini, 2014; Hickok et al., 2016; Hagoort, 2019). In this section, I will introduce the major long-range fiber bundles containing the projecting axons that support the communication between brain areas (Catani and Thiebaut de Schotten, 2015). It should be noted that the anatomical segmentation of these fiber bundles as well as their cortical termination zones are a topic of ongoing debates (Dick and Tremblay, 2012; Tremblay and Dick, 2016).

One major fiber bundle that has been a major focus of evolutionary language research is the arcuate fasciculus (AF), which connects posterior temporal and parietal areas with the frontal lobe (Catani et al., 2005; Rilling et al., 2008; Eichert et al., 2019b). The AF is involved in a variety of language functions, such as language repetition (Wernicke, 1874; Bernal and Ardila, 2009), auditory-motor transformations (Hickok and Poeppel, 2004) as well as syntactic and phonological processes (Catani and Thiebaut de Schotten, 2008; Yeatman et al., 2011). Another long-range pathway that connects parietal and frontal lobe is the superior longitudinal fasciculus (SLF), which has three subdivisions (SLF1-3). The SLF is involved in several cognitive functions, notably in attention (De Schotten et al., 2011). Together, the SLF and the AF they have been described as the ‘dorsal’ language stream (Saur et al., 2008; Hickok et al., 2016).

The concept of a distinct dorsal and ventral processing stream has been borrowed from the visual system and also proved highly successful in understanding the functional anatomy of speech processing (Hickok and Poeppel, 2004). As opposed to the dorsal stream, the ventral language stream connects posterior temporal and parietal areas with the frontal lobe via the temporal lobe (Saur et al., 2008; Friederici, 2009). The following tracts have been suggested to form part of it: inferior fronto-occipital fasciculus (IFO), middle longitudinal fasciculus (MDLF), inferior longitudinal fasciculus (ILF) and the uncinate fasciculus (UF). The anatomical dissociation of the dorsal and ventral route supposedly forms the structural bases for a functional dissociation: The dorsal language stream supports mapping sound onto articulatory based representations (Hickok et al., 2016) and the ventral language stream is involved in lexical processing and accessing meaning (Saur et al., 2010; Lopez-Barroso et al., 2011).

Another important set of pathways are the pyramidal tracts, i.e. the cortico-bulbar and the cortico-spinal tract (CST). These tracts run through the medullary pyramids and bundle the descending projections from pyramidal neurons in primary motor cortex to the lower motoneurons (Kandel et al., 2013). Generally speaking, the cortico-spinal tract supplies brainstem motoneurons in the spinal cord, which control the muscles of the lower body and the corticobulbar tract connects to pools of lower motoneurons that control the head and neck. The motor neurons leave the brainstem in form of cranial nerves, which bundle both afferent and efferent fibers.

In addition to the major long-range fiber bundles described above, an abundance of other cortico-cortical fibers exist, such as the ones connecting Broca's area and primary motor cortex. These connections, however, are more diffuse and therefore more difficult to identify and characterize robustly.

1.3. Anatomy of the Larynx

The larynx, or voice box, is the central source for sound production during vocal communication in mammals. The larynx functions as a valve in the throat which connects inferiorly to the trachea and superiorly to the pharynx and the oral cavity (Figure 1.2). Several cartilages, muscles and ligaments form a complex arrangement, and their interactions contribute to the larynx' function as a vocal organ. Extrinsic muscles and ligaments connect the laryngeal cartilages to other skeletal structures while intrinsic muscles and ligaments connect the cartilages to each other.

cartilage sits a pair of small arytenoid cartilages. The cricoid, thyroid and arytenoid cartilages are connected via the cricothyroid ligament. This ligament has a thickened free upper margin, which has been termed the vocal ligament or true vocal cord. The bilateral pair of vocal ligaments forms a mucosal fold, which is known as the vocal fold and the space between the vocal folds is referred to as glottis or rima glottidis (Figure 1.2B).

The internal muscles of the larynx control the configuration of the cartilages and the vocal folds, which is vitally important during vocalization (Figure 1.2B). The lateral cricoarytenoid muscle (LCA) connects the lateral portion of the cricoid cartilage to the anterior tip of the arytenoid cartilages, while the posterior cricoarytenoid muscle (PCA) connects to the posterior side of the arytenoid cartilage. Transverse and oblique arytenoid muscles are unpaired and span the posterior surface of the arytenoid cartilages. The thyroarytenoid muscles form the body of the vocal folds and therefore run in parallel to the vocal ligament. The upper part of the thyroarytenoid muscle, close to the vocal ligament is a specialized part of muscle tissue, which is referred to as vocalis muscle. External laryngeal muscles, such as the thyrohyoid and the sternothyroid muscle can cause vertical movement of the larynx within the throat.

1.3.2. Laryngeal function during vocalization

The function of the larynx during vocalization is determined by the configuration of the cartilages and the vocal folds. During rest and normal breathing, the glottis is open, and the vocal folds are apart from each other, i.e. they are abducted, so that airflow from the trachea is not disturbed. During vocalization the vocal cords get pulled together, i.e. they are adducted, and the vocal ligament is stretched. Airflow during exhalation can then set the vocal folds in vibration, which generates a complex sound wave at a specific fundamental frequency, or pitch,

with harmonic overtones. The configuration of the vocal cords is mediated by a complex interplay of muscles and one specific muscle can be involved in both abduction and adduction of the vocal folds.

Adduction, i.e. stretching of the vocal folds can be induced by activity of several muscles. Contraction of the LCA causes a rotation of the arytenoid cartilages, which narrows the glottis resulting in a tension in the vocal ligaments. The position of the arytenoid cartilages is also influenced by contraction of the transverse arytenoid muscles at their posterior side. Contraction of these muscles pulls the arytenoid cartilages together, which moves the vocal folds inwards and downwards and closes the posterior portion of the glottis. A contraction of the thyroarytenoid muscles draws the arytenoid cartilages towards a more anterior position. This causes a shortening and relaxation of the vocal ligament, but also a closure of the glottis. The posterior cricoarytenoid muscle is the sole abductor of the vocal folds and the direct antagonist of LCA. Contraction of PCA causes an outward rotation of the arytenoid cartilages, which opens the widening of the glottis.

Generally speaking, increased tension in the vocal folds results in a higher frequency of the vocalization, or a higher pitch level. The tension can be changed by contraction of the internal laryngeal muscles, as described above, but also by activity of the external laryngeal muscles and by tongue movement. Such external changes to the position of the larynx affect the relative location of the cartilages, which modifies both the tension in the vocal ligament and the opening of the glottis.

1.3.3. Peripheral innervation of the larynx

The bodies of the motoneurons that innervate the laryngeal muscles are located in the nucleus ambiguus, a brainstem nucleus located within the medulla oblongata. All fibers that reach the larynx travel within the vagus nerve (10th cranial nerve, CN X) to the periphery (Figure 1.2C). The vagus nerve forms several major branches, two of which are the superior laryngeal nerve and the inferior laryngeal nerve. Close to the larynx, the superior laryngeal nerve subdivides into an internal and an external branch. While the internal branch contains mainly sensory afferences from the vocal folds, the external branch innervates the cricothyroid muscles on both sides. All intrinsic laryngeal muscles, other than the cricothyroid muscle, are innervated by the inferior laryngeal nerves. The inferior laryngeal nerves have a U-shaped course as they travel downwards and then curve upwards back to the larynx, where they connect to the muscles. Due to this shape, the inferior laryngeal nerve is also called recurrent laryngeal nerve. On the left side of the body, the recurrent laryngeal nerve has a notably longer course as it runs underneath the aorta before curving upwards. Changes in thickness of the nerves, however compensate for this difference, so that the electric potential reaches both sides of the innervated muscles at the same time (Walker, 1994).

While the peripheral innervation of the larynx, as described above, is accepted textbook knowledge, the cortical innervation of the motoneurons that control larynx activity is a highly controversial topic. The next section introduces the controversies regarding human laryngeal motor cortex, which have strong implications for our evolutionary understanding of the speech motor system.

1.4. Controversies Regarding Cortical Motor Control of the Larynx

Laryngeal motor control is one aspect of vocal behaviour, which has been a focus of evolutionary speech research (Fitch, 2010; Ackermann et al., 2014; Simonyan, 2014; Kumar et al., 2016; Belyk and Brown, 2017). Across the primate lineage, striking differences can be observed regarding the location of the cortical area that controls movement of the larynx (Belyk and Brown, 2017). In non-human primates, the representation of the larynx was determined using direct cortical micro-stimulation experiments, where activation of the laryngeal muscles was directly monitored. The larynx representation was localized robustly and consistently in an anterior ventral area in monkeys (macaque: Semon and Horsley, 1890; Hast et al., 1974; Simonyan and Jürgens, 2003b; squirrel monkey: Jürgens, 1976) and great apes (chimpanzee: Hines, 1940; Bailey et al., 1950; gibbon: Mott et al., 1911; gorilla and orangutan: Leyton and Sherrington, 1917). Histological examination and connectivity studies revealed that this area is located in premotor cortex in macaques (Simonyan and Jürgens, 2003a, 2003b).

Reports on the location of the neural representation of the larynx in the human brain, however, are inconsistent. Traditional human brain mapping studies (Penfield and Boldrey, 1937; Penfield and Rasmussen, 1950) did not directly record from the laryngeal muscles. The neurosurgeons rather assessed if cortical stimulation resulted in speech arrest or evoked a vocalization, which is an indirect assessment of laryngeal function. The precise location of the larynx representation therefore remained uncertain and it was broadly localized to the ventral portion of motor cortex. Non-invasive functional neuroimaging suggested that, in addition to this ventral area close to the Sylvian fissure, larynx movement activates a second distinct area in the human brain more dorsally located close to the representation of the lips and the hand (Brown et al., 2008). Such a dorsal larynx representation was reported consistently in humans using PET, fMRI and electrocorticography. Several studies demonstrated activity in both the

ventral and the dorsal larynx area. Figure 1.3 shows a visualization of the activation maxima from several studies and Table 1.1 lists a selection of previous findings from larynx localizers in humans. Both highlight the strong inconsistencies regarding the cortical location that was identified as larynx representation.

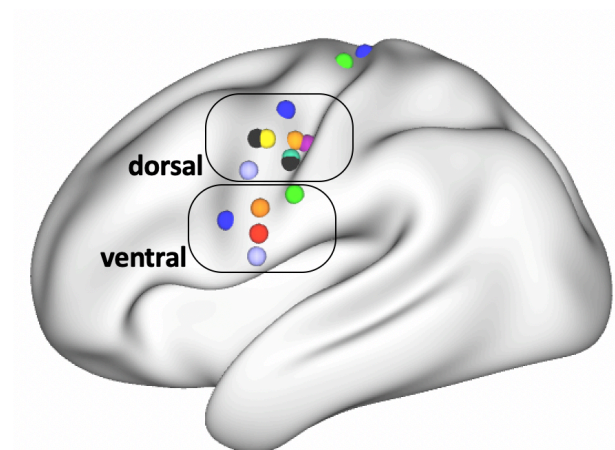


Figure 1.3 Inconsistent reports of the human larynx representation.

Activation maxima for the larynx representation in the human brain as defined in a selection of previous studies. The black boxes indicate what I refer to as dorsal and ventral portions of the central somatomotor region. The coloured circles represent the reported peaks of activation in the following studies: *green*: Belyk & Brown, 2014; *violet*: Bouchard, Mesgarani, Johnson, & Chang, 2013; *black*: Brown, Ngan, & Liotti, 2008; *yellow*: Dichter, Breshears, Leonard, & Chang, 2018; *red*: Grabski et al., 2012; *turquoise*: Kleber, Zeitouni, Friberg, & Zatorre, 2013; *pink*: Murphy et al., 1997; *blue*: Olthoff, Baudewig, Kruse, & Dechent, 2008; *orange*: Terumitsu, Fujii, Suzuki, Kwee, & Nakada, 2006.

Table 1.1 Overview of previous larynx localizers.

Reference	Method	Task	Main result
Penfield and Boldrey (1937)	DCES	induce vocalization and speech arrest by stimulation	mainly ventral region, but distributed across precentral gyrus

(table continued on following 2 pages)

Murphy et al. (1997)	PET	phrase production with and without voicing and articulation only	phonation: ventral region; breathing: dorsal region
Roedel et al. (2004)	TMS	MEPs of laryngeal muscles elicited by stimulation	two distinct activations in dorsal region
Schulz et al., (2005)	PET	voiced vs. whispered speech	Both activate precentral gyrus, but no difference between conditions
Haslinger et al. (2005)	fMRI	vowel production in patients with laryngeal dystonia	both groups activate precentral gyrus, but no group differences
Terumitsu et al. (2006)	fMRI	syllable production with and without articulation and articulation only	phonation activates both dorsal and ventral region
Sörös et al. (2006)	fMRI	vowel production vs. articulator movements	vowel production activates dorsal area, but no difference between conditions
Loucks & De Nil, (2006)	fMRI	phonation vs. controlled exhalation	both conditions activate ventral and dorsal region
Brown, Ngan, & Liotti (2008)	fMRI	vowel production and glottal stop	two separate activations in dorsal region
Olthoff et al. (2008)	fMRI	humming	activation in ventral, dorsal and supra-dorsal region

(table continued on following page)

Simonyan et al. (2009)	fMRI	glottal stop vs. controlled breathing	Both activate mid-portion of the precentral gyrus, but no difference between conditions
Grabski et al. (2012)	fMRI	vowel production	activation in ventral region
Bouchard et al. (2013)	ECoG	vowel production	activation in dorsal and ventral region
Kleber et al. (2013)	fMRI	anaesthesia of laryngeal folds in singers	deactivation in dorsal region
Belyk & Brown, (2014)	fMRI	vertical movements of the larynx	activation in ventral region
Dichter et al. (2018)	ECoG	pitch modulation during sentence reading	activity in dorsal region

Selection of previous investigations of the neural representation of the larynx in the human brain. Task descriptions refer to conditions relevant for laryngeal activity. Only results for the central somatomotor region in the left hemisphere are reported. DCES: direct current electrical stimulation, ECoG: electrocorticography, fMRI: functional magnetic resonance imaging, PET: positron emission tomography, TMS, transcranial magnetic stimulation.

1.5. Evolutionary Theories of Laryngeal Motor Control

Mapping the laryngeal motor cortex across the primate lineage in a comparable way faces two critical challenges: First, the laryngeal muscles are not readily accessible using surface electrodes and therefore invasive techniques are required to study the ultimate basis of laryngeal motor control in absence from any task-related confounds. Second, training non-human primates to produce vocalizations is notoriously difficult (but see Coudé et al. 2011;

Hage et al. 2013) and, to my knowledge, researchers have not yet succeeded in combining this with fMRI.

Nevertheless, the observed species differences across the primate lineage inspired evolutionary theories on laryngeal motor control to account for the neural differences among species and differences in vocal behaviour (Belyk and Brown, 2017; Mars et al., 2018a). One theory suggests that the dorsal larynx representation is a novel structure that evolved in humans in relation to our increased demands on the vocal motor system (Belyk and Brown, 2017). Support for a larynx motor cortex ‘duplication’ theory in humans comes from comparative research on avian species that display vocal learning abilities. The existence of two parallel, or duplicated, vocal motor control pathways has previously been described for the avian brain (Chakraborty and Jarvis, 2015). Furthermore, genetic expression profiles in the human dorsal and ventral larynx area are similar to those of analogous motor nuclei in the avian song system (Pfenning et al., 2014). It has therefore been argued that the evolutionary principle of brain pathway duplication underlies vocal learning behaviour in vocal learners, such as in song birds and humans, but not in non-human primates, who have relatively limited vocal learning capacities (Lameira, 2017a; Jarvis, 2019).

Another aspect that has been discussed in the context of evolution is the cortical location of the ventral larynx area. It has been suggested that the human ventral larynx area migrated posteriorly during human evolution, thereby moving from premotor cortex, as in non-human primates, to primary motor cortex (Belyk and Brown, 2017). Large scale relocations of brain areas to accommodate for local expansions of the cortical sheet have indeed been described in the primate brain. For example, due to expansion of the temporoparietal association cortex, the visual area MT+ complex is located much more ventrally in humans compared to monkeys

(Huk et al., 2002; Mars et al., 2016b). A cortical relocation of homologous areas into a different cytoarchitectonic area, however, has not been described before. Therefore, the ‘migration’ theory of the ventral larynx area, is currently lacking critical cytoarchitectonic evidence as well as mechanistic and evolutionary models.

Evolutionary adaptations underlying larynx motor control have further been studied in the context of brain connectivity. Much attention has been drawn to the connections between laryngeal motor cortex and phonatory brainstem nuclei. In humans, a direct, that is mono-synaptic, descending projection from primary laryngeal motor cortex to nucleus ambiguus has been described (Kuypers, 1958b; Iwatsubo et al., 1990). Such a direct projection is missing in monkeys (Simonyan and Jürgens, 2003b) and is weak in non-human apes (Kuypers, 1958c). While the primary evidence of these species differences does not remain without criticism (Ackermann et al., 2014), these connections have been described as uniquely human phenotype (Simonyan and Jürgens, 2003a). In the manual system, direct cortical projections from primary motor cortex to motoneurons have been shown to parallel the ability of hand dexterity in primates (Isa et al., 2013). It has therefore been suggested that these direct connections form the basis of increased fine control of the laryngeal muscles required for human speech.

Others have focussed on human specializations of long-range cortico-cortical connections supporting vocal motor control (reviewed in Mars et al., 2018). For example, the structural connectivity between laryngeal motor cortex and parietal cortex in humans is stronger than in macaques (Kumar et al., 2016). Furthermore, functional connectivity between laryngeal motor cortex and inferior frontal gyrus is left-lateralized, which indicates a relationship with hemispheric differences in language function (Greenlee et al., 2004; Simonyan et al., 2009). Note that most previous studies focussed on the network related to the dorsal laryngeal motor

area, but more recent work also examined the functional relevance of the structural network supporting the ventral larynx area in speech motor control (Neef et al., 2020).

It has also been suggested that human laryngeal motor control is embedded in a network with enhanced fronto-temporal connectivity (Jarvis, 2019). The fronto-temporal connections are known to play a critical role in a range of sensorimotor and cognitive processes enabling speech and language (Friederici, 2009). Expansion of the dorsal longitudinal connections and the AF is a hallmark of primate comparative neuroanatomy (Rilling et al., 2008; Ardesch et al., 2019; Eichert et al., 2019b; Balezeau et al., 2020). Although there is no evidence to date of a direct structural connection between laryngeal motor cortex and the temporal lobe, understanding the evolutionary changes in long-range fronto-temporal network organization provides critical clues about human brain specializations underlying vocal motor control.

In sum, previous literature suggested several human brain specializations critically related to laryngeal motor control and to the structural connectome underlying the speech system. In this thesis, I employed a systems-level approach using neuroimaging to address these controversies and to shed light on their evolutionary implications. First, I provided a more detailed neuroanatomical and functional characterization of human laryngeal motor cortex. Then, I investigated the relationships between morphological and functional variability in humans. Lastly, I studied principles of cortical reorganization systematically in a comparative framework across the primate lineage.

1.6. Research Methodology

In this thesis I used neuroimaging and comparative neuroanatomy to study the brain specializations underlying human vocal control. I employed magnetic resonance imaging

(MRI) to localize brain function related to human laryngeal motor control and to characterize microstructural properties of cortex underlying the activations (chapter 2). Using manual identification of sulcal segments, I characterized the anatomical morphology in the subcentral region of the brain and related these anatomical landmarks to functional activation peaks in individuals (chapter 3). Finally, I used a surface-based registration method to align maps of cortical myelin content across macaques, chimpanzees and humans (chapter 4). This registration was applied to surface tract maps derived from diffusion-MRI-based tractography. Here, I provide an introduction and justification of the research methodology employed throughout this thesis. Technical details for the implementation of the methods are provided in each chapter.

1.6.1. Structural and quantitative magnetic resonance imaging

MRI is an imaging technique that is widely applied in clinical and basic biomedical research (Baum, 2016). MRI scanners generate magnetic gradients and radio frequency waves that allow us to reconstruct a three-dimensional image of an object, such as a human body part, and since the 1970s, MRI has been used for imaging the human brain. Spatial image contrast in an MRI scan arises from variations in magnetic susceptibility of the tissue, which is governed by the macromolecular constitution of the specimen. The settings and timings of the scanner, i.e. the scanning sequence, determine the sensitivity of the MRI signal towards a certain biological tissue type. The MR signal is obtained at discrete samples in space, i.e. volumetric pixels or voxels. In a conventional anatomical brain scan, a so-called T1-weighted (T1w) scan, the cerebral cortex and deep brain nuclei (the 'grey matter' of the brain) appear darker than the remaining tissue of brain connections (the 'white matter' of the brain). When a different

scanning sequence is used, for example in a so-called T2w scan, grey matter appears brighter than white matter.

Histological studies demonstrated that the cellular make-up of the cerebral cortex differs vastly across different parts of the brain (Brodmann, 1905; Vogt and Vogt, 1919). Based on microstructural features, the cortex can be parcellated into architectonically definable and discrete areas. The boundaries between these brain areas, however, typically cannot be imaged using conventional MRI scanning sequences due to limitations in spatial resolution as well as signal sensitivity and specificity. Another limitation is the interpretability of raw intensity values. Highly complex interactions of technical and biological noise sources make it practically impossible to compare the numerical intensity values from an MRI scan between scanning sites and even within one scanner or one subject. MR signal intensities are therefore usually interpreted relative to a comparable baseline or reference scan.

One such relative measure is the ratio in signal intensity of a T1w and a T2w brain scans, which has been shown to correlate with myelin content in the brain (Glasser et al., 2012; Lazari and Lipp, 2020). The T1w/T2w ‘myelin maps’ can be informative about the location of primary sensorimotor regions, which have typically high myelin content (Fischl et al., 2008). This characteristic of primary brain regions is conserved across all mammals, which opens up the possibility to define homologous brain areas based on T1w/T2w myelin maps (Glasser et al., 2014; Donahue et al., 2018). The biophysical model underlying the signal intensities in the T1w/T2w myelin maps, however, is not fully understood and scanner- or scanning sequence-related confounds can affect the intensity values. The tissue properties in fixated post-mortem T2w brain scans, for example, differ substantially so that T1w/T2w myelin maps can only be obtained from living individuals.

To allow us to compare MR signal intensities numerically, so-called quantitative MRI (qMRI) sequences were developed (Weiskopf et al., 2014). A qMRI sequence allows one to reconstruct an anatomical scan that serves as biomarker for certain structural brain features, such as myelin content or iron concentration. The signal intensities in the reconstructed maps are numerically meaningful, reproducible and comparable across scanning sessions and sites (Weiskopf et al., 2013), which is a major benefit for longitudinal studies and data sharing across sites. By combining the information from multiple quantitative MRI maps, we can make estimates about the cyto- and myeloarchitecture of the cortex (Kuehn et al., 2017; Lipp et al., 2019). While qMRI has so far mainly been used in human brain imaging, the development of comparable sequences for *ex vivo* non-human primate brains is ongoing subject of research (Eichner et al., 2019; Papp et al., 2019).

1.6.2. Functional magnetic resonance imaging of speech production

In addition to structural aspects of brain architecture, the principles of magnetic resonance can be used to localise functional brain activity (Huettel et al., 2009). Functional magnetic resonance imaging (fMRI) is based on changes in the blood oxygenation level that results from increased metabolism in active brain areas. The metabolic processes affect the magnetic properties of the blood and therefore we speak of blood-oxygen-level-dependent (BOLD) imaging. The BOLD signal is typically sampled across time to determine where the signal changes as function of continuous brain activity during rest or during task.

Prior to the development of fMRI, functional imaging of the brain was performed using positron emission tomography (PET). One major drawback of PET, however, is that it requires

the administration of a radiotracer to the participant's blood system. Another limitation of PET is the lack of temporal detail as individual scans are obtained with a gap of several minutes. As opposed to PET, fMRI is a completely non-invasive technique that has to date not been shown to pose health risks and data can be acquired online during task performance at sub-second temporal resolution.

An MRI scanning sequence that is optimized to be sensitive to the BOLD contrast and to sample continuously at a high temporal resolution has the disadvantage of decreased spatial accuracy, compared to anatomical scans. In addition to the limited spatial specificity, the BOLD signal is highly prone to different sources of error, such as signal drop out and magnetic susceptibility artefacts. The signal can also be affected by physiological artefacts arising from, for example, head motion, cardiac pulsation, breathing and scanner-noise-induced auditory perceptions.

These artifacts complicate the characterization of vocalizations and overt speech production using fMRI. One way to handle several of these artifacts is to use a modified scanning sequence that acquires data in the period just after a vocalization, while the BOLD signal is still detectable in the activated brain areas - a so called sparse-sampled MRI acquisition (Hall et al., 1999; Sörös et al., 2006; Simonyan et al., 2009). A disadvantage of a sparse sampling sequence, however, is that it requires increased scanning time to obtain a robust number of scans, when compared to more conventional continuous imaging protocols.

Another way to handle the undesired side-effects is to control for possible confounds directly in the task design. In order to control for breathing-induced neural signals during a vocalization task, for example, a breathing condition can be introduced as baseline condition (Loucks et al.,

2007; Simonyan et al., 2009). To distinguish phonatory mechanisms from articulation during speech production, a previous PET study used a 2x2 factorial task design (Murphy et al., 1997). Participants produced a simple phrase in four ways: spoken aloud, mouthed silently, spoken without articulation and thought silently. Such a factorial design allows to isolate phonation and articulation as separate factors during speech production, while controlling for each other. Another consideration for fMRI acquisition in speech research is to account for effects on the auditory system. Active noise-cancelling headphones can reduce the perception of MRI noise and feedback the sound of the participant's vocalization with minimal delay, so that the auditory experience is more naturalistic.

Taken together, a range of factors need to be taken into account when designing functional task paradigms to localize brain activity related to speech production. In chapter 2, we implemented these considerations to develop a robust localizer for larynx-related brain activity with high specificity and sensitivity.

1.6.3. Tractography based on diffusion-weighted magnetic resonance imaging

In addition to the structural characteristics and functional activation of the brain, magnetic resonance can be used to image brain connectivity. Diffusion-weighted magnetic resonance imaging (dMRI) is based on the preferential diffusion direction of water molecules along the major fiber bundles in the brain's white matter (Bihan et al., 1985). In a dMRI scanning sequence, multiple magnetic gradients are applied at different spatial orientations distributed across the sphere. In a given voxel, the MR signal intensity for a given gradient depends on the white-matter geometry and on the diffusivity in the area captured by the voxel. By integrating this information across all gradients and voxels, we can reconstruct a whole-brain three-

dimensional vector image of preferential water diffusion direction. This map serves as approximation of the orientation in which the fibers in the brain run (Johansen-Berg and Rushworth, 2009).

In probabilistic tractography, this vectorized information is used to determine where the major pathways, or tracts, run in the brain (Behrens et al., 2007). Based on a pre-defined seed region the algorithm samples a distribution of streamlines following the local orientation estimates (Basser et al., 2000). Additional anatomical constraints can be defined to guide the course of the streamlines and the result is a reconstructed white-matter pathway. Tractography based on dMRI, however, has several limitations, which need to be taken into account when interpreting the results (Maier-Hein et al., 2017). Notably, if used without suitable anatomical constraints, tractography can generate false positive results and anatomically non-plausible results. Furthermore, the tracking algorithm can be biased by the cortical gyrification, which makes it difficult, if not impossible, to determine the exact location of a pathway's cortical termination (Jbabdi and Johansen-Berg, 2011; Reveley et al., 2015). Also, differences in the geometry of white matter tracts can affect the estimation of their trajectory: The algorithm performs poorly for sharp turns and it is challenging to model multiple crossing fibres within one voxel (Basser et al., 2000; Jones, 2010; Seunarine and Alexander, 2013).

Despite these limitations, dMRI has become a key technique to reconstruct the structural connections of the brain. DMRI data can be acquired in living individuals and *ex vivo* from fixated brains. This makes it possible to obtain brain connectivity information from a large range of species, which offers a great potential for comparative studies of evolutionary brain anatomy. Several major tracts in the brain have been shown to be homologous across primate species and tractography allows us to reconstruct the course of these tracts in multiple species

using a comparable technique (Mars et al., 2016c, 2018c; Bryant et al., 2020). Structural brain connectivity has therefore emerged as a key methodology for comparative neuroscience (Thiebaut de Schotten et al., 2019).

1.6.4. Individual variability in structural and functional brain anatomy

One aim of basic neuroscience is to determine fundamental principles of the brain that provide the basis of human cognition and behaviour. To derive patterns that are consistent across participants and therefore representative of the human brain, we aim to sample observations from a large study group. MRI is a suitable method to collect anatomical and functional data from large study groups because it is non-invasive and generally considered safe. The community has agreed on reference spaces for human volumetric and surface-based neuroimaging data to facilitate comparisons across studies. One commonly used standard template is the MNI-152 brain, which represents the average of 152 non-linearly registered scans (Fonov et al., 2011). To transform an individual volumetric brain scan to MRI stereotaxic space, a volumetric spatial registration is derived based on the MR signal intensities (Jenkinson et al., 2002; Andersson et al., 2010).

This approach, however, obscures details in individual brain structure and function. Brain anatomy and functional brain activity patterns are highly variable across individuals (van Horn et al., 2008). Anatomical variations can affect the size, shape and position of brain structures. Such variations are clearly observable in cortical gyrification, as each brain is folded slightly differently (Ono et al., 1990). In primary somatomotor regions, the link between neural anatomy and brain function is long-established and robust across the primate lineage (Penfield and Boldrey, 1937). Outside of primary areas, however, such as in the frontal lobe, the

structure-function relationship is controversial and less well defined, which is often attributed to increased inter-individual variability (Fischl et al., 2008; Keller et al., 2009). A thorough investigation of human brain function thus requires assessment of individual brain function in a shared context with individual neuroanatomy. Prominent sulcal landmarks, such as the central sulcus and the Sylvian fissure are readily identifiable in every brain, but the details of their morphology are in fact variable across participants (Sun et al., 2016; Germann et al., 2020). In other parts of the brain, the sulcal configuration itself is highly variable and different morphological types can be described for these regions (Sprung-Much and Petrides, 2018, 2020). In order to accurately identify sulcal landmarks that are comparable across participants it can therefore be necessary to annotate these structures manually.

Even when accounting for individual differences in anatomy, for example by applying a spatial registration to MNI standard space, individuals display variable functional activation patterns during task and rest (Glasser et al., 2016; Tavor et al., 2016). Inter-individual variability in brain function can manifest itself in different magnitudes of the BOLD signal change, variable spatial extent of the activated area or different topology of the activation pattern (van Horn et al., 2008). In cognitively demanding tasks these variations are expected due to factors such as varying performance, task strategy, compliance, and attention. But, even for basic motor localizers, based on stereotypical movements, a high variability can be observed. This variability constitutes a confound for conventional volumetric group-level fMRI analyses (Nieto-Castañón and Fedorenko, 2012). Differences in functional topology can lead to decreased sensitivity: a significant activation might not be detected at the group level when the activations do not overlap in MNI space. On the other hand, variability can result in decreased specificity or functional resolution: Spatially adjacent but functionally different neural responses might appear as one continuous cluster at the group level.

These problems call for an alternative characterization of group-level fMRI results that account for individual variability. A straight-forward way to illustrate inter-individual variability in brain function is to reduce the activation pattern within a specific region of interest to the voxel with the maximal statistical value, i.e. the activation peak. This allows for a visualization of the spatial spread of peaks (Takai et al., 2010) and to visualize the relation to individual anatomical landmarks intuitively (Bodin et al., 2018; Germann et al., 2020; Loh et al., 2020).

Another way to characterize inter-individual variability of brain function is to derive group count maps (Barch et al., 2013): First, individual statistical maps for a task contrast are mapped onto the individual's surface. Then the map is thresholded based on the individual significance level, which is taking into account the noise-level of the individual scan. This map is binarized and then transformed to a surface group space. By summing the binary significance maps of the entire study sample, a group-level count map is created. Areas that have a high overlap in the count maps are consistently activated across the sample, while areas with low count numbers are activated to a variable degree. One advantage of using surface-based activation maps is that it these can be used in conjunction with latest developments in surface-based inter-individual registration methods, as described in the next section.

1.6.5. Surface-based image registration

In addition to Cartesian volumetric registrations to a reference space, brains can be aligned using surface-based methods that leverage the sheet-like geometry of the cerebral cortex. In a cortical surface reconstruction, the convoluted brain surface is represented as two-dimensional closed mesh that consists of vertices, or nodes, and edges. Cortical surface features can be

represented as data associated with the vertices. In a surface-based registration, the vertices are shifted along the surface so that driving features at corresponding vertices are matched as determined by a cost-function. The registration of brain surfaces is thus a two-dimensional problem, rather than a three-dimensional problem as a volumetric registration. It is therefore computationally less expensive and requires fewer anatomical priors.

As for any registration, the nature of the research questions determines which input features are used to drive the registration. One common aim is to derive a registration that increases the overlap of functionally homologous areas across human participants. It is a matter of debate, however, which input features should form the basis of such a registration and how different modalities correlate. The commonly used FreeSurfer algorithm relies on sulcal depth maps and thereby it implicitly matches some aspects cortical folding (Fischl, 2012). Such a registration, however, might perform less well in parts of the cortex with complex morphology. Furthermore, it has been argued that alignment based on anatomy alone might be sub-optimal and that task-free brain activity during rest is superior in predicting task-based activations (Tavor et al., 2016). Others argue that microstructural features such as cortical myelin content and cortical thickness should drive an inter-individual registration (Glasser et al., 2016). To address this issue the multi-modal surface matching (MSM) algorithm has been developed (Robinson et al., 2014). MSM is a spherical registration approach, which is based on a multivariate cost function that can accommodate for several surface features in a flexible and adaptable framework.

The concepts of brain registration are tightly linked to the challenges of comparative primate neuroanatomy. By deriving a registration from one species to the other, we can explicitly transform brains into a common space, which allows us to perform comparisons on brain

anatomy (Mars et al., 2018a). However, there is no single ‘perfect’ registration that can map, for example, a monkey to a human brain. All aspects of brain architecture were modified during evolution and each individual aspect could provide us with a unique registration. Evolutionary pressures relating to our increased cognitive abilities, such as language function, resulted in the most prominent changes in the cerebral cortex (Falk and Gibson, 2001). Surface-based registration methods are therefore particularly suitable for characterizing evolutionary changes in the primate brain. By comparing where registrations based on different modalities disagree, we can shed light on brain evolution. Similarly, as in humans, we need to critically evaluate which input features are suitable for a cross-species registration and which priors are required.

1.7. Aims of the Thesis and Methodological Approach

The overarching goal of this thesis is to study the human brain specialisations underlying human vocal control. By using a combination of the research methods described above I addressed the question in a neuroanatomical, functional and a comparative perspective across primate species. First, I re-visited the question of the cortical representation of the larynx in the human brain using neuroimaging. I designed a novel task paradigm to localize brain activity evoked during vocalization using fMRI. The task design was based on a previous paradigm to dissociate laryngeal activity during vocalization from articulation (Murphy et al., 1997). Furthermore, the breathing instructions were matched across all conditions to control for breathing-related artifacts.

In the same participants, I derived spatial maps of cortical thickness, myelin-sensitive T1w/T2w maps, and multiple parameter maps from quantitative imaging. Based on a combination of these measures, I characterized the cortical microstructure underlying the dorsal and the ventral larynx area and compared them with those of other effectors. Such a

myeloarchitectonic approach gives us an indication of which activations are located in primary motor cortex and which are not.

The location and the existence of the ventral larynx area is controversial. Therefore, we assessed if inter-individual variability in sulcal morphology underlying the ventral larynx representation was related to variability in function. As part of this assessment, we provided a systematic description of the morphological patterns in the subcentral region. Based on manual labelling of the segments of the central sulcus and the sulci in the subcentral region, we demonstrated a tight link between structure and function on an individual participant level.

Moving from the functional human perspective to an evolutionary framework, I studied brain reorganization using a comparative approach. I developed an analysis framework that allows us to test whether different types of anatomical reorganization go hand in hand or whether they occurred independently. Specifically, I aimed to dissociate cortical expansion from independent changes to the connectivity profile. To model cortical expansion I derived a between-species cortical alignment based on cortical myelin content from T1w/T2w maps. Next, using tractography, I reconstructed several homologous white matter tracts. This allowed me to study where cortical relocation and expansion is sufficient to predict connectional reorganization and where we find evidence for additional evolutionary modifications to the tracts.

2

Mapping Human Laryngeal Motor Cortex During Vocalization

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2.1. Abstract

Human speech production requires fine neural control of the articulators and the larynx during vocalization. The representation of the larynx in the human brain is debated. Vocalizations can be elicited by stimulation of the ventral motor cortex, but neuroimaging reveals that producing vocalizations evokes activity in both a dorsal and a ventral area. We designed an fMRI study to isolate brain activity related to laryngeal activity during vocalization while controlling for breathing. We mapped the cortical motor representation of the larynx during vocalization and dissociated it from representations of supralaryngeal articulators (the lips and tongue). We also mapped the hand representation in the left hemisphere. Furthermore, we characterized the microstructure of activated cortical regions using structural and quantitative neuroimaging in individual participants. We found two separate activations during vocalization, which are in anatomically distinct parts of the brain. Individual participants show a consistent somatotopic arrangement of the movement-related activations. Quantifications of cortical microstructure suggest that the dorsal, but not the ventral larynx area, is located in primary motor cortex as indexed by high myelin content and thicker cortex. It remains unclear, however, whether and how these two brain areas differentially contribute to laryngeal motor control.

2.2. Introduction

The voluntary control of highly complex speech movements is regarded as one aspect of behaviour that is unique to humans (Fitch, 2017). Speech production requires the fine coordination of a large number of muscles to control the supralaryngeal articulators, respiration, and the vocal folds in the larynx during voice production (Jürgens, 2002). In addition to its role as the main source of vocal sound production, the larynx is implicated in various biological functions such as protection of the airways and swallowing (Ludlow, 2005). Several pairs of intrinsic and extrinsic muscles connect the laryngeal cartilages with each other

and to the skeleton. During vocalization, these muscles are controlled in a complex fashion, so that the tension in the vocal folds allows them to be set into vibration as air from the lungs passes through them. Several lines of evidence showed that the ventral part of the precentral gyrus and the central sulcus in the human brain are involved in speech motor control (Bohland and Guenther, 2006; Ackermann et al., 2014). The question as to which brain areas specifically control laryngeal activity during vocalization, however, remains debated.

Nearly 100 years ago, direct cortical stimulation of the ventral portion of the precentral gyrus in the human brain was shown to elicit vocalization (Foerster, 1936; Penfield and Boldrey, 1937). In more recent times, functional brain imaging studies show highly inconsistent results when mapping laryngeal activity during vocalization (reviewed in Belyk & Brown, 2017). Several studies report activity evoked by vocalization in both a ventral portion of the precentral gyrus located close to the Sylvian fissure and in a more dorsal portion of central sulcus and precentral gyrus (Terumitsu et al., 2006; Galgano and Froud, 2008; Olthoff et al., 2008; Grabski et al., 2012). Some studies report vocalization-evoked activity in the dorsal location only (Sörös et al., 2006; Brown et al., 2008; Kleber et al., 2013; Belyk and Brown, 2014; Belyk et al., 2018b). Only a few studies specifically isolated laryngeal activity during voice production by contrasting it with supralaryngeal articulation (Sörös et al., 2006; Terumitsu et al., 2006; Brown et al., 2009; Grabski et al., 2012), but the results were inconsistent across studies.

Another line of evidence comes from direct neurophysiological recordings from the cortical surface using implanted high-density electrode arrays in patients being prepared for epilepsy surgery. These studies also show activity in both a dorsal and a ventral region during speech production (Bouchard et al., 2013; Chang et al., 2013; Toyoda et al., 2014; Breshears et al.,

2015; Dichter et al., 2018). Interpretation of electrocorticography (ECoG) studies, however, is inherently limited due to the typically small sample size and potential neurological abnormalities in the patients' brains.

In addition to these inconsistent results, an additional confound in several studies mentioned above is breathing. Voice production and respiration functionally interact during speech production and volitional expiration has been shown to activate motor areas that are located close to the putative dorsal laryngeal representation (Ramsay et al., 1993; Evans et al., 1999; McKay et al., 2003). Several previous studies, however, did not control for breathing (e.g. Brown et al., 2008; Sörös et al., 2006). Those studies that specifically studied vocalization and breathing found largely overlapping activity during both conditions and a contrast showed no difference in motor cortex indicating that the activity was not specific to larynx activity (Loucks et al., 2007; Simonyan et al., 2009).

In addition to a lack of control for confounds such as breathing and supralaryngeal articulation, most neuroimaging studies mentioned above did not assess individual differences in the activation patterns. It is common to report group-level cluster-corrected results following volumetric nonlinear image registration of task-activation maps. Interpreting group-level results, however, might obscure subject-specific features and inter-individual variability, which limits sensitivity and functional resolution (Bennett and Miller, 2010; Nieto-Castañón and Fedorenko, 2012; Bouchard et al., 2013; Woo et al., 2014). This lack of individual detail might have caused a failure to detect one of the cortical larynx representations in previous studies. Moreover, averaging of small sample sizes with high variability, as often performed in ECoG studies, can show two distinct larynx representations, even when individual patients show activity in only one of the regions (Bouchard et al., 2013).

Comparisons of human brains and those of other primates indicate strong species differences in the neural organization underlying laryngeal control during vocalization (Ackermann et al., 2014; Simonyan, 2014; Kumar et al., 2016). Most notably, the location of the proposed human dorsal larynx representation in primary motor cortex is more dorsal-posterior than the non-human primate homolog, which is in a ventral premotor cortex area (Leyton and Sherrington, 1917; Hast et al., 1974; Jürgens, 1974; Simonyan and Jürgens, 2002; Coudé et al., 2011).

These various findings have led to the proposal of an evolutionary 'duplication and migration' hypothesis that the larynx motor cortex comprises two structures located dorsally and ventrally in the human brain (Belyk and Brown, 2017; Jarvis, 2019; reviewed in Mars et al., 2018). This theory proposes that the dorsal larynx representation is unique to humans and that it evolved in primary motor cortex due to our especially high demands on laryngeal motor control. The ancestral primate larynx representation in premotor cortex is presumed to be homologous to the human ventral larynx representation, which migrated posteriorly, potentially into human primary motor cortex. The cortical areas underlying the larynx representations, however, are currently unknown and it has not been tested whether the ventral larynx representation is in primary motor or in premotor cortex. A myeloarchitectonic approach, which has also become available for neuroimaging, enables us to describe some properties of cortex (Kuehn et al., 2017). Primary motor cortex, for example, is characterized by higher cortical myelin content and higher cortical thickness compared to adjacent premotor cortex and with somatosensory cortex located on the caudal bank of the central sulcus and postcentral gyrus (Fischl and Dale, 2000; Glasser and van Essen, 2011; Lutti et al., 2014). Describing the anatomical parcels underlying the larynx representations can inform evolutionary hypotheses and provide clues about their functional relevance.

This study sought to determine the anatomical location of larynx-related neural activity in individual participants and to characterize the cortical structure underlying these representations. Our experimental design aimed to isolate brain activity related to voice production by controlling for breathing-related movements and movements of articulators. In one task, we identified the (supralaryngeal) articulation and the (laryngeal) vocalization component of speech during syllable production using a factorial design described in a previous study (Murphy et al., 1997). We refer to the latter ‘vocalization’ component as an index for laryngeal activity during voice production, while other studies have referred to it as ‘phonation’ or ‘voicing’. In a second task, we localized the separate neural representations of lip, tongue and larynx using highly controlled basic movements, while breathing movements were matched across conditions. In order to characterize the microstructural properties underlying the larynx representation, we compared their myelin content and cortical thickness derived from structural and quantitative MRI measurements. These quantifications give an indication of the type of cortex underlying the activated regions, to inform our knowledge about the organization of the human larynx motor cortex.

2.3. Material and Methods

2.3.1. Participants

20 participants (12 females, 18 – 40 years [27.4 ± 5.6 , mean $\pm$ SD], 5 self-reported left-handers) took part in the study. All participants were self-reported native English speakers; two were raised bilingually and three were fluent in a second language. All reported normal hearing, normal or corrected-to-normal vision, no neurological impairments and no history or diagnoses of speech disorders. The study was approved by the Central University Research Ethics

Committee (CUREC, R55787/RE001) in accordance with the regulatory standards of the Code of Ethics of the World Medical Association (Declaration of Helsinki). All participants gave informed consent to their participation and were monetarily compensated for their participation.

2.3.2. Experimental design and task

We used two tasks to map the motor representations of the articulators and the larynx. Task 1 engaged the speech motor system in an ecologically valid way that required the participants to utter a short syllable sequence in different conditions. Task 2 required the participants to perform basic movements that are commonly used in other localizer studies. Participants practiced all tasks outside the scanner to be sure they understood the task requirements. Articulator movements and vocalizations as well as breathing were demonstrated by the experimenter and practised until the participants performed them as required.

Task 1 - syllable production task

Participants were instructed to produce the utterance “/la lei li la lei li/” in four different conditions (Figure 2.1B): speaking (overt speech), supralaryngeal articulation only (silent mouthing), overt vowel sound production of vowel /i/ six times but no articulation (vowel production) and thinking (covert speech). The vowel /i/ was chosen because it is a natural and familiar sound that requires laryngeal movement during vocalization, but involves minimal movement of the jaw muscles, lips and pharyngeal part of the tongue (Grabski et al., 2012). Participants were instructed not to whisper during the ‘silent mouthing’ or ‘covert speech’ conditions as this would involve laryngeal motor activity.

For all conditions, the breathing pattern was explicitly instructed using the fixation symbol on the screen (Figure 2.1A). Participants were instructed to inhale for 1.5 s (fixation was a square) and exhale for 4 s (fixation was a cross); the utterance in each condition was performed once during each 4-s exhalation. Each condition was performed in blocks lasting 22 s, which corresponded to four repetitions of the breathing cycle. Each block was followed by a rest period of 8 s with normal breathing (i.e., not explicitly instructed). This rest period allowed the participants to relax their breathing patterns and to maintain a comfortable respiratory state. The four conditions were presented in a fixed pseudo-random order following a balanced Latin-square design wherein each condition was repeated five times; each condition followed and preceded each of the other conditions once and the same condition was not presented consecutively. Participants were instructed to keep their mouth slightly open during the whole session and to keep the jaw relaxed.

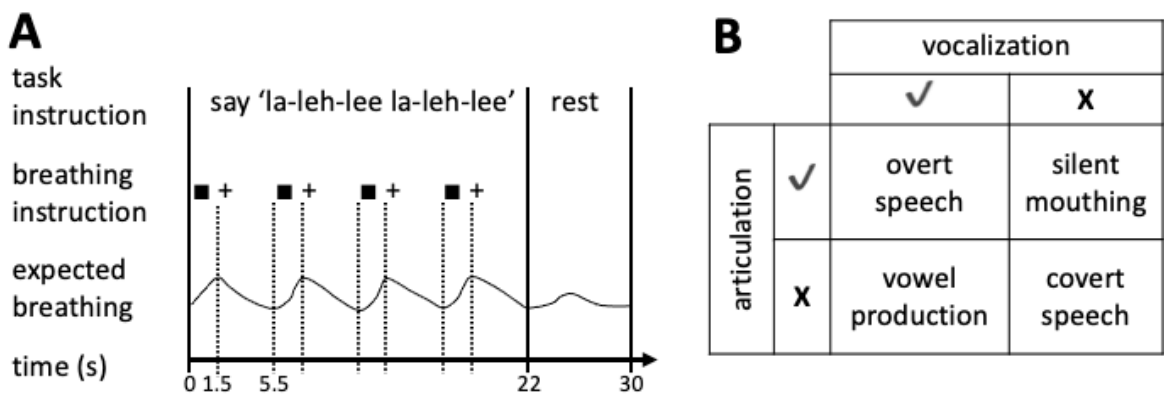


Figure 2.1 Design and paradigm for fMRI task.

A: Timing of the task paradigm for an example block of the ‘overt speech’ condition from the syllable production task (task 1). The utterance was repeated four times while breathing was instructed followed by a resting period with normal breathing. ‘■-symbol’: inhale, ‘+-symbol’: exhale. **B:** Factorial design used in the syllable production task. The four task conditions can be described according to orthogonal main factors for laryngeal activity during vocalization and supralaryngeal articulation. Breathing was instructed during all conditions as shown in A.

Task 2 - basic functional localizer

There were four task conditions: tongue retraction, lip protrusion, production of the vowel sound /i/, and a ‘breathing only’ condition. The three task conditions for basic speech movements were contrasted with the ‘breathing only’ condition in which breathing was explicitly instructed as described above. Articulator movements and vowel production were repeated during 4 s of exhalation at a rate of approximately 1 - 2 repetitions/s as described for task 1. Participants were instructed to keep the movement rate constant throughout the scan for all movement types. Breathing instructions, task timing and randomization of the blocks were the same as described for task 1 above, except that each condition was repeated four times during the scan run. Note that each 22-s block was followed by an 8-s period of rest with normal breathing, as above.

Hand localizer

During the scanning session, participants performed the basic localizer first and then the syllable production task. In between these two tasks involving vocalizations, the participants performed a phonological and semantic judgement task (Devlin et al., 2003). Participants had to indicate a yes/no response by pressing a button with the right index or the middle finger every 3 s. The task data were used here only to localize the hand representation in the left hemisphere; the language task data were not reported.

2.3.3. MRI data acquisition

MRI data were obtained at the Oxford Centre for Human Brain Activity (OHBA) using a 3-T Siemens Prisma scanner with a 32-channel head coil. Two structural images of the whole brain were acquired; a T1w image (MPRAGE sequence; 1 mm³ isotropic resolution, TR = 1900 ms,

TE = 3.97 ms, TI = 905 ms, 8° flip angle, bandwidth = 200 Hz/pixel, echo spacing = 9.2 ms, FOV = 192 × 192 × 174 mm³) and a T2w image (SPACE turbo-spin-echo sequence; 1 mm³ isotropic resolution, TR = 3200 ms, central TE = 451 ms, variable flip angle, bandwidth = 751 Hz/pixel, echo spacing = 3.34 ms, echo train duration = 919 ms, Turbo Factor = 282, FOV = 256 × 256 × 176 mm³, GRAPPA acceleration factor 2). For task fMRI, whole-head T2*-weighted echo-planar images (TE = 30 ms) were acquired using multiband sequence (factor 6, TR = 0.8) at 2.4 mm³ isotropic resolution. Task fMRI parameters were adopted from the ABCD study (Casey et al., 2018). Two task fMRI scans were conducted lasting 8 min (600 volumes, task 2) and 10 min (750 volumes, task 1). In between the two tasks, participants performed a phonological and semantic judgement task for 9 min, which did not involve vocalization.

Furthermore, a multi-parameter mapping (MPM) protocol was acquired (Weiskopf et al., 2013; Lutti et al., 2014). Proton-density-weighted (MPM_{PDw}), magnetization-transfer-weighted (MPM_{MTw}) and T1-weighted (MPM_{T1w}) images were acquired using a tailored pulse sequence (1 mm³ isotropic resolution, FOV = 256 × 224 × 176 mm³, TR = 25 ms, bandwidth = 488 Hz/pixels, first TE/echo spacing = 2.3/2.3 ms, 6° flip angle (MPM_{PDw}, MPM_{MTw}) or 21° (MPM_{T1w}), slab rotation = 30°, and number of echoes = 8/6/8 (MPM_{PDw}/MPM_{MTw}/MPM_{T1w} respectively), GRAPPA acceleration factor 2 × 2, 40 reference lines in each phase encoded direction. Quantitative R1 (= 1 / T1) maps were estimated from the MPM_{PDw} and MPM_{T1w} images as demonstrated in Weiskopf et al. (2013), which was extended by including a correction for radio frequency transmit field inhomogeneities (Lutti et al., 2010) and imperfect spoiling (Preibisch and Deichmann, 2009). Regression of the log-signal from the signal decay over echoes across all three MPM contrasts was used to calculate a map of R2* (= 1 / T2*) (Weiskopf et al., 2014). The transmit field map was calculated using a 3D echo-planar imaging (EPI) spin-echo/stimulated echo method (Lutti et al., 2012, 2010; FOV = 256 × 192 ×

192 mm³, matrix = 64 × 64 × 48 mm³, TE = 39.06, mixing time = 33.8 ms, TR = 500 ms, nominal α varying from 115° to 65° in steps of 5°, acquisition time 4 min 24 s) and was corrected for off-resonance effects using a standard B0 field map (double gradient echo FLASH, 3 × 3 × 2 mm³ resolution, whole-brain coverage). The MPM parameter maps took approximately 20 minutes to acquire. In addition to the MRI scans mentioned here, participants underwent a diffusion-weighted scan and a resting-state scan (data not reported here). The total duration of the entire scanning session was approximately 1.5 h, preceded by approximately 45 min of briefing and task practice.

Physiological recordings were carried out throughout scanning using a respiratory belt to measure the breathing pattern and a pulse oximeter to monitor the heart rhythm during the scan. Physiological data were recorded using a Biopac MP150 (Biopac, Goleta, CA, USA) at a sampling frequency of 500 Hz. Participants wore ear-plugs and MRI-compatible headphones (OptoActive-II, Optoacoustics Ltd, Moshav Mazor, Israel), which reduced scanner noise using electrodynamic noise-cancelling. At the beginning of the scanning session, the headphones were calibrated, and noise-cancelling performance was further monitored throughout the session. Prior to each functional scan the attenuation algorithm 'learned' the scanner noise for 16 s. During the two tasks involving vocalizations, participants were audio-recorded using an MRI-compatible microphone with noise cancelling (Dual Channel FOMRI-III, Optoacoustics Ltd, Moshav Mazor, Israel) at a sampling rate of 22,050 Hz.

2.3.4. Behavioural data analysis

The participants' vocal behaviour for the tasks was manually assessed using the audio recordings. The breathing patterns during the task blocks recorded using the Biopac were

inspected visually to verify that participants complied with the breathing instruction. Individual breathing traces were cropped into segments of 30 s, which consist of 22 s of instructed breathing during the task block and 8 s of subsequent rest period with normal breathing (Figure 2.3).

2.3.5. Structural MRI analysis

T1w and T2w scans were pre-processed using the Human Connectome Project (HCP)-pipeline (Glasser et al., 2013) cloned from the ‘OxfordStructural’ - fork (<https://github.com/lennartverhagen/Pipelines>). The processing pipeline includes anatomical surface reconstruction using FreeSurfer and automatic assignment of neuroanatomical labels (Fischl, 2012; Jenkinson et al., 2012). The T2w image was registered to the T1w image using FSL’s FLIRT using spline interpolation (Jenkinson et al., 2002).

The image of the T1w scan was divided by the image of the T2w scan to create a T1w/T2w-ratio image. The T1w/T2w-ratio was mapped onto the native midthickness surface and then resampled to the 164k standard surface mesh (fs_LR, approximately 164.000 vertices per hemisphere) using adaptive barycentric interpolation using Workbench Command (www.humanconnectome.org/software/connectome-workbench.html). Mapping was performed with the ‘-volume-to-surface-mapping’ command using the ‘-myelin-style’ option. This T1w/T2w map has been shown empirically to correlate with cortical myelin content (Glasser and van Essen, 2011; Glasser et al., 2014). In addition to T1w/T2w myelin maps, the HCP-pipeline provides automatic generation of cortical thickness surface maps.

MPM parameter maps were reconstructed and pre-processed using the hMRI-toolbox (Tabelow et al., 2019) embedded in the Statistical Parametric Mapping framework (SPM12, Wellcome Trust Centre for Neuroimaging, London, UK). For one participant, MPM data were excluded due to motion-induced blurring. MPM_{MT} , MPM_{R1} and MPM_{R2^*} maps were registered to the MPAGE T1w scan using FLIRT rigid-body transformation and spline interpolation and then mapped to the surface using the same steps as for the T1w/T2w myelin map. The three MPM parameter maps have been shown to correlate to different degrees with myelin content in white matter, subcortical structures and grey matter (Draganski et al., 2011; Callaghan et al., 2014; Lutti et al., 2014; Bagnato et al., 2018). Finally, one step of surface-based smoothing (FWHM = 4 mm) was applied to the five surface maps of interest - T1w/T2w map, three MPM parameter maps and cortical thickness map.

2.3.6. FMRI data analysis

Functional MRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). The following pre-statistics processing was applied: motion correction using MCFLIRT (Jenkinson et al., 2002); non-brain tissue removal using BET (Smith, 2002); spatial smoothing using a Gaussian kernel (FWHM = 5 mm); grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor; low-frequency drifts were removed using a temporal high-pass filter with a cut-off of 90 s for all three tasks. Motion corrected images were unwarped using a fieldmap and PRELUDE and FUGUE software running in FSL (Jenkinson, 2003). Registration to the high resolution structural scan and standard 2 mm MNI-152 template was carried out using FLIRT (Jenkinson and Smith, 2001; Jenkinson et al., 2002). Registration from high resolution structural to standard space was then further refined using FNIRT nonlinear

registration (Andersson et al., 2007). Time-series statistical analysis was based on a general linear model (GLM) implemented in FILM with local autocorrelation correction (Woolrich et al., 2001). Standard motion correction parameters and individual volumes that were motion outliers, determined using `fsl_motion_outliers`, were included as separate regressors at the first level for each participant.

For the syllable production task (task 1), the conditions were analysed in a factorial model that allowed the (supralaryngeal) articulation and the (laryngeal) vocalization component of the task to be separated (Figure 2.1B). Brain activity associated with the control of articulation was defined as ('overt speech' *minus* 'vowel production') *plus* ('silent mouthing' *minus* 'covert speech') and the main contrast for vocalization was derived by the contrast ('overt speech' *minus* 'silent mouthing') *plus* ('vowel production' *minus* 'covert speech'). The 8-s periods of rest with normal breathing between condition blocks served as baseline and were not modelled as a separate explanatory variable in the GLM; they were not included in contrast to any task condition in the analysis of this factorial design.

For the basic localizer task (task 2), activity during each condition for basic speech movements was assessed relative to the 'breathing only' condition with instructed breathing. The periods of rest with normal breathing in between conditions served as baseline (i.e. they were not modelled in the GLM as described for task 1). For the hand localizer task, we derived a contrast of all task conditions involving button presses relative to the rest blocks (note, the breathing instruction was not used in the hand localizer task). All contrasts reported in the results for both tasks were assessed relative to conditions with matched breathing. In addition, we report results of all individual task conditions relative to the resting condition with normal breathing for both tasks.

2.3.7. Volumetric group average activation maps

To obtain volumetric group average maps, each individual's statistical maps were transformed to standard space (MNI-152) using a nonlinear registration. Volumetric group mean activation maps were obtained using mixed effects in FLAME (FMRIB's Local Analysis of Mixed Effects, (Woolrich et al. 2004, Stage 1 only), where subjects are treated as random effects. Group-level z -statistic images were thresholded using a voxel-wise threshold of $z > 3.5$ ($p < 0.00025$, uncorrected). Note that the corrected threshold at $p < 0.05$ for a whole-brain analysis of these data is $z > 5$. Rather than use a small volume correction and region-of-interest (ROI) masking, we chose to display results at the lower threshold to better visualize the activity in the whole brain. The figures of the volumetric group-level results (Figure 2.4, Figure 2.6) are focused, i.e. centred, on the voxel of maximal z -value within the right hemisphere central somatomotor strip. For the vocalization contrast (Figure 2.4) and the vowel production condition (Figure 2.6), we generated two separate figures focused on the right dorsal and the ventral larynx representation.

2.3.8. Surface group count maps

In addition to volume-based analysis, task MRI activations were assessed using surface-based analyses. Surface space permits a better visualization of cortical brain activations. Furthermore, this allowed us to perform surface-based quantifications of cortical microstructures. Each participant's z -statistic images were thresholded voxel-wise at $p < 0.05$ (corrected using a z -value determined based on data smoothness and the RESEL count). This thresholded map was projected onto the individual's native midthickness surface using the '-ribbon-enclosed' option in `wb_command` '-volume-to-surface-mapping'. To derive group-level surface count maps, all

individual participant thresholded maps were resampled to the 32k standard (fs_LR) mesh based on the FreeSurfer registration, binarized and then summed at each vertex (Barch et al., 2013). The group count maps are shown on an inflated average brain surface, thresholded at $n > 4$ participants. They give an indication of the inter-individual variability in the areas activated by task, providing complementary information to maps of activity averaged across participants.

2.3.9. Individual surface activation maxima

Surface-based activations were studied further using an ROI-based approach to isolate and focus on activations in the central somatomotor strip. The two tasks provided complementary results for further analysis with task 1 serving as a robust localizer for the larynx representations, and task 2 providing an accurate localizer for lip and tongue representations. In order to assess intra-individual spatial variability of the motor representations, we derived the location of individual activation maxima in both hemispheres from selected task contrasts: For the larynx, we used the main contrast for vocalization during the syllable production task (task 1). For lip and tongue, we used the task contrasts from the basic localizer task (task 2) and for the hand, we used the hand localizer task (left hemisphere only).

An example of the volumetric ROIs in an individual is shown in Figure 2.2B. The central sulcus ROI used for the hand, lip and tongue was defined using FreeSurfer's automatic volumetric labelling based on the Destrieux Atlas. For the larynx, we identified two activation maxima in separate ROIs: One for the dorsal and one for the ventral larynx representation. The dorsal larynx ROI was a portion of the same central sulcus ROI used above but limited to z -coordinates in MNI space of 50 to 30. The limits were determined empirically, so that ROI did

not capture the ventral larynx representation or an unrelated supra-dorsal activation in the trunk area, which was observed in some individuals (Foerster, 1931).

As described in the results section below, the ventral larynx representation lay outside the central sulcus and was located ventrally in the subcentral part of cortex. Due to the high intra-individual morphological variability in this region (see below chapter 3), the ventral larynx ROI was derived manually based on individual anatomy in surface space. A liberal surface ROI was drawn on each individual's midthickness surface covering the ventral part of the central sulcus and adjacent gyri (Figure 2.2A). Anteriorly, the ROI was delineated by the inferior portion of the precentral sulcus and posteriorly the ROI spanned the postcentral gyrus. If present, the lateral portion of the ascending sulcus in the subcentral gyrus was included within the ROI. The dorsal limit of the ROI was defined by a horizontal plane across the gyrus at the level of the usual location of the posterior ramus of the inferior precentral sulcus. The ventral larynx surface ROI was converted into a volumetric ROI covering the underlying cortical ribbon using `wb_command`. We checked that the ventral larynx ROI did not overlap with subjacent auditory cortex in the temporal lobe or inferior frontal cortex.

In some participants, the main contrast for vocalization in the syllable production task had additional activity related to articulation of the tongue. To remove this, we transformed the coordinates for each individual's maximal voxel from the tongue contrast (from the basic localizer task) to the functional space of the syllable production task (task 1) using rigid-body transformation and then derived a spherical ROI (7 voxels diameter) around it. This sphere was used to mask the z -statistic image of the main contrast for vocalization prior to localizing the maxima for laryngeal activity in the dorsal and ventral ROIs described above.

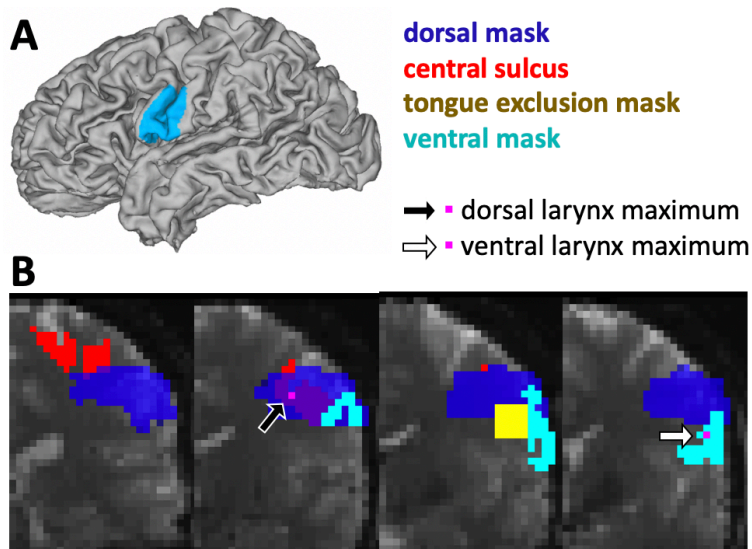


Figure 2.2 Regions-of-interest to derive task maxima.

A: Example of a manually drawn surface ROI for the ventral larynx representation (*light blue*). **B:** Volumetric ROIs overlaid onto an individual's functional scan in four central sections (left to right: posterior to anterior). The central sulcus ROI (*red*) was used to derive the voxel of maximal activation for the hand, lip and tongue. The intersection of the central sulcus ROI and a dorsal mask (*dark blue*) was used to derive the voxel of maximal activation for the dorsal larynx representation (*black arrow, pink voxel*). The ventral mask was projected from surface to volume space (ROI shown in A, *light blue*) to derive the voxel of maximal activation in the ventral larynx representation (*white arrow, pink voxel*). An ROI around the voxel of maximal activation in the tongue contrast (*yellow*) was masked out from the dorsal and ventral mask.

Individual volumetric ROIs were linearly transformed from FreeSurfer's anatomical to functional space of the respective task fMRI scan. Within the ROI, the voxel of maximal intensity was determined from the uncorrected z -statistics image. It should be noted that for some participants this local maximum did not achieve the corrected voxel-wise significance threshold, which was used to create the surface count maps (left hemisphere: hand $n = 3$, dorsal larynx $n = 6$, ventral larynx $n = 5$; right hemisphere: dorsal larynx $n = 5$, ventral larynx $n = 4$). Using a lower uncorrected threshold is justified given our goal to visualize and assess spatial variability of the activation maxima. Activation maxima were manually inspected in the participant's native volume space to confirm that the systematic approach described below captured task-related activations. The activation maxima were mapped to the individual's native midthickness surface, resampled to the 32k standard (fs_LR) surface mesh using the

FreeSurfer registration, smoothed (FWHM = 1 mm), and binarized to form a small circular patch.

Given that the two tasks had some conditions in common, we were able to examine intra-individual reliability of the location of activation maxima for larynx and tongue as described above compared with those derived based on the vowel condition and the main contrast for articulation. We computed the geodesic distance on the individual's native midthickness surface for pairs of maxima (tongue condition and main contrast for articulation; main contrast for vocalization and vowel condition).

2.3.10. Cortical surface features at activation maxima

We described the cortical microstructure at the individual activation peaks based on different surface measures. In order to assess cortical myelin content, we used the T1w/T2w map and the MPM parameter maps (MPM_{MT}, MPM_{R1} and MPM_{R2*}). We extracted the mean value for each individual participant's surface map within the area defined by the circular patch around the vertex of maximal activation for hand, lip, tongue, dorsal larynx and ventral larynx representation, i.e. at the peaks shown in Figure 2.9A. These measures were obtained from the participant's native surface mesh prior to resampling to the standard mesh. In order to account for the different ranges in intensities and to allow direct comparison of the maps, we computed *z*-scores based on the distribution of individual values in each map and each ROI. We assessed the differences in *z*-scores across the different ROIs using a linear mixed effects analyses as implemented in R's *lmer* function (Bates and Sarkar (2007), R Core Team and Foundation for Statistical Computing). The model included fixed effects for ROI (dorsal larynx, lip, tongue, ventral larynx), hemisphere (left, right) and map (T1w/T2w, MPM_{MT}, MPM_{R1}, MPM_{R2*}) and

random effects for subject. A Shapiro-Wilk test revealed a normal distribution of the data at a significance level of $p > 0.001$.

In addition to cortical myelin content, we also quantified cortical thickness values at the same individual surface peaks. To increase sensitivity of the quantification described above, we excluded activation maxima that were located in a part of cortex with lower cortical thickness and thus likely to be activity in somatosensory rather than motor cortex (Fischl and Dale, 2000). We used a heuristic lower threshold of 2 mm to exclude sensory activation maxima and then re-ran the statistical evaluation of myelin-values described above. A linear mixed effects analysis for the fixed effects of ROI and hemisphere and random subject effects was performed to assess cortical thickness values after exclusion of the sensory activation maxima.

In order to further characterize the differences in myelin content across the ROIs, we used an additional quantification: We computed the pair-wise Manhattan distance across the ROIs based on a vector of the four raw (i.e., non- z -transformed) values in each participant after excluding the sensory activation maxima. The differences across ROIs were visualized in form of a dissimilarity matrix, where we averaged Manhattan distances within each ROI first across participants and then across hemispheres.

A linear discriminant analysis (LDA) was run to explore which combination of z -transformed surface features (T1w/T2w, MPM_{MT}, MPM_{R1}, MPM_{R2*}), best discriminated the ventral from the dorsal larynx representation after excluding sensory activation maxima. The parameters of the LDA were estimated using a singular value decomposition with no shrinkage.

2.4. Results

We acquired fMRI data in 20 participants during performance of two tasks: (1) a syllable production task required participants to produce “/la lei li la lei li/” overtly, mouthed silently, and covertly, and to produce the vowel /i/, (2) a basic localizer task required participants to make small repetitive movements of the lips, tongue and larynx (vowel production); an additional task was used as functional localizer for movement of the right hand.

2.4.1. Auditory recordings

All participants vocalized as instructed during the conditions that involved vocalizations in both tasks. In all other conditions, participants remained silent, as instructed.

2.4.2. Breathing during vocalization tasks

We recorded the breathing traces using a breath belt during both vocalization tasks to confirm that the breathing patterns were comparable across different conditions (Figure 2.3). As expected for all conditions in both tasks, four breathing cycles were visible in the first 22 s, during which breathing was instructed. Note that in one participant, where an extension of the breathing belt was used, the breathing trace differed in overall shape, but the breathing cycles were still visible. Examination of these figures shows that the shape of the trace and variability were similar across all conditions, but some differences were observed. For example, in the vowel production condition of the basic localizer task, exhalation was more gradual and less rapid than in the other four conditions. All four conditions in the syllable production task showed a more gradual exhalation pattern than in the basic localizer task.

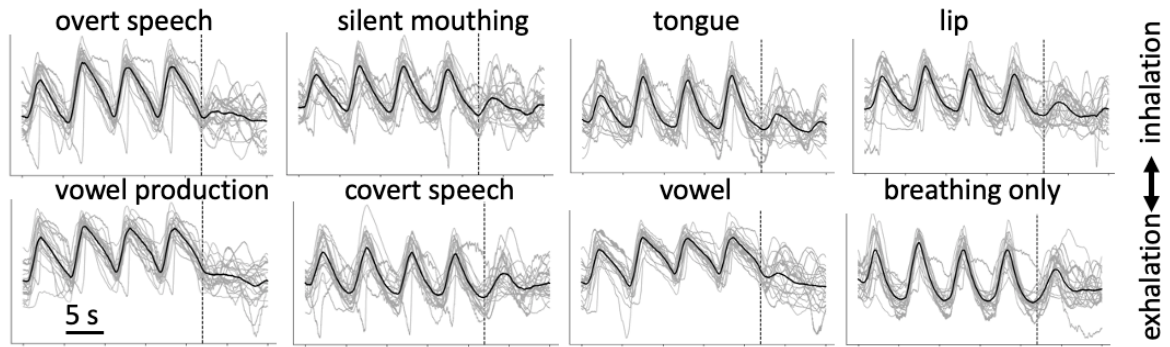


Figure 2.3 Breathing patterns.

Breathing traces during syllable production task (left two panels) and basic localizer task (right two panels). *Black lines*: Group mean after normalizing all individual breathing traces to the same amplitude; *grey lines*: average traces for individual participants ($n = 20$). *Dashed vertical line*: Indicates the end of the 22-s task block and the beginning of the 8-s resting period with normal breathing.

2.4.3. Syllable production task (task 1)

Syllable production task - volumetric results

We localized the cortical activations for movement control of (supralaryngeal) articulation and (laryngeal) vocalization during syllable production. MNI coordinates of activation maxima in the somatomotor strip are reported in Table 2.1.

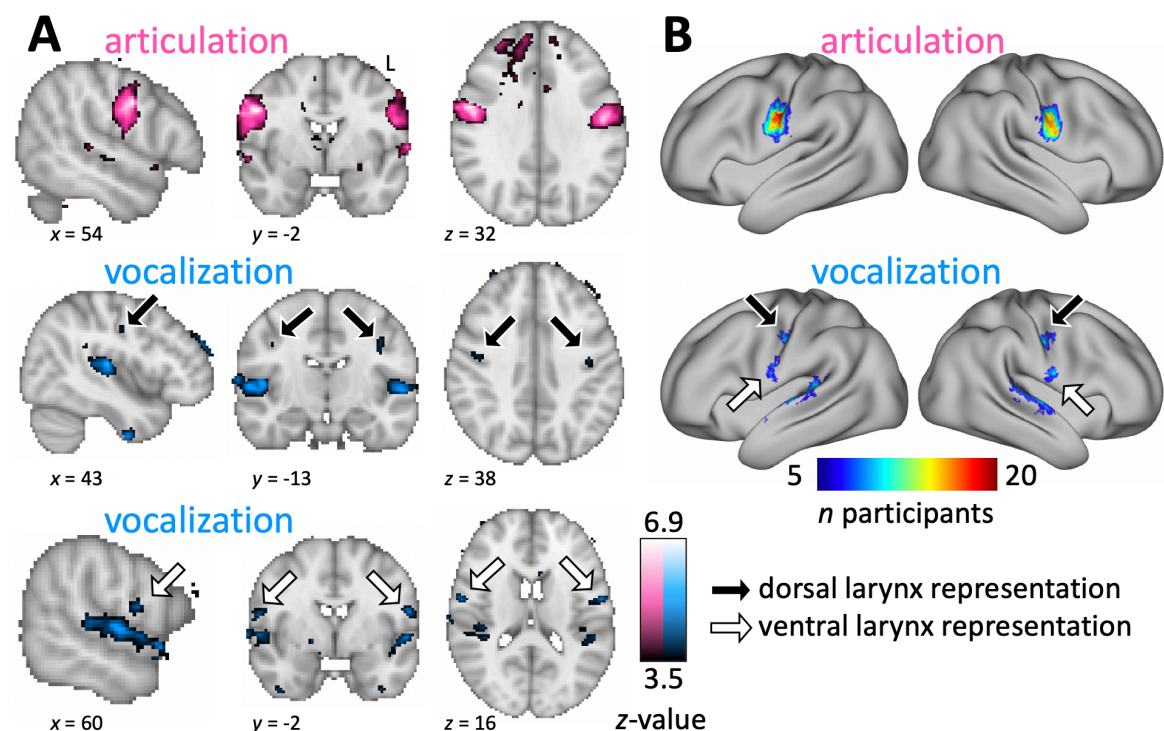
Table 2.1 Group-level task activation maxima.

Task	Main contrast	LH			RH		
		<i>x</i>	<i>y</i>	<i>z</i>	<i>x</i>	<i>y</i>	<i>z</i>
hand localizer	hand	-38	-21	57			
syllable production	articulation	-50	-12	32	54	-2	32
	vocalization (dorsal)	-40	-16	36	43	-13	38
	vocalization (ventral)	-58	-2	18	60	-2	16
basic localizer	lip	-48	-10	40	50	-8	36
	tongue	-52	-11	28	56	-4	30
	vowel (dorsal)	-42	-16	40	50	-6	40
	vowel (ventral)	-58	-2	20	58	-4	18

Reported are MNI coordinates of maxima in somatomotor cortex for the main contrasts reported in the manuscript (LH, RH: left and right hemisphere). For the main contrast for vocalization and vowel production, dorsal and ventral maxima are reported separately.

The main contrasts for vocalization and articulation were defined based on a factorial design that combined the data from all four task conditions in the syllable production task (Figure 2.1B). The main contrast for articulation showed activity in the mid-portion of the central gyrus (Figure 2.4A). Note that we also found spurious activity in prefrontal white matter, which we presumed was induced by task-correlated movement. The main contrast for vocalization showed activity in two somatomotor regions in both hemispheres: one located within a dorsal region of central sulcus, and a second located in a more anterior-ventral region. Portions of the superior temporal gyrus were also activated during vocalization. This is presumed to reflect auditory perception of self-generated vocalizations. The dorsal and ventral activations were separate and did not appear to be connected.

Vocalization and articulation also activated parts of cerebellum and SMA in a somatotopic fashion. Group-level volumetric results for cerebellum and SMA in both functional tasks are described below in section 2.4.5.



(figure continued on following page)

Figure 2.4 fMRI results of syllable production task.

A: Whole-brain group activation maps showing areas activated during articulation and vocalization (voxel-wise threshold: $z > 3.5$, $n = 20$). For the main contrast for vocalization (*blue*), both dorsal and ventral representation are shown in separate panels. *Black arrow*: Dorsal larynx representation; *white arrow*: ventral larynx representation. Note: In both tasks, vocalization also resulted in activation of the superior temporal gyrus, presumably reflecting auditory stimulation due to hearing oneself vocalizing (see also Figure 2.6B). **B:** Surface group count maps of the same contrasts as shown in A. Individual surface maps were thresholded at $p < 0.05$ (corrected voxel-wise), binarized, and resampled onto the fs_LR surface. These maps were summed across the group of 20 participants and thresholded at $n > 4$.

Syllable production task - surface results

Group count maps of the syllable production task projected to the surface showed that several vertices in the mid-portion of the central sulcus were commonly activated during articulation in 19 out of 20 participants (Figure 2.4B). The group count map for vocalization showed separate dorsal and ventral regions robustly activated across the group. In the main contrast for vocalization, there was greater variability in the exact location of activity above threshold and the areas activated in individuals were smaller than for the main contrast for articulation. This resulted in less overlap of activated vertices for the vocalization group maps. For some participants, vocalization-induced activity did not reach significance in relevant brain regions, which also resulted in lower values in the count map. In addition to the main contrasts for vocalisation and articulation derived from the factorial design, we show the group count maps of all individual task conditions relative to the resting condition with normal breathing in Figure 2.5.

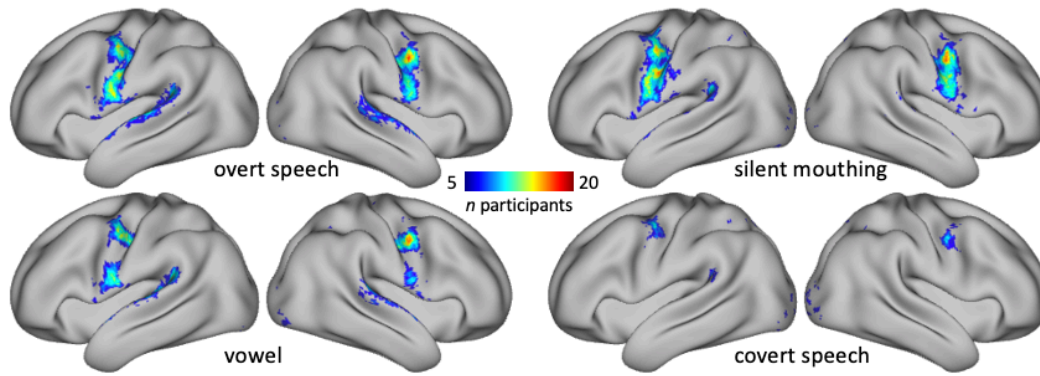


Figure 2.5 Surface count maps of individual task contrasts in syllable production task.

Shown are surface count maps of the four task conditions - overt speech, silent mouthing (articulation only), vowel production (vocalization only), covert speech (thinking) - when compared to the resting baseline with normal breathing.

2.4.4. Basic localizer task (task 2)

Basic localizer task - volumetric fMRI results

Brain activation during movement of the tongue and the lips and for laryngeal activity during vowel production was assessed by contrasting basic speech movements with a ‘breathing only’ condition, which was matched for controlled breathing. We found distinct activation peaks that followed the predicted somatotopic organization in the mid-portion of the central sulcus with the tongue representation more ventral than the lip representation in both hemispheres (Figure 2.6A) (Penfield and Rasmussen, 1950; Grabski et al., 2012; Carey et al., 2017). The location of the activity during tongue movement overlapped with the result of the main contrast for articulation in the syllable production task; as previously, we found spurious activity in the prefrontal lobe white matter that we presume is movement related.

Vowel production induced activity bilaterally in a central dorsal region, a ventral region anterior to the central sulcus, and in superior temporal cortex. Within the dorsal region,

activation is found both in the central sulcus and on the precentral gyrus. As for the syllable production task, activity in superior temporal cortex was presumed due to hearing oneself. In the group average map, and at the statistical threshold used, the dorsal and ventral larynx representations appeared to be connected, with residual activity along the ventral central sulcus.

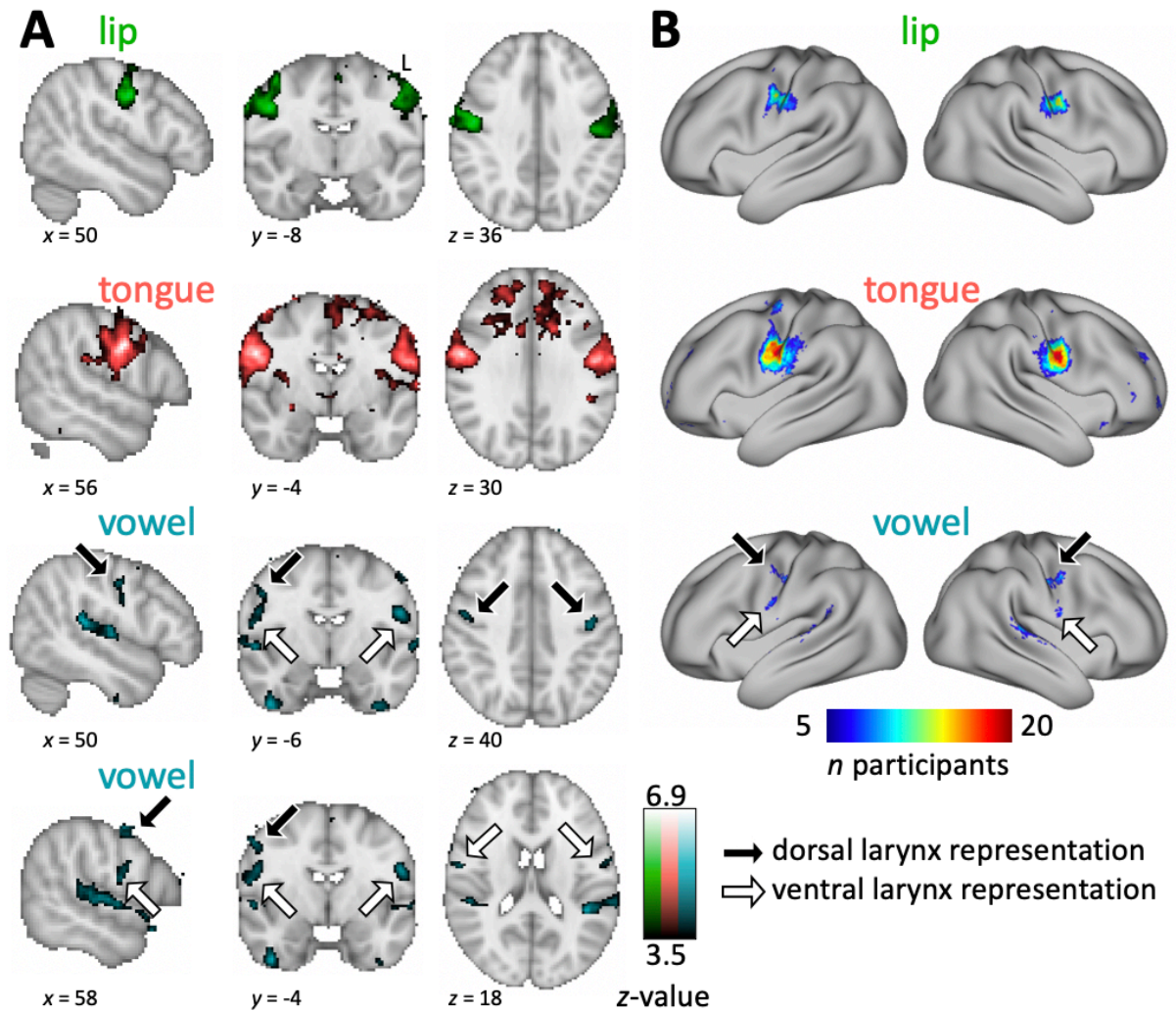


Figure 2.6 fMRI results of the basic localizer task.

A: Whole-brain group activation maps showing areas activated during lip and tongue movement and during vowel production (voxel-wise threshold: $z > 3.5$, $n = 20$). For the vowel production condition (*turquoise*), both a dorsal and a ventral representation are shown in separate panels. See legend to Figure 2.4 for details. **B:** Surface group count maps of the same conditions.

Basic localizer task - surface results

Group count maps of the basic localizer task projected to the surface revealed the same somatotopic activity pattern as seen in the volumetric results for group average activity (Figure 2.6B). The tongue condition showed highly consistent activation in the mid-portion of the central sulcus during articulation (maximum overlap was achieved for all 20 participants). The result for tongue movement was highly similar to the result of the main contrast for articulation, which is in line with the volumetric results. Complete overlap of activated vertices was not achieved for the lip condition, but the activated region was still highly consistent across participants (maximum: 15 participants). Individual task conditions relative to the resting condition with normal breathing are shown in Figure 2.7.

For the vowel production condition, the group count maps show a dorsal and a ventral cluster, similar to the pattern seen during the main contrast for vocalization (task 1). The dorsal cluster extended from the central sulcus to the precentral gyrus and in the right hemisphere a distinct dorsal gyral and a dorsal sulcal activation can be observed. We presumed that this dorsal gyral activity, but not the sulcal activity, is a residual breathing-related effect, because it overlaps with activity from the ‘breathing only’ condition (Figure 2.7). The ventral activation cluster appeared to extend into a part of cortex where we expected the tongue representation and may reflect residual tongue activity during vowel production. In contrast to the volumetric results for the group averaged activity, the count maps based on individually thresholded activation maps show the dorsal and ventral representation activated by vowel production to be clearly separate.

As described above for the main contrast for vocalization, the values in the group count maps for vowel production indicate that the location of this activity is less consistent than for the

other conditions; the area activated during vocalization is also smaller, both dorsally and ventrally, than for the articulators. The highest overlap was achieved in the right dorsal larynx representation in 11 participants.

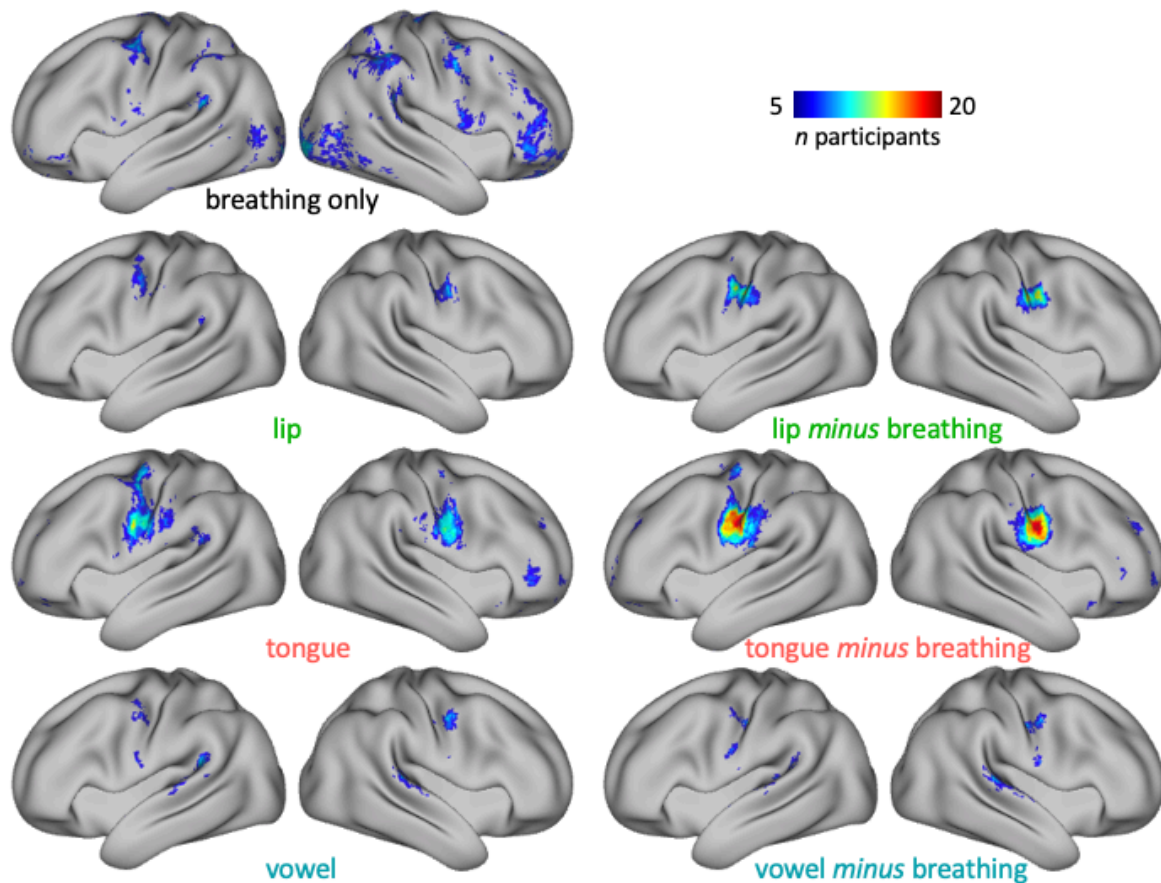


Figure 2.7 Surface count maps of individual task contrasts in the basic localizer task.

Left panel: Task conditions compared to the resting baseline with normal breathing. The ‘breathing only’ condition is a contrast between the breathing condition (where breathing was instructed) and the resting baseline with normal breathing. A lower threshold of $z = 1.96$ was used to threshold individual z -statistical images for this contrast due to generally lower activation. *Right panel:* Movement conditions compared to the breathing condition with explicit breathing instruction (these maps are also shown in Figure 2.6B).

2.4.5. Task activations in SMA and cerebellum

Task activations in SMA

The two main contrasts for articulation and vocalization - at a lower voxel-wise threshold of $z > 2$ - revealed activity bilaterally in SMA (Figure 2.8). A somatotopic arrangement was observed with vocalization activating a more anterior and more dorsal part of cortex. The representations of the effectors during the basic localizer task also show a clear somatotopy: in the dorsal-to-ventral direction, we first find the representation of the larynx, which is also most anterior, then of the lip and then of the tongue. Only one single representation of the larynx was found in SMA in both the main contrast for vocalization and in the vowel condition. The location of the representation of the speech effectors is in line with previous accounts in the literature (Picard and Strick, 1996). It has been suggested that the vertical line through the anterior commissure, the VAC, is a landmark for a division of SMA proper and pre-SMA (Picard and Strick, 1996; Rizzolatti et al., 1996). Our results thus suggest that lip and tongue representations are located just posterior to VAC in an anterior region of SMA proper. Laryngeal activity during vocalization, however, activates a part of cortex anterior to VAC, presumably in pre-SMA.

Task activations in cerebellum

Movement of the articulators and laryngeal activity also evoke activation of the cerebellum in a somatotopic fashion, which is mirroring the order observed for motor cortex (Figure 2.8B). Most ventrally, a representation of the larynx is observed, which activates during vowel production and vocalization. This is followed dorsally by a representation of the lips and the tongue and then by a second representation of the larynx.

According to a probabilistic atlas of the human cerebellum (Diedrichsen et al., 2009), all the activations observed are located in the anterior cerebellar lobule VI. This finding is consistent with previous neuroimaging studies that found activation of the anterior-superior portion of the cerebellum during speech movements (Petersen et al., 1989; Fiez and Raichle, 1997). A previous resting-state functional connectivity study demonstrated that cerebellar representations mirror the topography in cerebral motor cortex (Buckner et al., 2011). It is therefore presumed that the ventral-most representation of the larynx in cerebellum, which is subjacent to the lip representation, is related to the dorsal larynx representation in motor cortex (also adjacent to the lips) and vice versa.

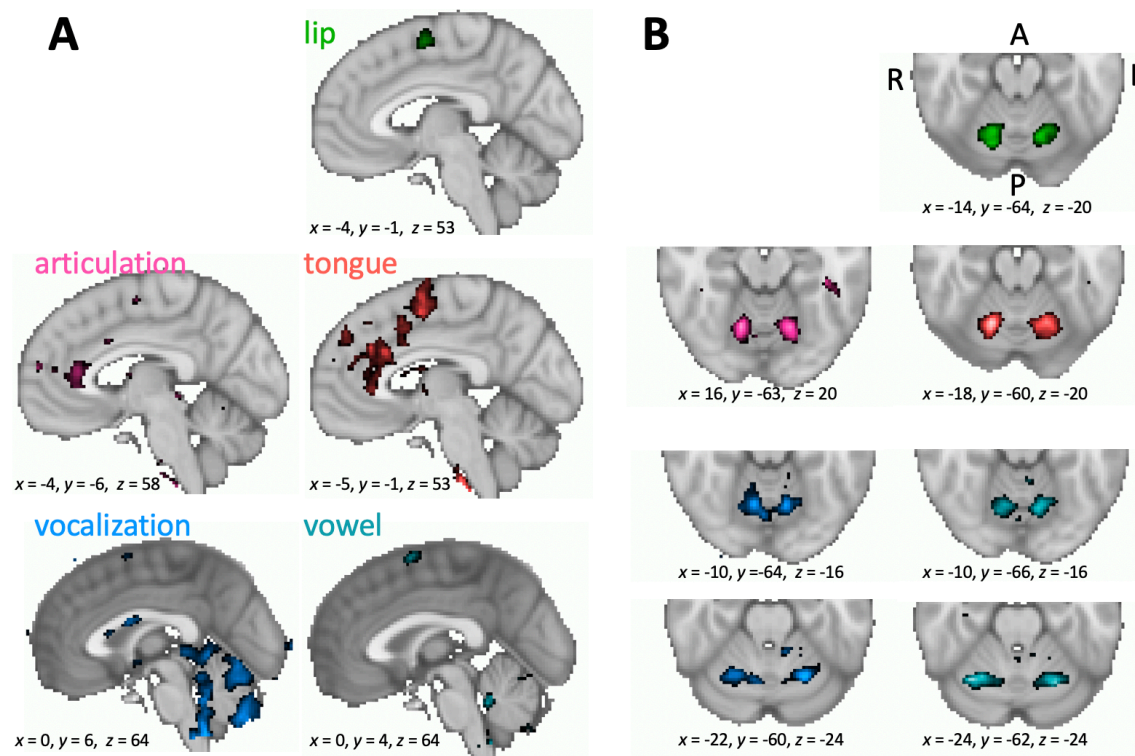


Figure 2.8 Task activations in SMA and cerebellum.

Whole-brain group activation maps showing areas activated during syllable production task (*left panels*) and basic localizer task (*right panels*) (voxel-wise threshold: $z > 3.5$, $n = 20$). Coordinates are given for the voxel of maximal activation in the left hemisphere. **A:** Activity on the medial brain surface centred on the voxel of maximal activity in the left SMA (note that for the main contrast for vocalization the threshold was lowered to $z > 2$). **B:** Cerebellar activity in the same task contrasts and shown in the same colours as in A overlaid onto an axial slice through the cerebellum of the MNI-152 brain. For the main contrast for vocalization and for the vowel production contrast, two separate activations are shown in different slices 8 mm apart.

2.4.6. Individual surface activation maxima

In order to characterize the variability of brain activity across participants, we derived individual activation peaks for the hand movement (based on the hand-localizer, only in the left hemisphere), lip and tongue movement (based on the basic localizer task) and vocalization (based on the syllable production task). The main contrast for vocalization was used to localize the dorsal and a ventral larynx representation. Figure 2.9A shows the spatial distribution of individual activation peaks on an inflated brain surface. Overall, the location of the peaks for the different movement types is highly consistent with similar inter-individual variability. For the ventral larynx representation, particularly in the left hemisphere, however, the location of the maxima appears to be more variable.

Inter-individual reliability of the activation maxima across the two tasks was compared for ‘vocalization’ and ‘vowel production’ for both dorsal and ventral larynx ROI as well as for ‘articulation’ and ‘tongue movement’ in the tongue ROI. Reliability was high with a median distance across participants of less than 10 mm between the pairs in the three ROIs in both hemispheres.

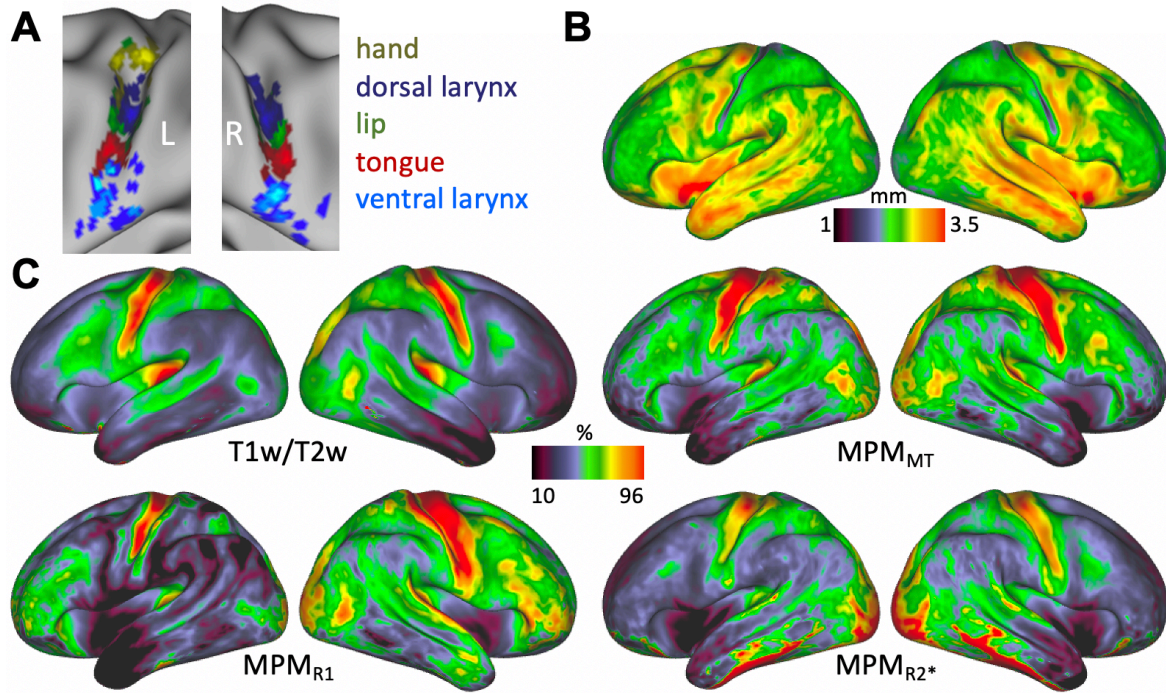


Figure 2.9 Task maxima and microstructural maps.

A: Individual activation maxima derived for hand movement (only left hemisphere), lip movement, tongue movement and vocalization (i.e., larynx activity during voice production) ($n = 20$). For vocalization, activation maxima in two separate brain regions were derived to localize the dorsal and ventral larynx representations. **B:** Average cortical thickness map ($n = 20$). **C:** Average T1w/T2w myelin map ($n = 20$) and MPM parameter maps ($n = 19$).

2.4.7. Cortical surface maps of microstructural features

We derived whole-brain average surface maps for different measures related to cortical microstructure (Figure 2.9B, C). Overall, the T1w/T2w map and the three MPM parameter maps (MPM_{MT}, MPM_{R1}, MPM_{R2*}) show a similar pattern of myelin content across the cortex (Figure 2.9C). In all four maps, the central sulcus as well as precentral and postcentral gyrus are characteristically high in myelin, which is considered to be a defining feature of primary motor and sensory cortex (Glasser and van Essen, 2011). The location of the ventral boundary of the somatomotor cortex, indicated by a steep gradient of myelin values, slightly varies across the four maps, but this boundary is consistently more ventrally located in the right compared with the left hemisphere.

The four maps that correlate with cortical myelin (T1w/T2w, MPM_{MT}, MPM_{R1} and MPM_{R2*}) are sensitive to different biophysical properties of the myelin, but it should be noted that their sensitivity profiles are not completely independent (Callaghan et al., 2016). Therefore, some dissimilarities regarding the distribution of myelin along the cortex can be observed across the maps. MPM_{MT} and MPM_{R1} have a stronger signal in the frontal lobe compared to the T1w/T2w and the MPM_{R2*} map. In addition to myelin, the R2* signal is influenced by cortical properties such as iron content and calcium (Wu et al., 2009; Bagnato et al., 2018). For the T1w/Tw2 map, the underlying biophysical model is less well understood. The high R2* values in the ventral temporal lobe are likely a susceptibility artifact caused by signal loss at the air-tissue boundary.

In the cortical thickness map, a prominent strip of low values (i.e. thinner cortex) can be observed at the posterior bank of the central sulcus, indicating the location of primary sensory cortex (Fischl and Dale, 2000) (Figure 2.9B). Cortical thickness values are high (i.e. thicker cortex) in the anterior bank of the central sulcus and in the precentral gyrus, which is indicating the location of primary motor cortex. In the cortical thickness map, the ventral boundary of primary sensory cortex can be determined by a sharp gradient at the level of the subcentral gyrus. The location of this boundary is comparable with the location described above in the other maps. The same hemispheric difference can be observed with the somatomotor boundary being located further ventrally in the right hemisphere. The whole-brain maps demonstrate that these cortical surface measures are informative about the cortical microstructure underlying our functional activation maxima, which we describe in the next section. The result of the reconstructed MPM parameter maps *per se* is not a primary result of the paper.

2.4.8. Cortical surface features at activation maxima

Next, we determined the intensity value of the microstructural surface maps at the individual participants' activation maxima for the different movement types (Figure 2.10). Figure 2.10A shows z-transformed intensity values for T1w/T2w, MPM_{MT}, MPM_{R1} and MPM_{R2*} maps at hand, lip, tongue as well as dorsal and ventral larynx maxima, i.e. at the peaks presented in Figure 2.9A. Mixed effects analyses with the factors ROI (excluding the hand ROI), hemisphere and map demonstrated that there were highly significant effects of ROI ($F(3, 600) = 60.69, p < 0.001$) and hemisphere ($F(1, 600) = 43.54, p < 0.001$) but no significant effect of map ($F(3, 600) < 1, n.s.$). The significant effect of ROI reflects that values for the ventral larynx are lower than in the other ROIs (post-hoc pairwise *t*-tests with Tukey adjustment, $p < 0.001$), but the values for dorsal larynx representation, lip and tongue were not significantly different. The main effect of hemisphere reflects higher values in the right hemisphere ($p < 0.001$). The interaction between ROI and hemisphere was significant ($F(3, 600) = 3.93, p = 0.009$) indicating that the difference between the ventral larynx representation and the other motor representations was greater on the right hemisphere than on the left.

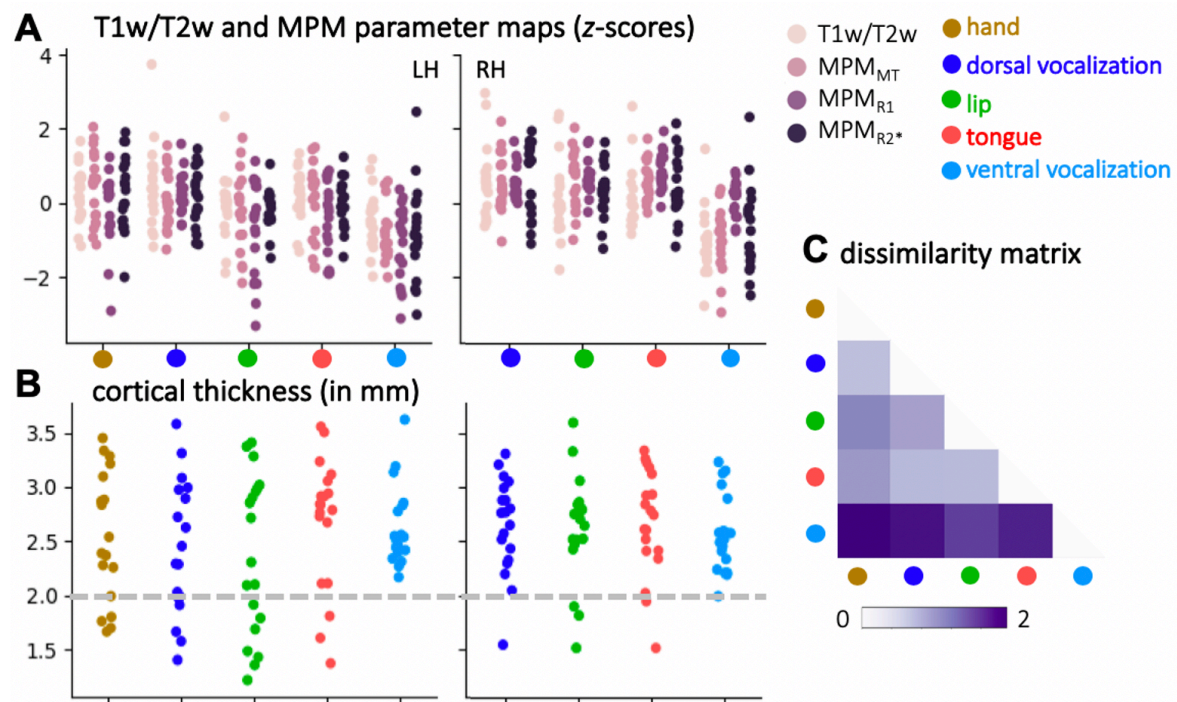


Figure 2.10 Measures of cortical microstructure derived at individual activation maxima.

A: Microstructural values that correlate with myelin (T1w/T2w, MPM_{MT}, MPM_{R1}, MPM_{R2*}) derived at activation maxima (for individual maxima see Figure 2.9A) ($n = 19$). **B:** Individual values of cortical thickness. *Dashed grey line:* indicates the threshold that was chosen to determine the exclusion of activation maxima that are presumed to be sensory due to their location in cortex < 2 mm thick. **C:** Dissimilarity matrix of the myelin values across ROIs. The matrix shows a quantitative comparison of the values shown in A after excluding sensory activation maxima. Pair-wise dissimilarity is defined as the Manhattan distance between each pair of ROIs.

Cortical thickness values show a high inter-individual variability for hand, dorsal larynx, tongue and lip ROIs (Figure 2.10B). This effect can be attributed to the fact that some maxima are located in the depth of the central sulcus, where values are low, while others are located on the anterior bank of the central sulcus, where values are high. Variability in the right hemisphere is lower, which is consistent with the location of the maxima predominantly on the anterior bank (Figure 2.9A). This result potentially indicates that some of derived maximal activation peaks represent activity evoked by somatosensation rather than by motor control. If maxima with a cortical thickness level below a cut-off value of 2 mm (Fischl and Dale, 2000) are excluded, the comparison of cortical thickness values shows a marginally nonsignificant

effect of ROI ($F(3, 120) = 2.53, p < 0.061$), but not of hemisphere ($F(1, 120) < 1$). The marginal effect of ROI is driven by the ventral larynx representation having lower cortical thickness values compared to the other ROIs ($p < 0.068$).

To increase sensitivity in the quantification of myelin values described above, we re-ran the linear mixed model while excluding the sensory activation maxima as defined by a cortical thickness below 2 mm. The significant main effects of ROI ($F(3, 480) = 73.62, p < 0.001$) and hemisphere ($F(1, 480) = 20.81, p < 0.001$) were stronger than reported above. As reported above, the main effect of ROI is due to decreased values in the ventral larynx representation and the hemispheric effect due to increased values in the right hemisphere. The interaction effect of ROI and hemisphere ($F(3, 480) = 2.26, p = 0.08$) was no longer significant, indicating that the difference between ventral larynx representation and the other motor representations did not differ between hemispheres.

To assess effects based on the non-transformed values of the four maps (T1w/T2w, MPM_{MT}, MPM_{R1}, MPM_{R2*}) rather than the z-scores, we also derived a ‘dissimilarity matrix’ based on the pair-wise Manhattan distances between ROIs after excluding the sensory activation maxima (Figure 2.10C). The values were averaged across hemispheres, because no significant interaction effect was found. The dissimilarity matrix reflects the effect of the ventral larynx representation being most dissimilar from the other motor representations based on these quantitative measures of cortical myelin.

A linear discriminant analysis (LDA) revealed that the following equation can be used to discriminate the ventral larynx representation from the dorsal larynx representation:

$$y = -0.28 \cdot T1w/T2w - 2.82 \cdot MPM_{MT} - 3.03 \cdot MPM_{R1} - 0.47 \cdot MPM_{R2*} - 1.19$$

When calculating the y using the z -transformed values of the surface maps at a specific location, a resulting value of $y > 0$ indicates that the profile of values is more similar to the ventral larynx representation than to the dorsal larynx representation. The formula indicates that MPM_{MT} and MPM_{R1} are the most informative measures to discriminate the two larynx representations (largest weighting). The mean performance accuracy of the classifier is 0.94 (while 0.5 indicates chance level performance).

Taken together, these quantifications show that the ventral larynx representation is located in cortex that has lower myelin content and lower cortical thickness compared to primary motor cortex, where the other movement representations including the dorsal larynx representation, are located.

2.5. Discussion

The goal of this study was to characterize the cortical microstructure underlying the laryngeal representations in the human brain. We localized brain activity evoked by voluntary control of laryngeal movements during vocalization using a novel paradigm. We showed that even when controlling for breathing, vocalization elicits brain activity in two separate parts of the somatomotor cortex: a dorsal region in the central sulcus and a ventral region close to the Sylvian fissure. On an individual level, the laryngeal activations and the activations during movement of hand, lips, and tongue show a consistent somatotopic arrangement. Characterization of cortical microstructure based on structural and quantitative MRI shows that the dorsal larynx representation has a similar profile to other movement representations primary motor cortex, while the ventral larynx representation has lower myelin content and

cortical thickness. These results suggest that the dorsal larynx representation is the primary locus of laryngeal motor control in primary motor cortex, while the ventral larynx representation relates more to secondary activations in non-primary motor cortex.

Our experimental task was designed to separate laryngeal activity from that evoked by supralaryngeal articulation and breathing. We were able to dissociate the supralaryngeal and laryngeal components of syllable production using a factorial design and orthogonal task contrasts (Murphy et al., 1997). Covert speech is known to engage similar somatomotor brain areas to overt speech production (Kleber et al., 2007). For our purpose, however, using a ‘covert speech’ condition was essential to allow us to construct a fully factorial model aimed at isolating activity related to the execution of movements involved in articulation and vocalization while controlling for other processes such as selection and planning of movement sequences. The result of a factorial design is statistically more robust than a subtraction design, because the main contrasts include data from all task conditions improving the mathematical estimates and associated statistics. The main contrast for vocalization showed some residual activity of the tongue representation. Using the neutral vowel schwa or glottal stops instead (Gick, 2002; Loucks et al., 2007; Brown et al., 2008; Grabski et al., 2012; Belyk et al., 2018b), however, might have caused pharynx activity that would have been more difficult to dissociate.

In addition to the syllable production task, we used a basic localizer task that involved movement of the lips, and tongue, and vowel production. Results of the basic localizer task were consistent with the syllable production task, and intra-individual variability across the comparable contrasts was low. The areas activated for the main contrast of articulation overlap with the result from the tongue localizer, which is expected given that the syllables produced mainly rely on tongue movement. The areas activated for the main contrast for vocalization

overlap with the result from the vowel production condition, but some differences could be observed. Residual tongue activity resulting from producing the vowel /i/ is more apparent in the vowel production condition, suggesting that this was better controlled for in the factorial design.

When studying vocalizations, control for breathing is essential given that human vocal speech sounds are mostly produced during exhalation. Several previous neuroimaging studies, however, did not control for breathing. Some previous studies used an instructed exhalation condition for comparison with vocalization (Loucks et al., 2007; Galgano et al., 2019), but a difference in activation of regions in motor cortex was not found consistently. One likely explanation is that explicitly instructing participants to exhale might engage laryngeal muscles in such a way that no difference to laryngeal activity during vocalization can be observed. In this study, the breathing pattern and rate were matched for all task conditions. Inhalation and exhalation were explicitly instructed on a screen and monitored using a breath belt. Although slight deviations in the shape of the breathing trace can be observed, the overall breathing traces were comparable across participants and conditions. The deviations in breathing patterns observed in the basic localizer task suggest that breathing is less well controlled for compared with breathing during the syllable production task, where participants produced the same utterance in different conditions. It should be noted that a similar functional paradigm, employing a factorial design and controlling for breathing patterns, has recently been used in an fMRI study to dissociate phonatory and articulatory representations in the cortex (Correia et al., 2020). The concepts presented in this chapter, however, have been presented prior to that publication in form of a conference abstract (Eichert et al., 2019a) and a preprint (Eichert et al., 2020b).

Our results suggest that controversial findings within the neuroimaging literature can largely be explained by differences in experimental design. Several studies focused on the dorsal region as location of the ‘laryngeal motor cortex’ or ‘larynx phonation area’ (Sörös et al., 2006; Brown et al., 2008; Kleber et al., 2013; Belyk and Brown, 2014; Belyk et al., 2018b). Brown et al. (2008) suggested the presence of a ‘dorsolateral’ larynx representation on the crown of the precentral gyrus ($x = 50, y = -2, z = 37$) and a ‘ventromedial’ larynx representation in the central sulcus ($x = 44, y = -8, z = 34$). Our results suggest, however, that activity in the gyral portion of the dorsal region is a residual breathing-related signal and not specific for laryngeal activity during vocalization. When contrasting the ‘breathing only’ condition, during which breathing was explicitly instructed, to the resting baseline with normal breathing, we found activity in a dorsal gyral region ($x = 55, y = 0, z = 43$), which is presumably related to voluntary control of the diaphragm during instructed inhalation and exhalation (Figure 2.7) (Ramsay et al., 1993; Olthoff et al., 2008). Similarly, we found the dorsal gyral activation when contrasting the ‘covert speech’ condition, which involved instructed breathing, but with no overt movement, to the resting baseline with normal breathing (Figure 2.5). We also find this dorsal gyral activity during vowel production, but only when we assess it relative to the resting baseline with normal breathing (Figure 2.7). When we assess vowel production relative to the ‘breathing only’ activity, only the sulcal portion of the dorsal activity, which we also found in the main contrast for vocalization, remains ($x = 43, y = -13, z = 38$). The activation that Brown et al. (2008) refer to as ‘ventromedial’ larynx area, overlaps most closely with the region that we focus on in this paper as dorsal (sulcal) larynx representation.

In addition to activation of the dorsal larynx representation, multiple neuroimaging studies reported activation in a region that we refer to as ventral larynx representation (Terumitsu et al., 2006; Olthoff et al., 2008; Grabski et al., 2012). Strong evidence for the involvement of a

ventral larynx representation in laryngeal motor control also comes from electrical recording from the cortical surface during vocalization and microsimulation studies (Galgano and Froud, 2008; Bouchard et al., 2013; Chang et al., 2013; Breshears et al., 2015; Dichter et al., 2018). Electrocorticography demonstrated the presence of both a dorsal and a ventral larynx representation much more consistently than the fMRI literature (reviewed in Conant et al., 2014). Direct electrical stimulation of the dorsal larynx representation causes vertical larynx movement that correlates with stimulation magnitude and evokes vocalization (Dichter et al., 2018); stimulation of the ventral larynx representation causes speech arrest (Chang et al., 2017).

In addition to our novel experimental design, this study focuses on investigating individual differences in laryngeal activation patterns. The task count maps demonstrate that laryngeal activity is much less consistent across participants compared with other movement types. In some participants the dorsal or ventral activity did not reach the individual significance threshold. The location of the ventral larynx representation, in particular in the left hemisphere, show a much wider spatial spread across the cortex indicating large intra-individual variability. A recent anatomical study based on the dataset presented here, showed that inter-individual morphological variability in the ventral end of the central sulcus is higher than in the rest of the central sulcus (see below chapter 3). It was shown that variability in sulcal morphology can explain, in part, spatial variability in functional activation peaks. As a result of the variability and the low z -statistical values, group level fMRI analysis in previous studies might have failed to detect one of the larynx representations or the task paradigm was not optimized to localize the larynx representations.

The existence of two separate brain regions correlating with laryngeal activity in the central sulcus raises the question as to whether both of them are involved in motor control of the larynx. Given that the larynx area in the macaque is located in a ventral premotor area (Hast et al., 1974; Jürgens, 1974; Simonyan and Jürgens, 2002; Coudé et al., 2011), an evolutionary ‘migration and duplication’ hypothesis was proposed (Belyk and Brown, 2017; Jarvis, 2019; reviewed in Mars et al., 2018). The theory states that the human ventral larynx representation migrated posteriorly during evolution, possibly into a different cytoarchitectonic area. The dorsal larynx representation is thought to have evolved as human novelty in primary motor cortex.

Additional support for a duplication theory also comes from genetic profiling analyses comparing gene expressions in song-learning birds and humans (Pfenning et al., 2014; Chakraborty and Jarvis, 2015). Gene expression in the avian vocal nuclei, that are involved in vocal learning, are similar to the expression profiles in both the dorsal and the ventral larynx representation of the human brain. In the context of this more general brain pathway duplication theory, it was recently suggested that there was an additional duplication in human vocal premotor cortex, leading to the emergence of a pre-larynx motor cortex (preLMC) just anterior to the ventral larynx representation (Jarvis, 2019). The proposed genetic mechanisms for the evolutionary brain pathway duplication theory in laryngeal motor control, however, remain to be verified experimentally.

An alternative interpretation of the non-human primate literature would suggest that the species differences are quantitative rather than qualitative. While traditional electrical mapping studies failed to find a focal larynx representation in primary motor cortex in non-human primates (Bailey et al., 1950; Hast et al., 1974; Simonyan and Jürgens, 2002), neuronal activity

correlating with laryngeal movement was observed, for example, during swallowing in macaques (Martin et al., 1997, 1999). Furthermore, tract-tracing studies showed that the premotor larynx representation in the macaque has direct connections to primary motor cortex (Künzle, 1978; Simonyan and Jürgens, 2002). These findings might indicate the presence of a non-human homology of the dorsal larynx representation, which is experimentally more challenging to localize due to its smaller spatial extent.

The present study is the first to characterize the microstructural properties of cortex underlying the dorsal and ventral larynx representation. The use of high-resolution quantitative neuroimaging allows us to characterize cortical architecture noninvasively (Weiskopf et al., 2013; Lutti et al., 2014). Measures from T1w/T2w maps and MPM parameter maps (MPM_{MT}, MPM_{R1}, MPM_{R2*}) are sensitive to different microstructural properties of the cortex, but all of them have been shown to correlate with myelin to varying degrees (Glasser and van Essen, 2011; Weiskopf et al., 2013; Callaghan et al., 2014, 2015; Bagnato et al., 2018). Age-related effects can confound measures of cortical microstructure (Gennatas et al., 2017; Grydeland et al., 2019), but the effects in the regions studied are expected to be minimal given the age range of our participants.

The cytoarchitectonic area underlying the larynx representations, remains to be determined histologically. Our quantification, however, suggests strongly that the dorsal larynx representation is located in primary motor cortex, which is characterized by high myelin content and thicker cortex (Fischl and Dale, 2000; Glasser and van Essen, 2011). The ventral larynx representation has lower myelin content and thinner cortex indicating that it is located in a different cortical territory. Based on a cytoarchitectonic map of the human brain (Brodmann, 1905), we suggest that the human ventral larynx representation is located in

Brodmann area (BA) 6 (premotor cortex). This interpretation is consistent with the idea that the primate brain contains multiple laryngeal representations in different cortical areas: The human ventral larynx representation is homologous to the premotor macaque larynx representation (Hast et al., 1974; Jürgens, 1974; Simonyan and Jürgens, 2003a; Coudé et al., 2011) and the human dorsal larynx representation is homologous to a macaque larynx representation in primary motor cortex, which receives projections from the premotor larynx representation (Künzle, 1978; Simonyan and Jürgens, 2002).

Many individual maxima for the ventral larynx representation were located in the subcentral part of cortex. Brodmann considered this a postcentral and therefore somatosensory cortical region (BA 43), subjacent and anterior to primary somatosensory cortex (BA 3, 1 and 2) (Brodmann, 1905). A magnetoencephalography study showed that air-puff stimulation of the larynx evokes activity in a ventral region (Miyaji et al., 2014). Using fMRI, BA 43 was also found to be activated by movements of the tympanic membrane associated with changes in pressure in the oropharyngeal cavity such as those that occur during gustation and swallowing and in vocalization (Job et al., 2011). These results suggest that the ventral larynx activity could be a sensory rather than a motor representation.

The question regarding the distinct functional contributions of the two larynx representations during voice productions remains unanswered. Based on our results, we formulate a hypothesis regarding the causal role of the two laryngeal representations during voice production: We propose that, as for vocalizations in non-human primates, the ventral larynx representation is involved in basic control of the vocal folds so that a vocal sound can be produced. Rapid adduction (tensioning) and abduction (relaxation) of the vocal folds at the onset and offset of a vocalization is primarily modulated by the intrinsic laryngeal muscles (Jürgens, 1974;

Simonyan and Jürgens, 2003a). Fine motor control, which is required for pitch modulations in human speech and singing, however, also relies on the vertical movement of the larynx within the trachea, which is mediated by the extrinsic muscles. We suggest that pitch control is facilitated by the dorsal larynx representation, which is located in primary motor cortex (Kleber et al., 2013; Dichter et al., 2018; Finkel et al., 2019). Activity in both intrinsic and extrinsic laryngeal muscles, however, are tightly coupled and might not be controlled by distinct brain areas (Belyk and Brown, 2014). Our hypothesis is in line with an evolutionary theory suggesting that the dorsal larynx representation is unique to the human brain (Belyk and Brown, 2017). Such a functional dissociation of dorsal and ventral larynx representation during vocalization, however, still needs to be tested directly using, for example, non-invasive brain stimulation.

In sum, we used neuroimaging to localize neural activity related to laryngeal motor control during vocalization, while controlling for confounding factors such as breathing and supralaryngeal articulation. We found two activated regions for laryngeal activity during vocalization, which are in anatomically distinct brain areas. Quantification of cortical microstructure suggests that the dorsal representation, but not the ventral representation, is located in primary motor cortex. It remains open, whether and how these two representations differentially contribute to laryngeal motor control.

3

Morphological and Functional Variability in Central and Subcentral Motor Cortex

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3.1. Abstract

There is a long-established link between anatomy and function in the somatomotor system in the mammalian cerebral cortex. The morphology of the central sulcus is predictive of the location of functional activation peaks relating to movement of different effectors in individuals. By contrast, morphological variation in the subcentral region and its relationship to function is, as yet, unknown. Investigating the subcentral region is particularly important in the context of speech, since control of the larynx during human speech production is related to activity in this region. Here, we examined the relationship between morphology in the central and subcentral region and the location of functional activity during movement of the hand, lips, tongue, and larynx at the individual participant level. We provide a systematic description of the sulcal patterns of the subcentral and adjacent opercular cortex, including the inter-individual variability in sulcal morphology. We show that, in the majority of participants, the anterior subcentral sulcus is not continuous, but consists of two distinct segments. A robust relationship between morphology of the central and subcentral sulcal segments and movement of different effectors is demonstrated. Inter-individual variability of underlying anatomy might thus explain previous inconsistent findings, in particular regarding the ventral larynx area in subcentral cortex. A surface registration based on sulcal labels indicated that such anatomical information can improve the alignment of functional data for group studies.

3.2. Introduction

A fundamental challenge in neuroscience is to establish meaningful links between brain structure and function. One of the clearest cases of such a structure-to-function relationship is found in the somatomotor system in the central strip of the cerebral cortex (Jackson, 1863; Fritsch and Hitzig, 1870; Vogt and Vogt, 1919; Penfield and Boldrey, 1937). Different parts

of the body are represented in an orderly and consistent fashion, following a somatotopic organization, the schematic visualization of which is known as Penfield's 'homunculus'.

In contrast to electrical brain mapping studies, neuroimaging studies typically report average activations from larger groups of participants. This approach demonstrates effects that are representative of the human brain in general, but obscures participant-specific features and inter-individual variability, limiting sensitivity and functional resolution (Bennett and Miller, 2010; Nieto-Castañón and Fedorenko, 2012; Woo et al., 2014). Consequently, a growing number of neuroimaging studies now tend to report activation patterns in individuals (Miller et al., 2002; Barch et al., 2013; Carey et al., 2017). A deeper understanding of structure-to-function relationships, however, requires examination of inter-individual functional variability together with anatomical variability.

Several studies have established a coupling between the sulcal/gyral brain anatomy and functional activation in individuals (e.g., Amiez et al. 2006, 2013; Derrfuss et al. 2012; Zlatkina et al. 2016; Bodin et al. 2018). A recent neuroimaging study demonstrated that the location of individual somatomotor activation peaks held consistent relationships between the effector being moved and the morphologically defined segments of the central sulcus (Germann et al., 2020). The inter-individual variability of the central sulcus segments, however, was not quantified and this investigation focused exclusively on the central sulcus.

Speech-related movements in particular activate portions of the ventral central somatomotor strip and subcentral gyrus (Penfield and Boldrey, 1937; Olthoff et al., 2008; Grabski et al., 2012; Bouchard et al., 2013). Voluntary control of laryngeal movements during vocalization evokes brain activity in two distinct regions in lateral motor cortex: a dorsal region close to the

representation of the lips and a ventral region close to the lateral fissure (Belyk and Brown, 2017). The representation of the larynx in the human brain, however, remains controversial. The inconsistency among reports in the literature might be explained by inter-individual variability in structure and function in this region. Thus, examining the functional anatomy of the speech motor system requires investigation both of the central sulcus and the subcentral gyral region at an individual participant level.

To the best of our knowledge, there has been no systematic examination of the morphological variability in the subcentral gyrus and the adjacent opercular cortex. There are two distinct sulci in the subcentral region: the anterior subcentral sulcus (*ascs*) and the posterior subcentral sulcus (*pscs*) (Ono et al., 1990; Petrides, 2012, 2019). The variability of these two sulci in relation to the segments of the central sulcus across individuals, and the relationship of morphological variability to functional brain activity during movement, specifically vocalization, remain to be established.

Here, we assessed the structure-to-function relationship in the human motor system on an individual participant level by examining the morphology of the central sulcus, the subcentral gyrus and the adjacent central operculum. We performed sulcal labelling in surface space, rather than in volume space, which allows for better visualization and surface-based registration with respect to the 2D topology of the cerebral cortex (Fischl et al., 1999b, 1999a; Ghosh et al., 2010).

To visualize and quantify inter-individual variability of the examined sulcal segments, we derived spatial probability maps in both standard surface and volume stereotaxic space. In a subset of participants, we acquired functional magnetic resonance imaging (fMRI) data to

localize brain activity during movement of the hand, lips and tongue, as well as the larynx during vocalization (see chapter 2). To investigate how this structure-to-function relationship can help to improve alignment of data, we registered the brains of all participants based on the sulcal surface labels. A decrease in distances across activation peaks after applying the registration would indicate that anatomical variability can explain some of the functional variability.

3.3. Material and Methods

3.3.1. Participants

Data from two groups of participants were used. In one study group, both structural and functional MRI data were acquired ($n = 20$), but in the other study group, only structural MRI data were available ($n = 30$). Structural and functional MRI data from the performance of motor tasks were acquired from 20 participants (12 females, 18 – 40 years [27.4 ± 5.6 , mean $\pm$ SD], 5 self-reported left-handers). All participants were self-reported native English speakers (two were raised bilingually from infancy and three were fluent in a language acquired second) and had no history or diagnosis of speech disorders. All had normal hearing, normal or corrected-to-normal vision, and no neurological impairments. The participants were part of a study that had been approved by the Central University Research Ethics Committee of the University of Oxford (CUREC, R55787/RE001) in accordance with the regulatory standards of the Code of Ethics of the World Medical Association (Declaration of Helsinki). All participants gave informed consent for their participation and were monetarily compensated.

In addition, we used cortical brain surface reconstructions from 30 participants provided by the Human Connectome Project (HCP), 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 (Van Essen et al. 2013). The minimally pre-processed datasets of the first 31 participants (16 female, age range 22-35 years, handedness information not accessed) of the Q2 release were used. One participant was excluded because of a technical problem in the automatic FreeSurfer parcellation.

3.3.2. MRI data acquisition

MRI data acquisition parameters differed for the two groups of participants. Data from the participants that took part in the functional study were obtained at the Oxford Centre for Human Brain Activity (OHBA) using a 3T Siemens Prisma scanner with a 32-channel head coil. Two structural images of the whole brain had been acquired at 1 mm isotropic resolution: a T1w image (MPRAGE sequence) and a T2w image (SPACE sequence). For task-fMRI, whole head T2*-weighted echo planar images were acquired at 2.4 mm³ isotropic resolution (TE = 30 ms, multiband factor 6, TR = 0.8 s, Casey et al. 2018). Details of data acquisition and preprocessing methods of the HCP participants are provided in Glasser et al. (2013) and Ugurbil et al. (2013). T1w images had been acquired using an MPRAGE sequence at 0.7 mm isotropic resolution.

3.3.3. Structural MRI data analysis

Data from the participants who took part in the functional study were pre-processed using the HCP-pipeline (Glasser et al., 2013). The automatic processing pipeline includes cortical surface reconstruction using FreeSurfer based on the contrast from the T1w and the T2w images and automatic assignment of neuroanatomical labels. Cortical surface reconstructions

of the HCP participants were derived using FreeSurfer based on the T1w scans and directly provided by the database. A linear transformation (12 degrees of freedom) from the high-resolution T1w anatomical scans to standard MNI space (nonlinear 6th generation atlas, Fonov et al. 2011) was derived using FSL's FLIRT (Jenkinson and Smith, 2001; Jenkinson et al., 2002) and further refined using FNIRT nonlinear registration (Andersson et al., 2007).

3.3.4. Sulcal labelling

We identified the following sulci and sulcal segments in the structural data from all 50 participants: five segments of the central sulcus from dorsal to ventral (*cs_1* to *cs_5*), the lateral and opercular segments of the anterior subcentral sulcus (*ascs_lat*, *ascs_op*) and the posterior subcentral sulcus (*pscs*) (Figure 3.1A). Sulcal labels for one example participant are shown in Figure 3.1B and Figure 3.1C. Sulcal labels were drawn manually onto the native surface mesh (approximately 136,000 vertices) in Connectome Workbench's *wb_view* (www.humanconnectome.org/software/connectome-workbench.html). Surface features of both pial and white matter surfaces were inspected in conjunction with the participant's T1w scan. The identification of the central sulcus segments was based on changes in direction of the sulcus, and on the location of gyral 'plis de passage', which are small gyral bridges connecting the postcentral with the precentral gyrus or based on more subtle 'wall pinches'. These landmarks can be most easily identified on the white matter surface and are not always visible on the pial surface alone (Germann et al., 2020). The following description of morphological criteria was sufficient to identify the sulci in all 50 participants.

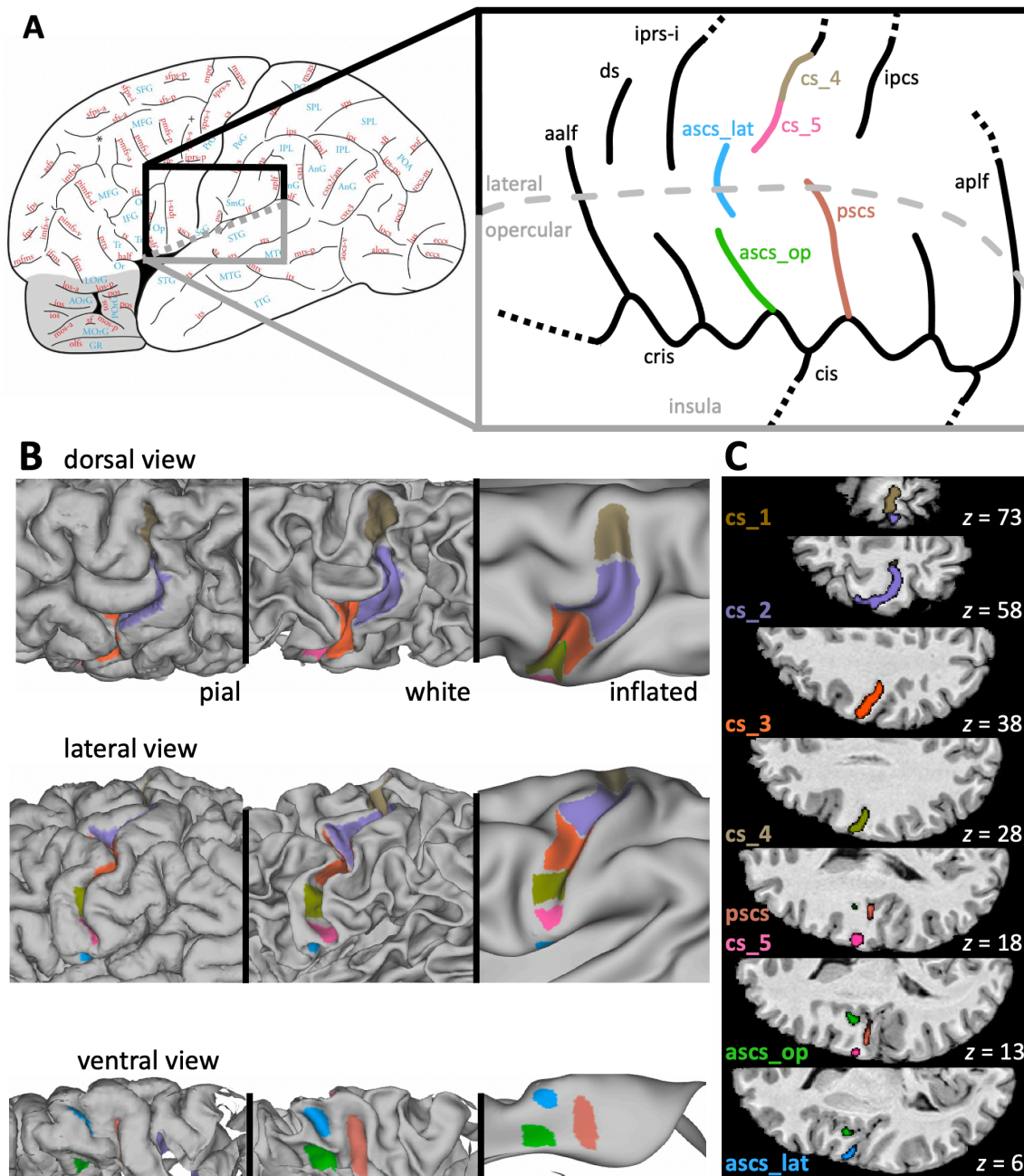


Figure 3.1 Sulcal morphology in the central and the subcentral region.

A: *Left:* Sulcal map of the human cerebral cortex (reprinted from Petrides M. (2019) Atlas of the morphology of the human cerebral cortex on the average MNI brain. New York: Academic Press, with permission from Elsevier). The region shown in grey depicts the orbito-frontal cortex. *Right:* Typical configuration of sulci in the subcentral region. This configuration was classified as morphological Type 1 (Figure 3.2). Anatomical variability was assessed in the sulci marked with colour: *ascsc_lat*: lateral segment of the anterior subcentral sulcus, *ascsc_op*: opercular segment of the anterior subcentral sulcus, *cs_1* to *cs_5*: segments of the central sulcus (only *cs_4* and *cs_5* are shown in the highlighted region), *pscs*: posterior subcentral sulcus. *Dashed grey line:* Visible surface boundary between lateral and opercular cortex. *aalf*: anterior ascending ramus of the lateral fissure, *aplf*: ascending posterior ramus of the lateral fissure, *cis*: central insular sulcus, *cris*: circular insular sulcus, *ds*: diagonal sulcus, *iprs*: inferior precentral sulcus. **B:** Sulcal labels in one individual displayed onto the native pial surface, white matter surface, and inflated surface. **C:** Sulcal labels of the same participant in MNI volume space.

Cs_1 is the most dorsal segment of the central sulcus, which runs more or less in a vertical straight direction. Its ventral boundary was drawn at the location where a gyral bridge provides a prominent landmark on the posterior bank of the central sulcus. *Cs_2* has a characteristic curvature in the shape of the Greek omega letter (see Figure 3.1B, C), which is known as the ‘hand knob’ (Yousry et al., 1997). The hand knob can be split into two smaller knob-like curves, the so-called epsilon configuration (Yousry et al., 1997). If this configuration were present, both knobs were labelled together as *cs_2* and the pinch on the posterior wall of the central sulcus in the middle was not treated as a boundary between these labels. The boundary between *cs_2* and *cs_3* was drawn at the location where the central sulcus changes direction and where a gyral passage can be observed on the posterior bank. In some brains, an additional convexity of the central sulcus can be observed on the posterior bank in the middle of *cs_3*. The ventral boundary of *cs_3* was drawn ventral to this convexity, if present, at the location where a small gyral bridge forms a landmark on the anterior bank of the central sulcus. The last two segments of the central sulcus, *cs_4* and *cs_5*, are smaller in extent, shallower and more variable in their morphology (see Results section for a more detailed anatomical characterization). The boundary between *cs_4* and *cs_5* was defined based on a gyral bridge on the posterior bank of the central sulcus. *Cs_5*, which is the most ventral part of the central sulcus, can form an additional curve or run straight in a variable direction.

The labels for *ascs* and *pscs* were assigned based on an atlas of human brain morphology (Petrides, 2019). For *ascs*, we labelled two distinct segments: a lateral and an opercular segment (*ascs_lat*, *ascs_op*). The course of *ascs_lat*, *ascs_op* and *pscs* was highly variable and a detailed description of the sulcal anatomy in the subcentral region is reported in the results section.

The morphological patterns of the ventral subcentral region were categorized into five types depending on the configuration of the *ascs_lat*. The classification was based on the location of the *ascs_lat* on the cortex and its spatial relation to other sulci. Sulcal segments were considered as ‘merged’, when there was a clear continuation on the pial surface, although, in some cases, a discontinuity between the merged sulci was still observed on the white matter surface. Occurrences of morphological subtypes were reported for each hemisphere separately, but a contingency test was not performed because of small count numbers.

3.3.5. Spatial probability maps

In order to characterize the inter-individual morphological variability of the labelled sulci, we generated probability maps in surface and volume space. To obtain surface probability maps, all surface labels were resampled from native to a standard mesh (32k fs_LR) following FreeSurfer’s registration (Glasser et al., 2013). At each vertex, the 50 surface maps were binarized, summed up and then normalized to create a surface label with intensities ranging from 0 % to 100 % at the maximal possible overlap of all 50 participants. For visualization, the surface probability maps were displayed onto an inflated template surface.

For the generation of volumetric probability maps, individual surface labels were mapped to volume space. For surface-to-volume mapping, we used the participant’s native pial surface registered to 32k_fs_LR mesh and non-linearly transformed to MNI-space (i.e. the surfaces in the subfolder /MNINonLinear/fsaverage_LR32k/). We performed the mapping to 0.5 mm resolution volume space using `wb_command` and ‘-nearest-vertex’ option. Given that the volumetric labels spatially varied in 3 rather than in 2 dimensions, the labels were smoothed

(Gaussian kernel with FWHM of 2 mm) to enhance overlap. The smoothed labels were then thresholded at 0.1, binarized and then summed at each voxel. For visualization, volume probability maps were overlaid onto the MNI average brain.

3.3.6. Anatomical characterization of sulcal segments

We characterized the sulcal segments using several anatomical descriptors. Inter-individual variability was determined as the maximal value within the surface probability map. For the measure of sulcal depth, we computed the minimal value in each individual's sulcal segment labels using FreeSurfer's sulcal depth map. The depth value is based on an individual's pial surface and expressed as normalized value relative to a baseline running along the sulcal banks, rather than in real-world units. The depth values range from positive values at the crown of a gyrus to negative values at the fundus of a sulcus. In shallow parts of the brain, the minimal sulcal depth value of a sulcal label can therefore even be positive. Furthermore, we computed the mean cortical thickness in each label, as provided by FreeSurfer. As measure for the size of the sulcal segment, we computed the number of vertices that the surface label spanned on the native surface.

3.3.7. Functional MRI experimental design

The 20 participants who provided structural and functional data took part in an fMRI study on speech production and laryngeal motor control. The experimental design, processing, and fMRI results of this study have been reported in chapter 2 and are here only briefly described. In a functional localizer task, participants were asked to perform repeated lip protrusion or tongue retraction at a rate of approximately 1-2 reps/s. The participant's breathing pattern was

explicitly controlled using the fixation symbol on the screen, instructing them to inhale for 1.5 s and exhale for 4 s. A ‘breathing only’ condition, during which the participants followed the same breathing pattern, was acquired as baseline condition. Each task condition was performed in blocks lasting 22 s followed by a rest period of 8 s with normal breathing. The conditions were presented in a fixed pseudo-random order following a balanced Latin-square design wherein each condition was repeated four times (see chapter 2, Figure 2.1).

In a separate task, participants were instructed to produce a syllable sequence (/la lei li la lei li/) under four different conditions: overt speech, silent mouthing, only vowel production, and covert speech. Breathing instructions, task timing and randomization of the four blocks were the same as described for the localizer task, except that each condition was repeated five times. In a third task, participants performed a task that required both phonological and semantic judgements. Participants had to indicate a yes/no response by pressing a button with the right index or the middle finger every 3 s. This task was analysed as a localizer for the hand region in the left hemisphere.

3.3.8. Functional MRI data analysis and statistical analysis

Functional MRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 6.00, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl) including motion correction of the images and unwarping using a fieldmap (Jenkinson, 2003). Time-series statistical analysis was based on a general linear model (GLM) implemented in FILM with local autocorrelation correction (Woolrich et al., 2001). Standard motion correction parameters and individual volumes that were motion outliers, determined using `fsl_motion_outliers`, were included as separate regressors at the first level for each participant.

Registration to the high-resolution structural scan and standard 2-mm MNI template was carried out using FLIRT. Registration from high resolution structural to MNI space was then further refined using FNIRT nonlinear registration (Andersson et al., 2007).

In the functional localizer task for lip and tongue movements, activity during each condition was assessed relative to the ‘breathing only’ condition. For the syllable production task, the conditions were analysed in a factorial model that allowed separation of the (supra-laryngeal) articulation and the (laryngeal) vocalization components of the task. Brain activity associated with the control of supra-laryngeal articulation was defined as (‘overt speech’ *minus* ‘vowel production’) *plus* (‘silent mouthing’ *minus* ‘covert speech’) and the main contrast for vocalization was derived from the contrast (‘overt speech’ *minus* ‘silent mouthing’) *plus* (‘vowel production’ *minus* ‘covert speech’). The task activations from the ‘articulation’ contrast are not further discussed in this manuscript.

In both tasks described above, the rest blocks with normal breathing served as baseline, which means that they were not modelled in the GLM. For the hand localizer task, we derived a contrast of all conditions involving button presses relative to a resting baseline. Note that this task only provided data to analyse the hand representation in the left hemisphere.

3.3.9. Individual surface activation maxima

To assess inter-individual variability of the fMRI results, we derived the location of individual activation maxima for hand, lip, and tongue movements, and larynx activity during vocalization. Activation maxima were derived using the steps described in chapter 2 (section 2.3.9) and are reported here only briefly. Different volumetric ROI masks were used for the

different motor representations based on individual anatomy in both hemispheres. We used an ROI of the whole central sulcus for hand, lip and tongue, based on the Destrieux Atlas. For the dorsal larynx representation, we used a more limited portion of the central sulcus ROI (MNI z-coordinates: 50 to 30). For the ventral larynx representation, we manually defined an ROI based on individual surface landmarks.

Individual volumetric ROIs were linearly transformed from FreeSurfer's anatomical to functional space of the respective task fMRI scan. Within the ROI, the voxel of maximal intensity was determined from the uncorrected z-statistics image. Activation maxima were manually inspected in the participant's native volume space to confirm that the systematic approach described below captured task-related activations. It should be noted that, for some participants, this local maximum did not achieve the corrected voxel-wise significance threshold (left hemisphere: hand $n = 3$, dorsal larynx $n = 6$, ventral larynx $n = 5$; right hemisphere: dorsal larynx $n = 5$, ventral larynx $n = 4$). Using a lower uncorrected threshold is justified given our goal to visualize and assess spatial variability of the activation maxima. The activation maxima were mapped to the individual's native mid-thickness surface, resampled to the 32k standard (fs_LR) surface mesh using the FreeSurfer registration, smoothed (FWHM = 1 mm), and binarized to form a small circular patch.

3.3.10. Structure-to-function relationships

Next, we examined the spatial relationship between the sulcal segments, and the task activation peaks at the individual participant level. This analysis was performed in the 20 participants who contributed both task activation and structural data. Individual task activation peaks were mapped onto the individual's cortical surface as described above for sulcal segments. In order

to characterize the structure-to-function relationship at the group level, we aligned all individual surfaces based on the anatomical surface labels and then we applied the same registration to individual task activation peaks. This approach allowed us to visualize individual variability in the spatial distribution of task activation peaks in a common group-level space.

The registration of sulcal labels was driven by the binary labels for *cs_1*, *cs_2*, *cs_3*, *cs_4*, *cs_5*, *ascs_lat*, *ascs_op* and *pscs* and performed using the multi-modal surface matching tool (MSM, Robinson et al. 2014), which is part of FSL. As target, or reference, for the MSM-based registration, we used the normalized and thresholded (> 0.4) average labels after projecting all of them to the same regular sphere (approximately 32,000 vertices) without using any other anatomical priors. Each participant's sulcal maps and the reference sulcal maps were merged into a combined file with six data arrays, i.e. they were provided as metric 'func.gii' file with six maps. Then, we derived a registration using MSM for each participant. We used default settings for MSM, and the following configuration parameters were empirically determined: levels=3; sigma_in=10,5,2; sigma_ref=10,5,2; lambda=0.1,0.01,0.01; it=10,10,3; opt=DISCRETE,DISCRETE,DISCRETE; CPgrid=2,3,4; SGgrid=4,5,6; datagrid=4,5,6.

We explored how manual labelling of sulcal segments can help to align data across participants. Thus, we compared our registration based on sulcal segments with the FreeSurfer registration and a registration based on sulcal depth maps. The FreeSurfer registration and sulcal depth maps were provided by the HCP-processing pipeline. To derive a registration based on sulcal depth, we used MSM default settings. To characterize the effect of the registrations, we quantified the spatial spread of the fMRI activation peaks: For each effector, we computed the median geodesic distances across all 20 activation peaks. The median distance for each effector

was, therefore, based on 190 datapoints. A smaller spatial spread of activation peaks indicates a better registration performance.

3.4. Results

3.4.1. Sulcal patterns in the subcentral region

The sulcal anatomy of the subcentral region was examined in a total of 100 hemispheres ($n = 50$ brains) and the morphological patterns encountered were classified into five types (Figure 3.2). The most commonly observed configuration of subcentral sulci (Type 1, 44 % of hemispheres) is shown in Figure 3.1B, C. In the Type 1 configuration, the central sulcus does not extend ventrally to meet the lateral fissure. The fifth and most ventral segment of the central sulcus (*cs_5*) is typically short and forms a curve in an anterior direction. The lateral segment of *ascs* (*ascs_lat*) is visible on the lateral surface of the brain and is clearly separate from the central sulcus. Dorsally, the *ascs_lat* extends into the precentral gyrus with variable length. Ventrally, the *ascs_lat* continues into the opercular cortex, which is hidden within the lateral fissure of the brain. A small gyral bridge separates the *ascs_lat* from the opercular segment of the *ascs* (*ascs_op*) which continues medially until it reaches the circular insular sulcus (*cris*). The medial origin of the *ascs_op* can be identified at a curve of *cris*, which is formed by the posterior short insular gyrus just anterior to the central insular sulcus (*cis*). Posterior to the *ascs_op*, the posterior subcentral gyrus (*pscs*) can be found, which extends laterally towards the lateral fissure. The medial origin of *pscs* can be identified at a curve of *cris* posterior to *cis*, which is formed by the anterior long insular gyrus. The number of curvatures of *cris* and the number of subcentral sulci is variable, but the *ascs_op* and the *pscs* could be reliably identified in every participant examined. The morphology of all of the mentioned sulci is variable across

participants, but the description of morphological types reported below focusses on the configuration of the *ascs_lat*.

3.4.2. Morphological patterns in the subcentral region

The subcentral region exhibits high inter-individual variability regarding the configuration of the *ascs_lat*. The observed occurrences of morphological types are reported in Table 3.1. In addition to the canonical (Type 1, 44 % of hemispheres) configuration described above, it was commonly observed that the *ascs_lat* merged with the central sulcus (Type 2, 20 %) (Figure 3.2). In these cases, the *ascs_lat* remains mostly on the lateral brain surface and does not reach into the opercular cortex. The *ascs_op* extends further lateral and its extension is visible on the lateral brain surface. Another commonly observed type was identified by a more opercular position of the *ascs_lat* (Type 3, 18 %).

In Type 3, the *ascs_lat* is less visible on the lateral surface and the central sulcus extends further ventral towards the lateral fissure. Type 3 is also characterized by a reduced gyral bridge separating the *ascs_lat* from the *ascs_op*. In six cases, the opercular continuation of the *ascs_lat* curved posteriorly and merged with the *pscs* instead of the *ascs_op* (not classified as a separate type). In several cases, we also observed the *ascs_lat* to merge with the inferior ramus of the inferior precentral sulcus (*iprs-i*) (Type 4). When the *ascs_lat* and the *iprs-i* merge, the ventral continuation of the *ascs_lat* curves in an anterior rather than a posterior direction and the position of the *ascs_lat* is more lateral than opercular. In a few cases (Type 5), the position of the *ascs_lat* was notably further rostral so that it was positioned anterior to the *ascs_op* rather than posterior as in the other configurations.

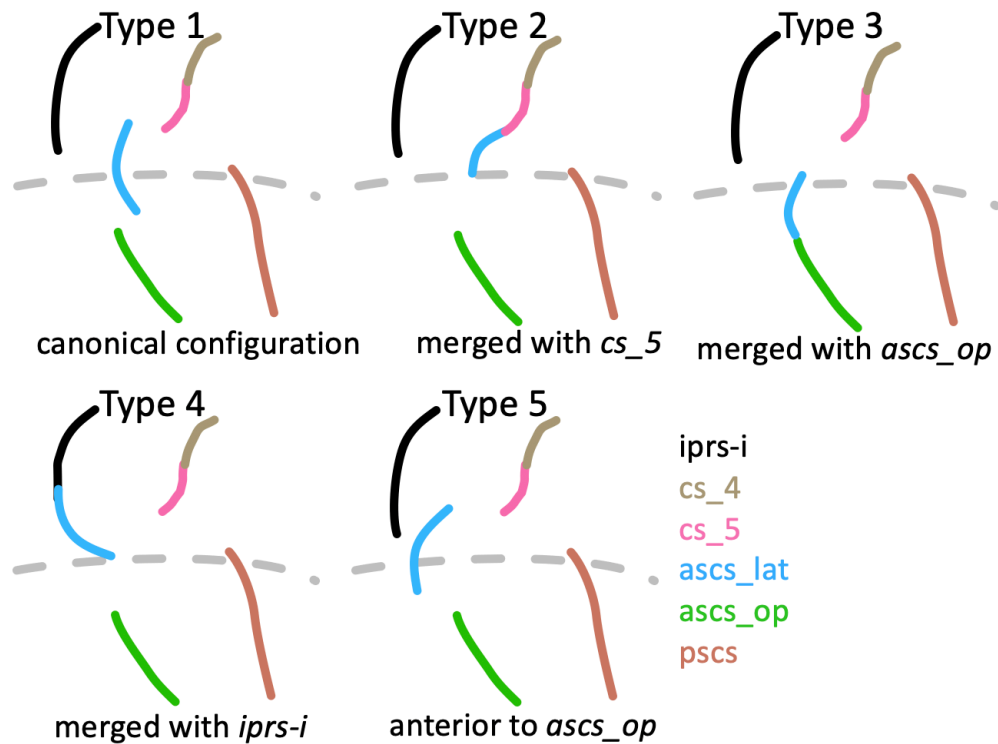


Figure 3.2 Morphological subtypes in the subcentral region.

Schematic drawing illustrating the main morphological patterns of the subcentral region formed by the *ascs_lat* with neighbouring sulci. Type 1: Canonical configuration observed in the majority of hemispheres (also shown in Figure 3.1B). Type 2: *ascs_lat* merging with the central sulcus. Type 3: *ascs_lat* merging with *ascs_op*. Type 4: *ascs_lat* merging with the inferior ramus of *iprs*. Type 5: *ascs_lat* located anterior to *ascs_op*.

Strong hemispheric differences were observed in the occurrences of some of the morphological types (Table 3.1). Type 2 (*ascs_lat* merging with *cs_5*) is much more common in left hemispheres (85 % vs. 15 %), while Type 4 (*ascs_lat* merging with *iprs*) and Type 5 (*ascs_lat* further anterior), are more common in right hemispheres (75 % vs. 25 % for Type 4; 83 % vs. 17 % for Type 5), although both types occur only infrequently. For Type 1 (canonical configuration) and Type 3 (*ascs_lat* merging with *ascs_op*), no pronounced hemispheric differences were observed.

Table 3.1 Morphological types in the subcentral region.

	Type 1		Type 2		Type 3		Type 4		Type 5	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
LH	20	45	17	85	9	50	3	25	1	17
RH	24	55	3	15	9	50	9	75	5	83
total (LH+RH)	44		20		18		12		6	

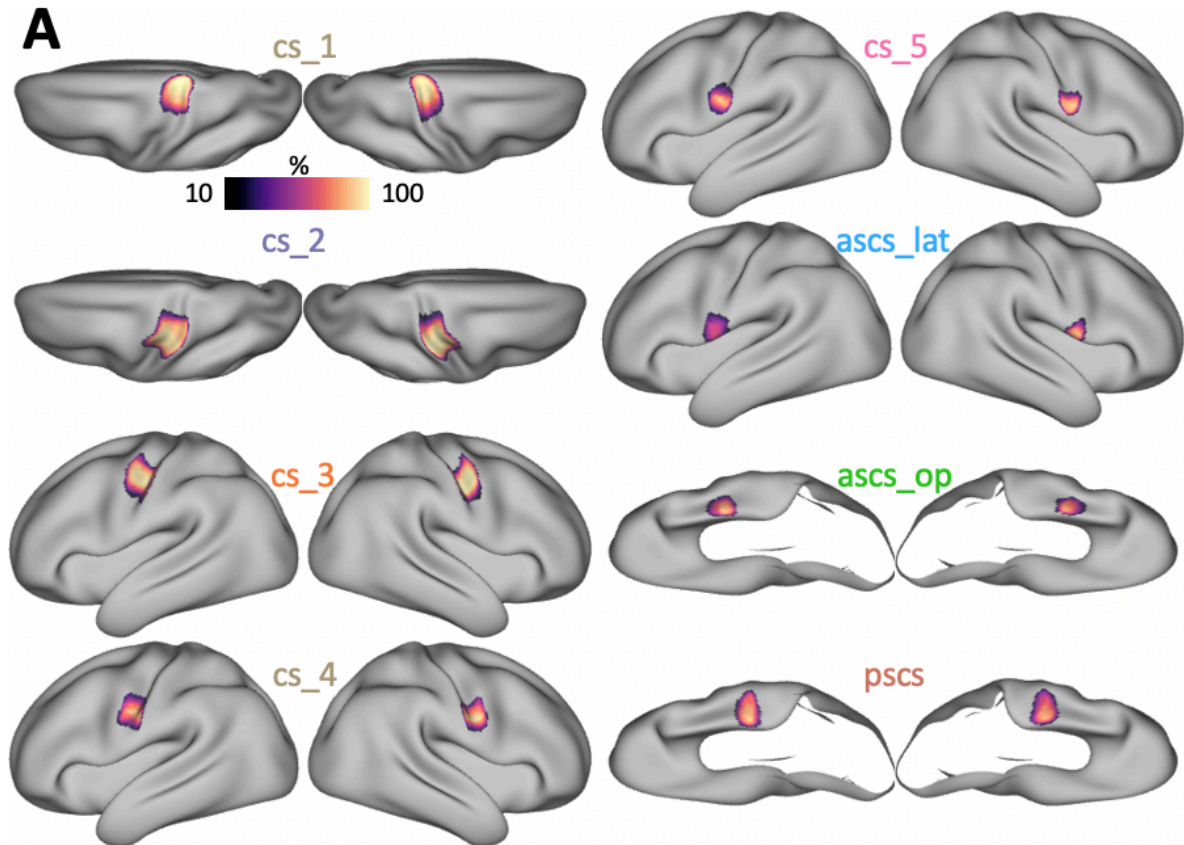
Observed frequencies of morphological patterns based on 100 examined hemispheres (LH, RH: left and right hemisphere). Provided are absolute numbers of occurrence (*n*) and relative occurrence of the type between the two hemispheres (%).

3.4.3. Spatial probability maps

The morphological variability of the central sulcus segments and the subcentral sulci was quantified and visualized using spatial probability maps in 2D surface space and 3D volumetric space (Figure 3.3). For both surface and volume probability maps, intensity values decrease from the centre of the map towards the edges, which is typical for overlap maps. Values in the volumetric probability maps are overall lower given that they capture variability in three spatial dimensions. The pattern of values across sulci and hemispheres, however, is consistent across surface and volumetric probability maps. For all sulci, hemispheric differences were observed with regard to the location in volume space (Figure 3.3B). All sulcal segments in the left hemisphere are located consistently further posterior compared to the segments on the right hemisphere, which is in line with the Yakovlevian anticlockwise torque of the two hemispheres (see Table 3.2 for coordinates of the voxel of maximal probability and for the centre of gravity for each label).

The first three segments of the central sulcus (*cs_1* to *cs_3*) show high inter-individual spatial consistency, which is characterized by high values, i.e. overlap, in the probability maps. Consistency is lower for *cs_4* and *cs_5*. Maximal values in the probability map for quantitative comparisons are visualized in Figure 3.4A. Consistency is also low for the subcentral sulci (*ascs_lat*, *ascs_op*, *pacs*) with lowest consistency for *ascs_lat*, especially in the left hemisphere.

The hemispheric effect for the *ascs_lat* is evident in both surface and volumetric probability maps. It was observed that the *ascs_lat* is more variable in the left hemisphere (see for example Figure 3.3B, slice $y = 1$). The low consistency in the *ascs_lat* is in line with the variable morphological types that were described above. The two subcentral sulci, *ascs_op* and *pscs*, are less consistent than the central sulcus segments, but more consistent than the *ascs_lat*.



(figure continued on following page)

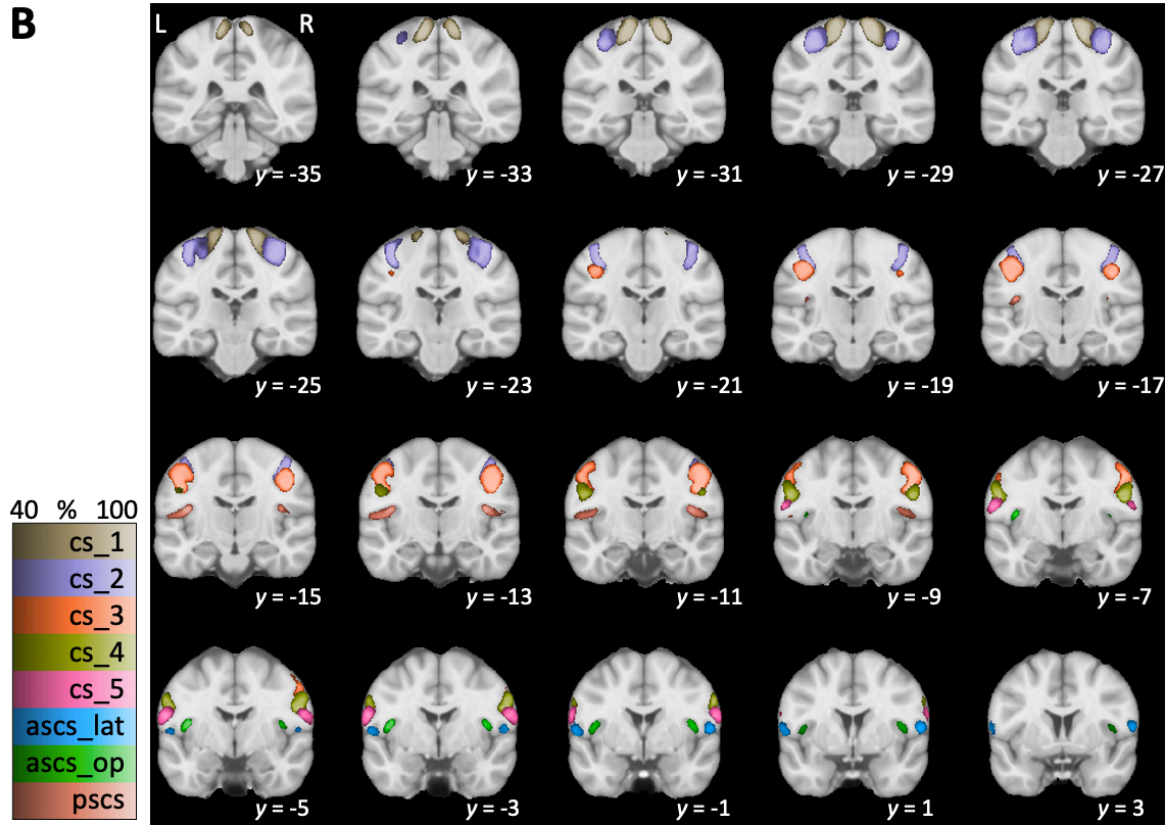


Figure 3.3 Spatial probability maps.

A: Surface probability maps of central and subcentral sulcal segments shown on an inflated average surface ($n = 50$). Labels for *cs_1* and *cs_2* are shown from a dorsal perspective. Labels for *ascs_op* and *pscs* are shown from a ventral perspective with the temporal lobe removed for better visibility of the opercular cortex. Intensity values show the extent of overlap with 100 % indicating an overlap in all participants. **B:** Volumetric probability maps overlaid onto the standard MNI template ($n = 50$). L: left hemisphere shown on left side of the image. Intensity values show the extent of overlap across participants. Colour maps for the different sulci have been matched in luminance so that brighter colours indicate higher overlap.

Table 3.2 Coordinates of spatial probability maps.

segment	LH						RH					
	maximum			centre of gravity			maximum			centre of gravity		
	x	y	z	x	y	z	x	y	z	x	y	z
<i>cs_1</i>	-14	-30	69	-17	-30	-69	16	-28	68	18	-28	69
<i>cs_2</i>	-36	-26	58	-36	-24	59	38	-23	60	36	-21	58
<i>cs_3</i>	-38	-18	42	-47	-15	46	44	-14	40	48	-12	46
<i>cs_4</i>	-54	-8	30	-55	-7	32	58	-4	32	56	-5	32
<i>cs_5</i>	-58	-6	22	-60	-4	22	62	-2	21	62	-2	22
<i>ascs_lat</i>	-60	2	12	-58	0	12	58	1	10	58	2	12
<i>ascs_op</i>	-41	-4	14	-44	-3	10	42	-3	14	44	-2	11
<i>pscs</i>	-39	-16	18	-48	-14	15	41	-14	18	48	-13	16

For each sulcal segment in left and right hemispheres, we provide the MNI stereotaxic space coordinates (x, y, z) for the voxel of maximal overlap and the centre of gravity in the volumetric probability maps.

3.4.4. Anatomical characterization of sulcal segments

In addition to probability maps, we characterized the sulcal segments based on sulcal depth, cortical thickness, and sulcal area of the surface label (Figure 3.4). It can be observed that the first three central sulcus segments (*cs_1* to *cs_3*) are deeper than *cs_4* and *cs_5*. Note that the sulcal depth measures are expressed in normalized FreeSurfer units, rather than in real-world values. The *ascs_lat* is notably shallower than the other segments. Cortical thickness increases from dorsal to ventral and is highest in the two subcentral sulcal segments (*ascs_op*, *pacs*). The largest sulcal segment, as indicated by sulcal area, is *cs_2*. It can be observed that *cs_2* is larger in the left hemisphere, which is consistent with previous reports of a structural asymmetry in the size of the ‘hand knob’ (Yousry et al., 1997; Germann et al., 2019). *cs_4* and *cs_5* are notably smaller than the other central sulcus segments. None of the three measures described show a hemispheric difference for *ascs_lat*, unlike the surface probability maps, which demonstrate greater variability, i.e. less overlap, in the left hemisphere. Given that handedness of the Human Connectome Project (HCP) participants was not accessed, we did not assess how this might have affected the measures described above. Regardless of handedness, however, it must be assumed that the majority of the 50 participants would be left lateralized for language. In addition, we report the anatomical measures depending on the morphological type of the subcentral region (Figure 3.4B). Although minor deviations from the patterns described in Figure 3.4A can be observed, overall, the anatomical characteristics for the sulcal segments are similar across all morphological types. Large confidence intervals for *ascs_lat* in sulcal depth depending on the morphological type are related to high spatial variability and small count numbers.

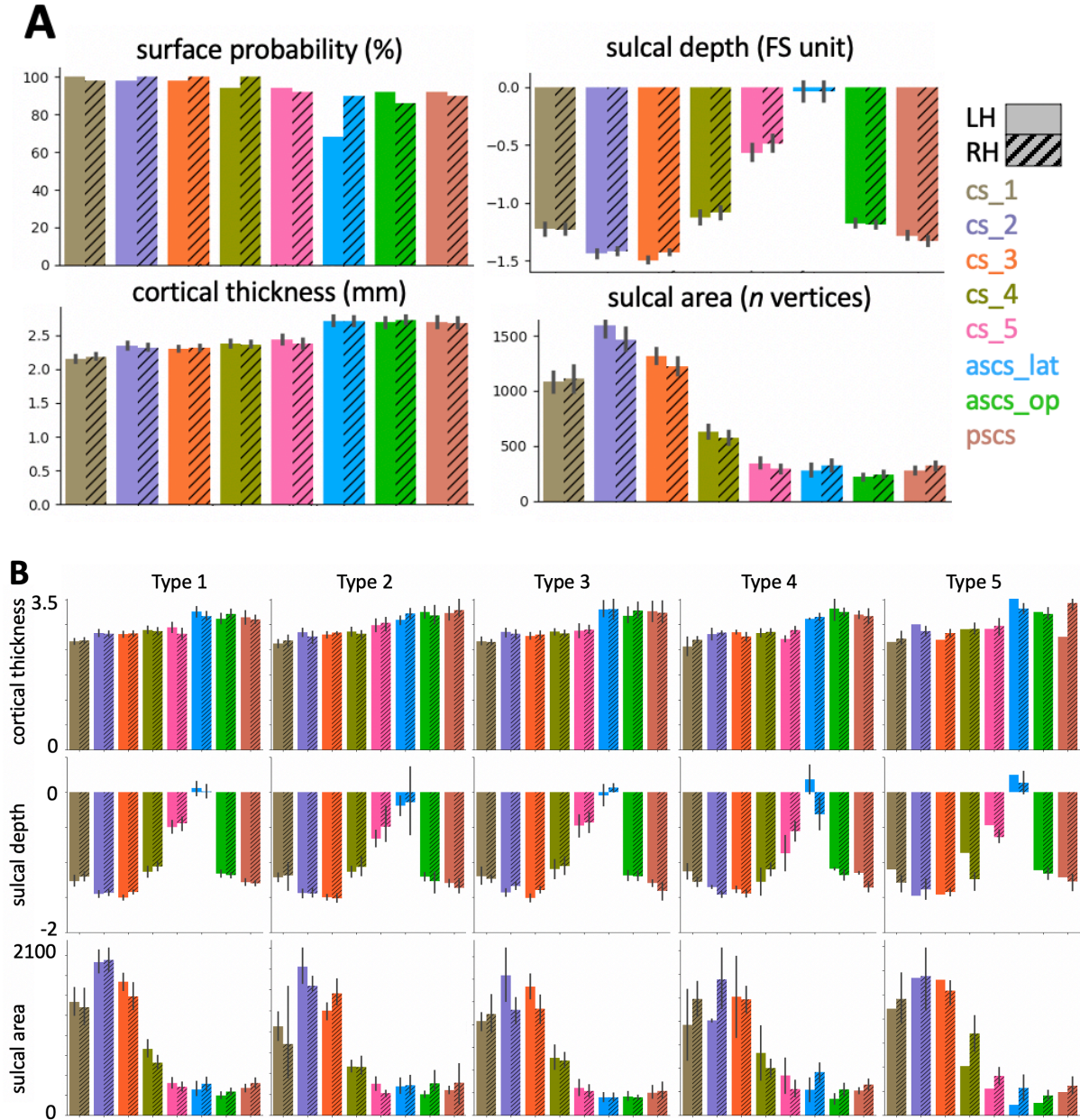


Figure 3.4 Anatomical measures for sulcal segments.

A: Surface probability quantifies the absolute value of maximal percentage for the overlap of sulcal labels as shown in Figure 3.3A. A low probability indicates high inter-individual variability. A more negative sulcal depth indicates a deeper sulcus. Bars for sulcal depth, cortical thickness and sulcal area show mean values $\pm$ 95 % confidence intervals ($n = 50$ for each hemisphere). **B:** Anatomical measures for sulcal segments in different morphological types. Morphological types refer to the patterns in the subcentral region as shown in Figure 3.2 (n depends on morphological type, see Table 3.1 for numbers). Colour labels as in A.

3.4.5. Sulcal registration and structure-to-function relationships

All individual sulcal labels and functional activation peaks are shown in Figure 3.5A. To visualize the spatial relationships at the group level, we first registered all individual surfaces based on the binary sulcal labels using MSM. After registering the sulcal labels, we averaged all transformed sulcal labels and thresholded (> 0.4) the average labels to obtain the outlines of registered sulcal segments. Figure 3.5B shows the outlines of the registered sulci, which demonstrates an orderly dorsal-ventral configuration of the sulcal segments.

Next, we applied the registration that was used to transform individual surface labels to the group space to the functional activation peaks of different effectors in individual participants. Figure 3.5B shows the location of the resampled activation maxima in relation to the outlines of the registered segments. Given that the same registration was applied to individual sulcal labels and the functional peaks, the location of resampled peaks with respect to the registered sulci reflects the original spatial relationship on the native surfaces of the individuals. The location of the peaks is therefore similar, but not identical to the location obtained from a group-level FreeSurfer-based registration (as shown in Figure 2.4A from chapter 2).

Overall, a strong correspondence between sulcal labels and functional peaks was observed. The observed relationships between labels and peaks are reported in Table 3.3. The majority of activation peaks from the hand localizer fall inside the centre of *cs_2*. The dorsal peaks for larynx activity are found on the anterior bank of *cs_3*. Peaks from the lip localizer also fall within *cs_3* and overlap with the location of the dorsal larynx peaks. Those for the lip, however, are reliably located at the more ventral extent of *cs_3* whereas those for the dorsal larynx peaks span the segment. Activation peaks from the tongue localizer fall inside *cs_4* with high

consistency. The ventral peaks for larynx activity are associated with *cs_5* and *ascs_lat* or with the gyrus in between, but the relationship is less consistent than for the other functional peaks. The sub-threshold maxima were similar in terms of their location to the other maxima.

The relationship between sulcal label and effector is very similar across hemispheres (Table 3.3). The only exception is the lip cluster in the right hemisphere, where several peaks are located in *cs_4* rather than in *cs_3*. The group-level representation (Figure 3.5B), however, demonstrates that this difference is driven by minor variations in the boundary between *cs_4* and *cs_3*, rather than being a systematic hemispheric difference in the cortical location of the activity. The sulcal label underlying the ventral larynx peak (either *cs_5* or *ascs_lat*) was the same in both hemispheres for the majority of participants ($n > 12$). Furthermore, we examined the relationship between morphological type of the subcentral region and the location of the ventral larynx peak. We observed no systematic relationship, i.e. all combinations of type and sulcal label are present. Given the low count number for each combination, a robust quantification of this observation is not feasible.

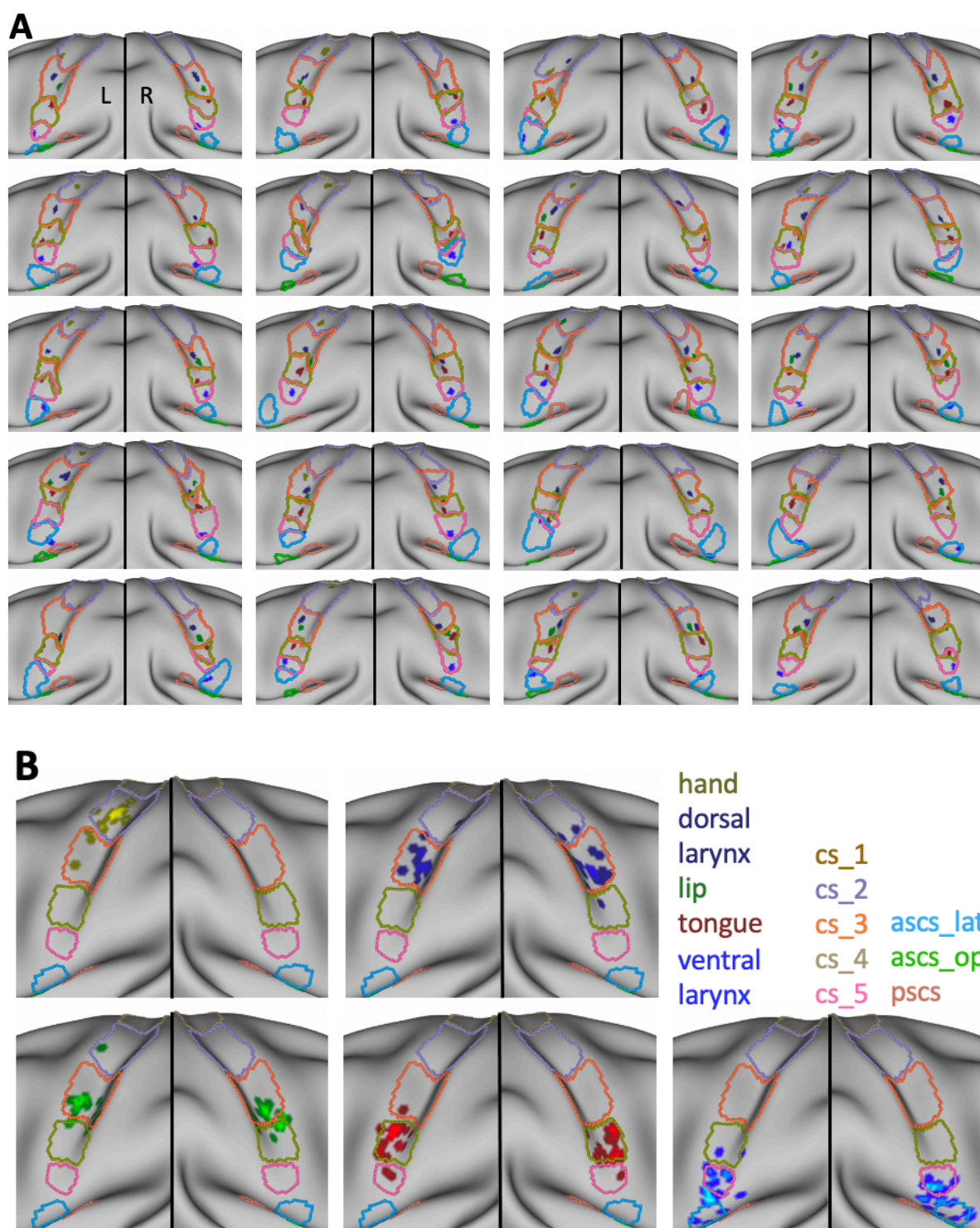


Figure 3.5 Structure-to-function relationships.

A: Individual sulcal labels and activation maxima for participants in cases where both anatomical and functional data were available ($n = 20$). Only the central strip of the brain is shown. For color legend see subfigure B. **B:** Location of individual activation peaks with respect to the sulcal segment outline after applying the sulcal registration (*ascs_op* is not visible). The sulcal segment outlines show the averaged, normalized and thresholded (> 0.4) sulcal labels after registration. Brighter colors indicate overlapping peaks.

Table 3.3 Structure-to-function relationships.

	LH					RH			
	hand	dorsal larynx	lip	tongue	ventral larynx	dorsal larynx	lip	tongue	ventral larynx
<i>cs_1</i>	0	0	0	0	0	0	0	0	0
<i>cs_2</i>	16	1	1	2	0	1	0	0	0
<i>cs_3</i>	4	19	19	1	0	18	15	0	0
<i>cs_4</i>	0	0	0	17	2	1	5	19	0
<i>cs_5</i>	0	0	0	0	12	0	0	1	12
<i>ascs lat</i>	0	0	0	0	6	0	0	0	8

Reported is the observed count of a sulcal label underlying the functional activation peaks ($n = 20$) depending on the hemisphere.

To characterize the effect of the sulcal registration on the spatial variability of functional peaks, we computed the median distance among all peaks (one each from 20 participants) for each effector for each hemisphere. We compared this measure of spatial spread with that obtained from the FreeSurfer registration and a registration based on sulcal depth maps. Overall, the three registrations have a similar effect on the spatial variability (Figure 3.6A). Some differences can be observed across effectors but given the sample size none of the effects are significant. For the lip cluster in the left hemisphere, spatial spread is increased for the registration based on sulcal labels. Spatial variability of the ventral larynx cluster in the left hemisphere was reduced the most by using the sulcal-segment registration, but the effect is small and not statistically significant. Figure 3.6B shows the spatial distribution of the fMRI peaks for the ventral larynx representation after a FreeSurfer-based registration and after registration based on sulcal segments. The peaks in the left hemisphere appear more focal when sulcal segments are driving the registration, which is in line with a reduction of the median distance among them. In the right ventral larynx cluster, however, the median distance was overall increased, and the overlap of fMRI peaks decreased. The spatial map, however, demonstrates that the registration brings several outlier peaks closer to the remaining cluster.

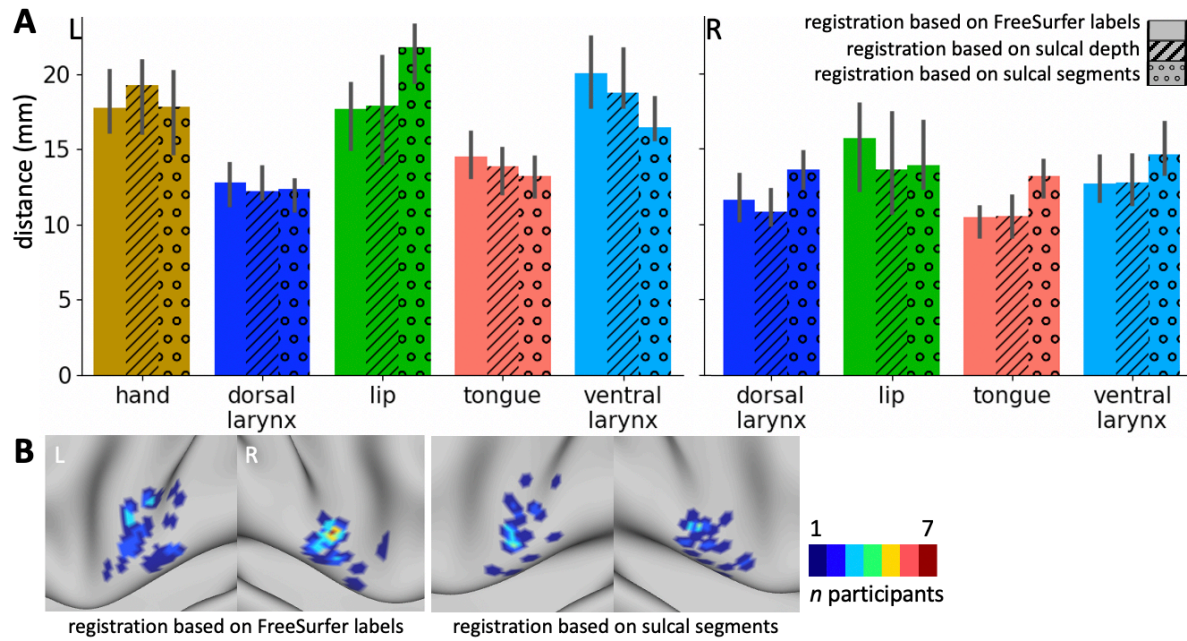


Figure 3.6 Effect of anatomical registrations on functional variability.

A: Distance of task activation peaks after registration based on FreeSurfer labels (*blank bars*), after registration based on whole-brain sulcal depth maps (*striped bars*) and after registration based on manually drawn sulcal segment labels (*dotted bars*). The median distance $\pm$ 95% confidence interval is shown across all peaks for each task cluster ($n = 20$, 190 pairs of points for each movement type). **B:** Overlap of fMRI peaks of the ventral larynx representation after FreeSurfer registration and after registration based on sulcal segments.

3.5. Discussion

We examined the morphological patterns and the inter-individual variability of the sulci in the central and subcentral region of the cerebral cortex in the human brain and their relation to activity evoked by movement. A robust relationship between morphological features of the central and subcentral sulcal segments and movement of different effectors at the individual participant level was demonstrated. An inter-individual registration based on the individually drawn sulcal segments demonstrated that morphological variability can explain functional variability, in particular in the subcentral region, where morphology is complex.

3.5.1. Morphology of the subcentral cortex and the central operculum

The present study provided a systematic examination of the sulcal patterns of the subcentral and adjacent opercular cortical region in the human brain, including inter-individual variability of the sulcal morphology. In this region, one encounters an anterior and a posterior subcentral sulcus (*ascs*, *pscs*) (Petrides, 2012, 2019). We showed that, in the majority of participants, the *ascs* consists of a distinct lateral (*ascs_lat*) and a distinct opercular segment (*ascs_op*).

The morphological patterns of the subcentral region were classified into five distinct types, based on the configuration of the *ascs_lat*. In addition to the canonical configuration (Type 1, Figure 3.1B), the *ascs_lat* often merged with the central sulcus, so that the central sulcus complex extended further ventral towards the lateral fissure (Type 2). In Type 3, the position of the *ascs_lat* is notably more opercular and it merges with the *ascs_op*, so that the *ascs_lat* and *ascs_op* form one continuous sulcus. In Type 4, the *ascs_lat* merges with the inferior precentral sulcus (*iprs*). Type 5 is characterized by a more anterior position of the *ascs_lat*, compared with its position in the other types. The variability in the configuration of the lateral and opercular segments explains why the *ascs* appears as one continuous sulcus in a volumetric average of structural brain images in MNI stereotaxic space (Petrides, 2019).

3.5.2. Sulcal labelling in surface space

We aimed to reproduce the segmentation of the central sulcus, as described in a volumetric labelling study (Germann et al., 2020). We labelled sulcal segments directly on the participant's native cortical surface, rather than in volume space as traditionally done in several MRI-based labelling studies (Germann et al., 2005, 2020; Zlatkina and Petrides, 2010; Amiez et al., 2013;

Sprung-Much and Petrides, 2018, 2020). We demonstrated the advantages of working in surface space as this can explicitly take into account the geometry of the cortical convolutions. The anatomical landmarks critical for labelling are conveniently visualized in surface space and, therefore, are more easily reproducible (Figure 3.1B). We characterized the sulcal segments using several surface-based anatomical features.

Furthermore, quantifications of geometrical and topological properties of interest for sulcus morphometry are readily available for surface labels (Mangin et al., 2004). Also, using surface labels allowed us to perform a surface-based registration, where distances are represented as geodesic distances along the cortex rather than as Euclidean distances between voxels (Fischl et al., 1999b; Klein et al., 2010). In the current study, the labels were drawn manually, but the development of automatic labelling algorithms for sulcal segments is subject of ongoing research (Clouchoux et al., 2006; Takerkart et al., 2015; Borne et al., 2020; Machlouzarides-Shalit et al., 2020).

3.5.3. Spatial probability maps

We visualized the morphological variability and spatial extent of the five segments of the central sulcus (*cs_1* to *cs_5*) and the subcentral sulci in surface and volumetric probability maps. The first three central sulcus segments show the highest spatial consistency across participants. Consistency decreases for the fourth and even further for the most ventral (fifth) segment of the central sulcus, which is in line with previous observations (Germann et al., 2020). The decrease in probability values for *cs_4* and *cs_5* can also be attributed to their smaller spatial extent compared with the sulcal labels for *cs_1* to *cs_3*. Consistency for the

subcentral sulci *ascs_lat*, *ascs_op* and *pscs* is also notably reduced, which is in line with the inter-participant variability in the morphological patterns as described above.

3.5.4. Hemispheric differences

The classification of morphological types and the spatial probability maps demonstrated hemispheric differences in the subcentral cortical region, namely that the position of the *ascs_lat* along the rostro-caudal axis differs between hemispheres, but not its dorso-ventral position. The *ascs_lat* in the left hemisphere tends to be located further posteriorly, which frequently results in a merge with the central sulcus, while the *ascs_lat* in the right hemisphere is located more anteriorly. The present quantifications showed that the *ascs_lat* is more variable in the left hemisphere, although several anatomical measures of the *ascs_lat* are not different across hemispheres, such as the sulcal depth and sulcal area.

The hemispheric difference in variability can be interpreted in relation to sulcal variability of the ventro-lateral cortex (Germann et al., 2005; Sprung-Much and Petrides, 2018, 2020). Language processing is lateralized most frequently to the left hemisphere (language dominant hemisphere) and the role of the left inferior frontal cortex in language function is widely established (Broca, 1861; Price, 2000). Functional language lateralization is associated with structural asymmetries (Foundas et al., 1996; Josse and Tzourio-Mazoyer, 2004) and increased regional variability (Croxson et al., 2018), but the structure-to-function relationships remain controversial (Dorsaint-Pierre et al., 2006; Sprung-Much and Petrides, 2018). It is possible that that increased surface area in the left inferior frontal cortex affects the neighbouring sulci so that the *ascs_lat* is ‘pushed’ to a relatively more posterior location in the left hemisphere. Despite the hemispheric differences in morphology, the structure-to-function relationships for

basic movements of different effectors described below, did not exhibit notable differences between hemispheres.

Several previous studies indicated that structural asymmetries in the human motor cortex are related to handedness and gender (Amunts et al., 2000; Mangin et al., 2004; Sun et al., 2012; Germann et al., 2019). These effects are not limited to the hand knob but affect the shape of the entire central sulcus. Future investigations could test for a systematic relationship between handedness and the morphological variations described here for the subcentral cortical region. Linking functional and morphological variability to behavioural measures would improve our understanding of hemispheric specializations underlying human language and handedness.

3.5.5. Functional activation peaks and morphology

Here, we demonstrated a tight link between individual morphological features of the cortex and activation peaks for the different effectors examined. We replicated the relationships between segments of the central sulcus and functional localizers for the hand, lip, tongue, and larynx reported in Germann et al. (2020). We identified two larynx peaks, consistent with more recent studies suggesting that two separate regions in the central region correlate with larynx activity (Belyk and Brown, 2017; Jarvis, 2019). Germann et al. (2020) however, found only a ventral larynx peak. The difference across studies might be because our functional localizer differed substantially from the one used by Germann and colleagues. In the latter study, ‘humming’ was used as task instruction to localize larynx activity. Germann et al. found the ventral larynx peak to be associated with *cs_5*, and did not identify *ascs_lat*. We showed that it was also localized in the *ascs_lat* in a large proportion of participants (14 out of 40 hemispheres). This difference might be due to the significantly larger sample size in the current

study that allowed a more robust assessment of the relationships. A systematic location of the dorsal larynx peak relative to morphological features has been predicted before (Sun et al., 2016), but this is the first study that describes consistent structure-function relationships for both the dorsal and the ventral larynx representations and how they relate to the representations of the other articulators.

It should be noted that the functional contribution of the two larynx representations in motor control during vocalization is still debated (Simonyan, 2014; Belyk and Brown, 2017). Quantifications of cortical microstructure, such as myelin content, indicate that the ventral larynx region is not located in primary motor cortex (see above chapter 2). The focus of the current study, however, is not the interpretation of the functional activation peaks *per se*, but rather the assess of the relationship between their location and the underlying morphology. The individual variability in the cortical location of the ventral larynx representation could be one factor in explaining inconsistent reports in the literature. The morphological type was not predictive of the sulcal label underlying the ventral larynx peak and vice versa. At the given sample size of 20 participants, the total counts for each combination of morphological type and sulcal label is low and, therefore, it is not practical to quantify the robustness of this effect. The current dataset, however, suggests that the sulcal label predicts the location of the maximal functional activation, regardless of the morphological configuration formed by the sulci. Future research could elaborate on the functional significance of the morphological types (Sun et al., 2016). For example, using an fMRI-paradigm that activates the opercular region more directly might reveal effects on the size of the activated area or the activation strength.

3.5.6. Relationship between variability in structure and function

We observed a robust relationship between sulcal landmarks and the location of functional activation peaks for speech-related effectors. The somatomotor system, however, is only one example system, where a link between sulcal morphology and function at the individual participant level has been established (Boling et al., 1999; Coulon et al., 2011; Zlatkina et al., 2016; Germann et al., 2020). Detailed anatomical and functional studies have also revealed such relationships for other parts of the cortex (e.g., Amiez et al. 2006; Bodin et al. 2018). A registration based on individually drawn sulci reduced the spatial spread of task peaks for the left ventral larynx representation. This indicates an improved registration in this part of the brain, when compared to a registration based on sulcal depth and based on FreeSurfer. The effect, however, was small and not statistically significant, and for other effectors the registration even increased the spatial spread. Reducing the whole-brain statistical task activation maps to a peak might have obscured greater benefits of a registration based on sulcal segments.

In many contexts, such as brain development or cross-species comparisons, the manual identification of sulcal landmarks is vital to constrain the registration algorithm when automatic labelling is inaccurate or not feasible (Van Essen and Dierker 2007; Lebenberg et al. 2018, and see below chapter 4 for a comparative study). Here we show that manual identification of sulcal landmarks is also critical for registrations in the human brain, particularly in brain regions that are highly variable and have low gyrification. The ventral larynx peaks were not consistently co-localized with any specific sulcal segment, but were located in both *cs_5* and *ascs_lat*. The anatomical information of the labels, however, was still helpful for the alignment within the subcentral opercular region. The current study emphasizes the importance of detailed

anatomical investigations of sulcal segments and tertiary sulci in understanding the structure-to-function relationships in the cortex (Weiner et al., 2018; Miller et al., 2020).

In the current study, we examined gross anatomical features of the brain surface, an approach that is directly applicable to neuronavigation and neurosurgical planning. Recent advances in neuroimaging, however, allow us to acquire multiple image modalities in the same participants, some of which correlate with the underlying cellular microstructure (Fischl and Dale, 2000; Zhang et al., 2012; Weiskopf et al., 2013). Although the histological analysis of post-mortem brains remains the gold standard to define anatomical parcels of the brain, neuroimaging has become a versatile tool for parcellating the cortex and to establish relationships between different modalities (van den Heuvel and Yeo 2017; Jakobsen et al. 2018; Smith et al. 2019, and see chapter 4 for a comparative study). Future work will show how detailed morphological labelling and segmentation studies can benefit from other neuroimaging modalities.

In summary, the current study demonstrated a robust relationship between morphological features of the central and subcentral sulcal segments and movement of different effectors at the individual participant level. We described the morphological patterns of the sulci in the subcentral and central opercular cortical region, their inter-individual variability and several anatomical properties. Laryngeal activity during vocalization activates the subcentral region, which we found to be highly variable across participants. This variability could explain inconsistencies in previous reports about a ventral larynx representation.

4

Cross-species Cortical Alignment Identifies Different types of Anatomical Reorganization in the Primate Temporal Lobe

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4.1. Abstract

Evolutionary adaptations of temporo-parietal cortex are considered to be a critical specialization of the human brain. Cortical adaptations, however, can affect different aspects of brain architecture, including local expansion of the cortical sheet or changes in connectivity between cortical areas. We distinguish different types of changes in brain architecture using a computational neuroanatomy approach. We investigate the extent to which between-species alignment, based on cortical myelin, can predict changes in connectivity patterns across macaque, chimpanzee, and human. We show that expansion and relocation of brain areas can predict terminations of several white matter tracts in temporo-parietal cortex, including the middle and superior longitudinal fasciculus, but not the arcuate fasciculus. This demonstrates that the arcuate fasciculus underwent additional evolutionary modifications affecting the temporal lobe connectivity pattern. This approach can flexibly be extended to include other features of cortical organization and other species, allowing direct tests of comparative hypotheses of brain organization.

4.2. Introduction

The temporal lobe is a morphological adaptation of the brain that is unique to primates (Bryant and Preuss, 2018). Its origins likely include expansion of higher-order visual areas to accompany the primate reliance on vision (Allman, 1982). Temporal association cortex contains areas devoted to higher-level visual processing and social information processing (Sallet et al., 2011; Rushworth et al., 2013) that, in turn, rely strongly on visual information in primates (Perrett et al., 1992). The expanded temporal cortex in apes and humans contains several multi-modal areas and areas associated with semantics and language (Price, 2000; Dronkers et al., 2004; Hickok and Poeppel, 2007). As such, understanding the evolution of

temporal cortex across the primate order is a vital step to understanding primate behavioural adaptations.

Two lines of evidence are often brought to bear on differences in temporal lobe organization across humans and other primates. The first line emphasizes selective local expansions of temporal cortex and subsequent relocation of areas. Morphologically, great apes possess an extra sulcus in the temporal cortex, suggesting at the very least expansion of this part of cortex. Mars and colleagues (2013) reported a region in the middle part of the superior temporal sulcus of the macaque that shares anatomical features of the human temporo-parietal junction area located at the caudal end of the temporal cortex, suggesting a major relocation of this area. In a similar vein, Patel and colleagues (2019) suggest that expansion of the temporo-parietal junction and superior temporal sulcus gave rise to a modified ventral visual processing stream to support increased social abilities in humans. The second line of evidence emphasizes changes in the connectivity of the temporal lobe. Rilling and colleagues (2008) first suggested dramatic expansion of the arcuate fasciculus temporal cortex projections in the human, but more recent studies also emphasize increased projections of the middle longitudinal and inferior fronto-occipital fasciculi and their role in language-related processes in the human (Saur et al., 2008; Makris and Pandya, 2009; Makris et al., 2013; Catani and Bambini, 2014).

These different schools place different emphasis on what happened to temporal cortex across different primate lineages. Their results, however, should be interpreted in relation to one another as species differences in brain organization can come in many forms that can interact in unpredictable ways (Krubitzer and Kaas, 2005; Mars et al., 2016b, 2018a). Dissociating such different types of species differences is challenging (Figure 4.1A,B). For instance, given an ancestral or reference state, local expansions of the cortical sheet can lead to the relocation of

homologous areas between two species. As a case in point, human MT+ complex is located much more ventrally in posterior temporal cortex than its macaque homolog (Huk et al., 2002). Such cortical relocations also affect the location of connections of these areas, but this situation is distinct from the scenario in which a tract extends into new cortical territory.

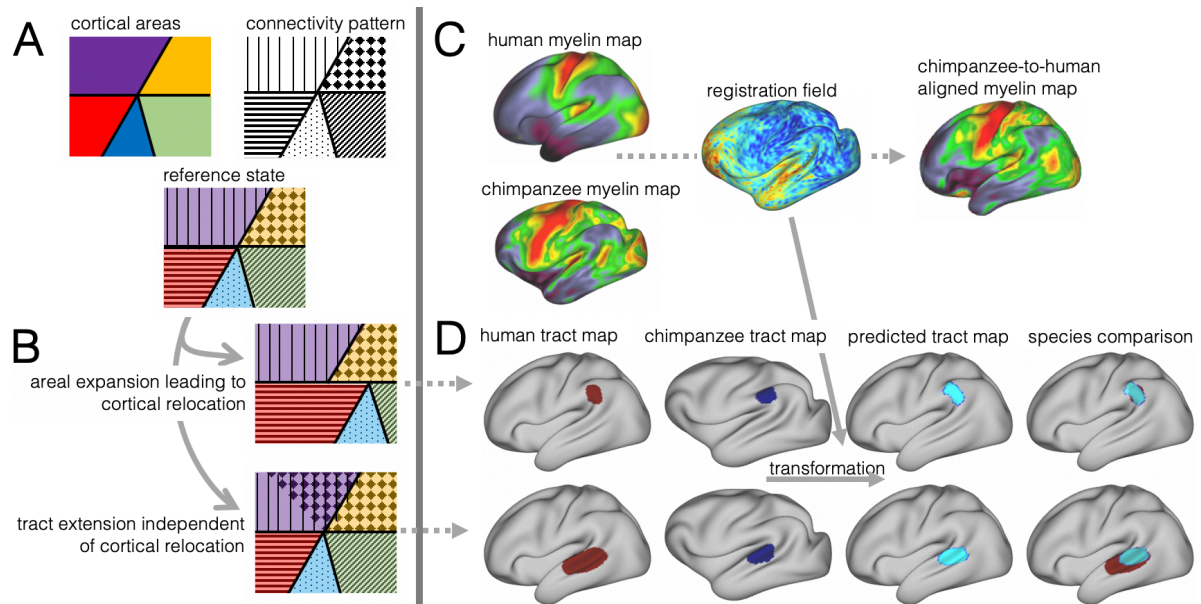


Figure 4.1 Cortical specializations.

A: Cortical brain organization can be described using different modalities such as brain areas defined by myelin content (*top left*) or the pattern of brain connections (*top right*). **B:** Anatomical changes can affect both modalities differentially. Top and bottom panel show different evolutionary scenarios. **C:** Alignment of homologous brain areas derived from myelin maps can model cortical expansion across species, here shown for human and chimpanzee. **D:** Applying the cross-species registration field to surface tract maps allows us to distinguish evolutionary scenarios. Here shown are toy example maps of one tract that extended due to areal expansion alone (*top panel*) and one tract that additionally extended into new brain areas (*bottom panel*). Red: human tract map, dark blue: chimpanzee tract map, light blue: transformed chimpanzee tract map.

These two scenarios are illustrated in Figure 4.1B. The red area in the top panel has expanded, leading to a relocation of the blue area with respect to the purple and yellow area. The connections of the areas do not change in this scenario, resulting in a relocation of the connections of the blue area. In the bottom panel, a yellow area's tract terminations have invaded the neighbouring purple territory, but this change is independent from cortical

expansion. Thus, in both cases connections are located in a different place from those in the reference state, but the causes are different.

In this study we investigate to which extent species differences in temporal lobe organization are due to cortical relocation and tract extension. To be able to do this, we propose a framework to test among different forms of cortical reorganization by registering brains together into a single shared coordinate system (Figure 4.1C,D). Such an approach allows us to place different brains into a common space based on one feature and then compare the results to registration based on another feature. This ‘common space’ concept proved feasible in a previous study testing whether the extension of the human arcuate fasciculus (AF) compared with the macaque AF could be accounted for by differential cortical expansion between the two brains (Eichert et al., 2019b). In the present study, we generalize this approach to develop a cross-species registration based on a multi-modal surface matching algorithm (MSM, Robinson et al., 2018, 2014, 2013) to derive a cortical registration between different species.

We based our registration framework on whole-brain neuroimaging data of macaque, chimpanzee and human brains. Neuroimaging allows one to acquire high-resolution data from the same brains using different modalities within a short time. The digital nature of the data allows easy manipulation, making it ideal for the present purposes (Lauterbur, 1973; Grannell and Mansfield, 1975; Bihan et al., 1985; Le Bihan et al., 1986; Thiebaut de Schotten et al., 2019). As primary modality we use surface maps derived from the cortical ribbon of T1- and T2-weighted scans, which have been shown to correlate well with cortical myelinization and which are available for all three species (Glasser and van Essen, 2011; Glasser et al., 2014). Such ‘myelin maps’ can be used to identify homologous areas across brains and species, such as primary sensory and motor cortex, which is high in myelin, and association cortex which is

low in myelin (Glasser et al., 2014; Large et al., 2016). As second modality, we use diffusion-weighted MRI tractography to reconstruct long range white matter fibers of the temporal and parietal lobes to establish its connections (Mars et al., 2018c; Bryant et al., 2019).

Given data from these two modalities, we developed an approach to reveal different types of cortical reorganization. We argue this approach is particularly suitable to study the temporal lobe, as it has well described myelin markers and cortical connections (Mars et al., 2013; Glasser et al., 2014; Ruschel et al., 2014; Large et al., 2016). First, we register the cortical surfaces of the different species to one another based on myelin maps (Figure 4.1C). This cortical alignment uses the distinction of primary and higher order areas in myelin maps as anchor points across all three species. Next, we apply this registration to overlay homologous parts of the cortex and to calculate the underlying distortions of the cortical sheet. This registration field effectively models areal expansion or contraction underlying cortical relocation of homologous areas. We then apply this registration to the cortical projection maps of temporal and inferior parietal lobe white matter tracts to assess how well the myelin-based registration can predict changes in tract projection patterns across species (Figure 4.1D). A good prediction, i.e. a high overlap of the tract maps, indicates that cortical expansion and relocation of targets zones alone can predict tract projections (Figure 4.1D, top), a poor prediction indicates that the tract is reaching new cortical territory (Figure 4.1D, bottom).

Here, we distinguish different scenarios of cortical evolution for a set of temporal and parietal white matter tracts. By applying a cross-species registration we can infer if only cortical relocation was affecting a tract's connectivity profile or if a tract is reaching into new cortical territory. A deeper understanding of species differences in brain reorganization is essential for

our understanding how evolutionary specializations of the temporal lobe underlie uniquely human cognitive functions.

4.3. Material and Methods

4.3.1. Human data and preprocessing

Human data were acquired in 20 subjects (12 females, 18-40 years) on a 3T Siemens Prisma scanner with a 32-channel head coil. The study was approved by the Central University (of Oxford) Research Ethics Committee (CUREC, R55787/RE001) in accordance with the regulatory standards of the Code of Ethics of the World Medical Association (Declaration of Helsinki). All participants gave informed consent to their participation and were monetarily compensated for their participation.

High-resolution structural images were acquired using a (MPRAGE) T1w sequence (TR = 1900 ms; TE = 3.97 ms; flip angle = 8°; 192 mm FoV; voxel size 1 mm isotropic) and (SPC) T2w sequence (TR = 3200 ms; TE = 451 ms; 256 mm FoV; voxel size 1 mm isotropic; Grappa factor = 2). Diffusion-weighted MRI (dMRI) data were acquired in the same subjects using a sequence from the UK Biobank Project (Miller et al., 2016). In brief, we used a monopolar Stejskal-Tanner diffusion encoding scheme (Stejskal and Tanner, 1965). Sampling in q -space included 2 shells at $b = 1000$ and 2000 s/mm^2 (voxel size 2 mm, MB = 3). For each shell, 50 distinct diffusion-encoding directions were acquired (covering 100 distinct directions over the two b -values). Five $b = 0$ images were obtained together with additional three $b = 0$ images with the phase-encoding direction reversed.

T1w and T2w scans were preprocessed using the HCP-pipeline (Glasser et al., 2013) cloned from the ‘OxfordStructural’ - fork (<https://github.com/lennartverhagen/Pipelines>). The processing pipeline includes automatic anatomical surface reconstruction using FreeSurfer and provides measures of sulcal depth and surface maps of cortical myelin content (Fischl, 2012; Jenkinson et al., 2012). The mean image of the T1w scans was divided by the mean image of the T2w scans to create a T1w/T2w image. The bias corrected T1w/T2w-ratio was mapped onto the mid-thickness surface using Connectome Workbench command-line tools. We refer to this surface map as T1w/T2w ‘myelin map’ (Glasser and van Essen, 2011; Glasser et al., 2014). In order to create a human average myelin map, the subject’s individual myelin maps were aligned using MSM. The myelin alignment was initialized using alignment based on maps of sulcal depth (a table with MSM parameters is provided in Appendix 1). To create the species average maps, we used an implementation of MSM that optimizes based on a first-order (pairwise) cost function to penalize against distortions, given that no excessive distortions were expected. Human volume data were registered to the Montreal Neurological Institute standard space (MNI-152) and surface data were transformed to a surface template space (fs_LR).

4.3.2. Chimpanzee data and preprocessing

In vivo chimpanzee structural MRI and dMRI data were obtained from the National Chimpanzee Brain Resource (www.chimpanzeebrain.org). Data were acquired at the Yerkes National Primate Research Center (YNPRC) at Emory University through separate studies covered by animal research protocols approved by YNPRC and the Emory University Institutional Animal Care and Use Committee (approval no. YER-2001206). Both structural MRI and dMRI data were collected on a Siemens 3T Trio Scanner (Siemens Medical System, Malvern, PA, USA). These chimpanzee MRI scans were obtained from a data archive of scans

obtained prior to the 2015 implementation of U.S. Fish and Wildlife Service and National Institutes of Health regulations governing research with chimpanzees. All the scans reported in this publication were completed by the end of 2012.

T1w/T2w myelin maps were obtained from a group of 29 adult chimpanzees (all female), scanned at 0.8 mm isotropic resolution (Glasser et al., 2012, 2014; Donahue et al., 2018). dMRI data were obtained in a subset of five individuals. Acquisition and preprocessing was previously described (Li et al., 2013; Mars et al., 2019). Two diffusion-weighted images (TR = 5900 ms; TE = 86 ms; 41 slices; 1.8 mm isotropic resolution) were acquired using a single-shot spin-echo echo planar sequence for each of 60 diffusion directions ($b = 1000 \text{ s/mm}^2$), each with one of the possible left–right phase-encoding directions and 4 repeats, allowing for correction of susceptibility-related distortion. For each repeat of diffusion-weighted images, five images without diffusion weighting ($b = 0 \text{ s/mm}^2$) were also acquired with matching imaging parameters.

Chimpanzee volume and surface data were registered to a standard space template based on 29 chimpanzee scans acquired at the YNPRC (Donahue et al., 2018). A species average myelin map from the 29 chimpanzees was derived using MSM as described for the human.

4.3.3. Macaque data and preprocessing

Ex vivo dMRI data were obtained from four rhesus macaques (one female, age at death: range 4-14 years) using a 7T magnet with Agilent DirectiveTM (Agilent Technologies, Santa Clara, CA, USA). Data acquisition and dMRI preprocessing have been previously described in detail (Eichert et al., 2019b). Data were acquired using a 2D diffusion-weighted spin echo multi-slice

protocol with single line readout (DW-SEMS; TE = 25 ms; TR = 10 s; matrix size: 128 x 128; resolution 0.6 mm; number of slices: 128; slice thickness: 0.6 mm). Nine non-diffusion-weighted ($b = 0$ s/mm²) and 131 diffusion-weighted ($b = 4000$ s/mm²) volumes were acquired with diffusion encoding directions evenly distributed over the whole sphere, except in one monkey where 7 non-diffusion-weighted images and 128 diffusion directions were collected. This protocol and similar ones have previously shown to be sufficient for comparison with *in vivo* human data (D'Arceuil et al., 2007; Dyrby et al., 2011; Eichert et al., 2019b; Mars et al., 2016).

Additionally, *ex vivo* data from one male macaque were obtained (de Crespigny et al., 2005) and preprocessed as described previously (Jbabdi et al., 2013). Relevant imaging parameters for dMRI data were: 4.7T Oxford magnet equipped with BGA 12 gradients; 3D segmented spin-echo EPI 430 μ m isotropic resolution, 8 shots, TE = 33 ms, TR = 350 ms, 120 isotropically distributed diffusion directions, $b = 8000$ s/mm². Despite the different scanning parameters, data quality was appropriate to allow pooling of the *ex vivo* data sets. *In vivo* data from the same macaque subjects were not available.

To obtain macaque T1w/T2w myelin maps, *in vivo* T1w and T2w scans data were obtained from a previous study on five separate rhesus macaques (4 females, age range 3.4 years - 11.75 years). Data acquisition and preprocessing of the macaque data have been described previously (Large et al., 2016). Procedures of the *in vivo* macaque data acquisition were carried out in accordance with Home Office (UK) Regulations and European Union guidelines (EU directive 86/609/EEC; EU Directive 2010/63/EU).

Macaque surface reconstruction and average myelin maps were derived as described for the human. Macaque volume and surface data were registered to a standard space, which is based on data from 19 macaques acquired at YNPRC (Donahue et al., 2016, 2018).

4.3.4. Myelin-based surface registration

Our aim was to derive a cross-species registration to model expansion and relocation of cortical brain areas. Therefore, we performed registration based on average surface myelin maps in the three species using MSM (Ishikawa, 2014; Robinson et al., 2018). MSM is a cortical surface registration algorithm, which works by projecting sheet-like models of the cortical surface to spheres; then aligning these by driving deformation of an input (or moving) mesh until features on the surface (i.e., myelin map intensities) increase in similarity with those represented on a fixed (or reference) mesh. We used a version of MSM that uses higher-order smoothness constraints and strain-based regularization for the regularization mode. MSM can work with multi-dimensional feature maps and use cross correlation across features to drive a registration. Multiple registration steps can be combined by using the output of a previous registration as initialization

The main inputs of MSM were the average species myelin maps (Figure 4.2A[1]). All surface and metric files were resampled to a regular 20k-vertices mesh (radius of the sphere: 10 cm). For all species, the same sphere was used for resampling, so that the vertices had correspondence across species (Figure 4.2A[2]). Given that substantial distortions are required to match myelin landmarks across species, we initialized the myelin-based registration using a region-of-interest-(ROI)-driven registration. Three binary ROIs were manually drawn in Connectome Workbench's `wb_view` onto each species' myelin map (Figure 4.2A[3]). The

value inside the ROI was 1 and outside 0. Two ROIs captured the highly myelinated precentral motor cortex (MC) and MT+ complex and a third ROI covered the medial wall (MW).

The first initialization step aligned a single ROI for MT+ complex between species to facilitate alignment of the remaining two ROIs (Figure 4.2A[5]). Next, the three ROIs were combined in a multi-dimensional file, i.e., in a metric file that contained three data-arrays, or columns (Figure 4.2A[6]). MSM alignment of these sets of ROIs was initialized by the MT+ ROI registration (Figure 4.2A[7]). Initialization was performed using the ‘--trans=X.sphere.reg.surf.gii’-setting in the MSM-command. In the following step, the whole-hemisphere myelin maps were aligned by using the three-ROI registration step as initialization (Figure 4.2A[10]).

To derive a macaque-to-human registration, we resampled the macaque myelin map first to chimpanzee space using the MSM-derived macaque-to-chimpanzee registration. Then we registered the resampled macaque map to the human map while using the chimpanzee-to-human registration as initialization. This approach allowed refinement of the macaque-to-human mapping rather than just applying the chimpanzee-to-human registration to the resampled map.

The surface registration was derived for both hemispheres separately with mirrored versions of the ROIs. For registration steps involving binary ROIs, the registration sphere derived from the left hemisphere was flipped to the right hemisphere. For the steps using myelin maps, we derived the registration separately for both hemispheres. Configuration parameters for all MSM-steps were determined empirically and were kept constant for the two hemispheres (a table with all parameters is provided in Appendix 2).

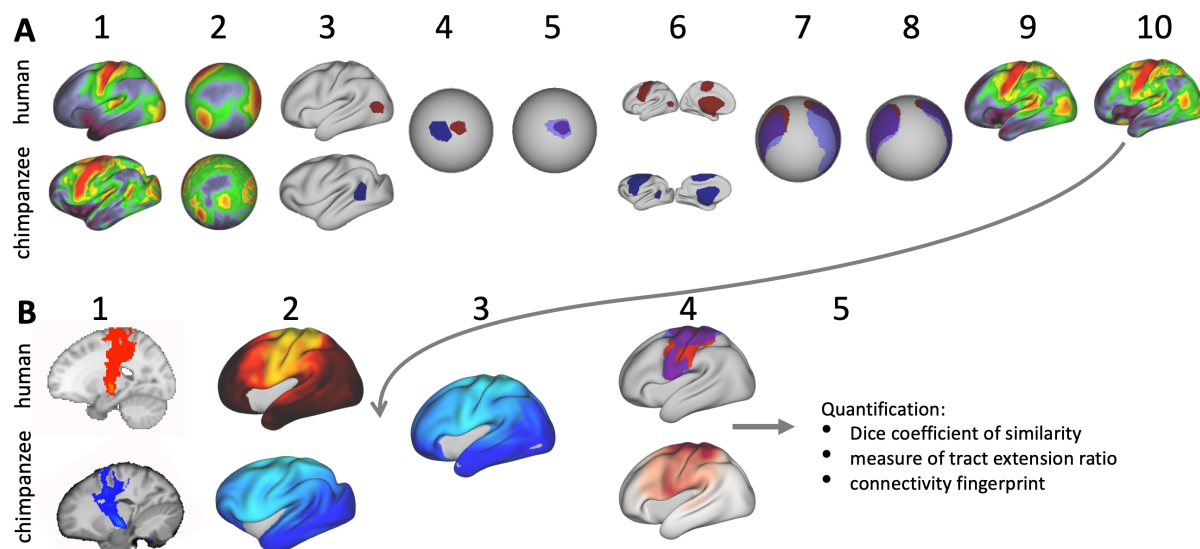


Figure 4.2 Pipeline to derive the myelin-based registration and tract maps.

A: MSM registration between chimpanzee and human myelin maps. [1] Average species myelin maps. [2] Myelin maps displayed on a common sphere. [3] ROI for MT+ complex drawn on the native surface. [4] MT+ ROI displayed on common sphere. [5] Chimpanzee MT+ ROI (*blue*) registered to human (*red*) using MSM. [6] Set of three ROIs (motor cortex, MT+, medial wall). [7] Effect of MT+ ROI-derived initialization on the set of ROIs (only motor cortex and medial wall visible). [8] Registration of the set of chimpanzee ROIs to human ROIs using MSM. [9] Effect of ROI-derived registration on whole hemisphere chimpanzee myelin map. [10] Registration of whole-brain myelin maps initialized by the set of ROIs using MSM.

B: Tract surface analysis. [1] Tractography result for an example tract (CST). [2] Tract map obtained by matrix multiplication method. [3] The myelin-derived cross-species registration (A[10]) is applied to transform the actual chimpanzee tract map to human space. [4] Having both tract maps in the same space allows for a direct species comparison visually in form of overlay maps (*top*) and spatial correlation maps (*bottom*). These comparisons can also be quantified numerically [5].

4.3.5. Spatial correlation of myelin maps and expansion maps

Spatial correlation of myelin maps

The quality of the registration performance was visualized by computing a local spatial correlation between the human myelin map and the result of the chimpanzee and macaque registration. The correlation was computed using a sliding window around every vertex on the sphere using MATLAB's `corrcoef`-function (MathWorks, Natick, MA). We used a search

kernel of 40° that corresponds to a circular search window with a radius of approximately 7 cm.

Expansion maps

To visualize the shifts underlying the three cross-species registrations, we derived a map of surface distortions. We computed the areal distortion between original and distorted mesh, as implemented in ‘wb_command -surface-distortion’. Here, distortions for each mesh face are estimated as $\log_2(A_1/A_0)$, where A_0 is the area of the original mesh face and A_1 is the area of a deformed mesh face. The values per vertex are calculated as a weighted average, where weights are calculated from the relative size of the vertices adjoining mesh faces. We are representing distortions between original and distorted sphere thus the numerical values of the distortion map indicate relative expansion or contraction.

These expansions are underlying the shifts that lead to relocation of cortical areas. A value > 1 indicates a relative increase of the underlying mesh triangles and a value < 1 indicates a relative decrease in size. Note that the overall increase in brain size across species is not accounted for in this calculation so that some areas show a distortion value smaller than 1.

4.3.6. Tractography

Human and chimpanzee dMRI data were preprocessed using tools from FDT (FMRIB's Diffusion Toolbox, part of FSL 5.0 [Smith et al., 2004]). We applied the TOPUP distortion correction tool followed by eddy-current distortion and motion correction (Andersson et al., 2003; Andersson and Sotiropoulos, 2016) as implemented in FSL. Macaque *ex vivo* dMRI data

were processed using tools from FSL as implemented in an in-house MRI Comparative Anatomy Toolbox (Mr Cat, www.neuroecologylab.org).

Preprocessed dMRI images were processed by fitting diffusion tensors (FSL's DTIFIT [Behrens et al., 2003]) and by fitting a model of local fiber orientations including crossing fibers (FSL's BedpostX, Behrens et al., 2007; Jbabdi et al., 2012). Up to three fiber orientations per voxel were allowed. Tractography was performed using FSL's probtrackx2. Registration warp-fields between each subject's native space and standard space were created using FSL's FNIRT (Andersson et al., 2007).

We performed tractography of the following temporal lobe tracts: Middle longitudinal fasciculus (MDLF), inferior longitudinal fasciculus (ILF), the third branch of the superior longitudinal fasciculus (SLF3), the inferior fronto-occipital fasciculus (IFO), and the arcuate fasciculus (AF). To validate our approach for whole-brain analyses, the analyses described below were also performed for cortico-spinal tract (CST) and vertical occipital fasciculus (VOF) and results of the extended set of tracts are shown in separate result figures. Placement of seed, waypoint, and exclusion masks was based on previous studies, in order to reconstruct known pathways for these tracts in all three species (human and macaque: De Groot et al., 2013; Mars et al., 2018c, protocols for AF: Eichert et al., 2019b; chimpanzee: Bryant et al., 2018). Masks were drawn in standard space and warped to native subject dMRI space for probabilistic tractography. The resulting tractograms were normalized by dividing each voxel's value by the total number of streamlines that successfully traced the required route ('waytotal'). To decrease computational load for further processing all tractograms were down-sampled (human: 2 mm, chimpanzee: 1.5 mm, macaque: 1 mm).

4.3.7. Surface tract maps

To assess which part of the cortical grey matter might be reached by the tracts, we derived the surface representation of each individual tractogram using a matrix multiplication method described previously (Mars et al., 2018c, Figure 4.2B[2]). We calculated whole-hemisphere vertex-wise connectivity matrices, tracking from the 20k-vertices mid-thickness surface to all voxels in the brain. These matrices were computed for both hemispheres and each subject individually in the three species. In the macaque we used the five subject's average mid-thickness in standard space as input for the computation instead of individual surfaces.

To rebalance the weights in the tracts to be more homogenous, connectivity values were weighted by the distance between vertex and voxel. A distance matrix across all vertices of the mid-thickness surface and all brain voxels was computed using MATLAB's `pdist2`-function resulting in a matrix of the same size as the connectivity matrix. Each element in the connectivity matrix was then divided by the corresponding value in the vertex-to-voxel distance matrix. To decrease data storage load (approximately 10 GB per matrix) the weighted connectivity matrices of the five subjects were averaged for each hemisphere and species.

To visualize a tract's surface representation, we multiplied the averaged connectivity matrix with a tract's tractogram (FSL's output `'fdt_paths'`). We refer to the tract surface representation here as 'tract map'. The approach described above decreases gyral bias in the resulting tract map notably when compared to surface-based tractography or surface projections of the tractogram. However, the method introduced spurious effects on the medial wall and insular cortex, which are generally not well captured in the tract map. Given that both areas are not of interest in this study, they were masked out for further analysis. Tract maps were derived for

each subject and both hemispheres separately. Individual surface maps were smoothed on the mid-thickness surface (human: 4 mm kernel (sigma for the gaussian kernel function), smoothing on individual surface; chimpanzee: 3 mm kernel, smoothing on average surface; macaque: 2 mm kernel, smoothing on average surface), lognorm-transformed and averaged across subjects.

4.3.8. Predicted tract maps

Next, we tested if our myelin-based registration can be used to predict the tract maps across species. We resampled individual chimpanzee and macaque tract maps to human space using the macaque-to-human and the chimpanzee-to-human registration (Figure 4.2B[3]). Intensity values in actual and predicted tract maps ranged from 0 to 1. We averaged all predicted tract maps and displayed the average map onto a human average surface (Q1-Q6_R440). For visual inspection we also assessed and showed thresholded tract maps. Thresholds were chosen different for each tract, ranging from 0.6 to 0.85, so that the most characteristic termination is visible.

To visualize and quantify the prediction of macaque and chimpanzee tracts in human space, we derived weighted whole-hemisphere local correlation maps of the human tract map and the tract map predicted by macaque or chimpanzee, similar as described above for the correlation of myelin maps. The local correlation map was computed using a sliding window around every vertex on the sphere (diameter 10 cm for all three species) using MATLAB's `corrcoef`-function (MathWorks, Natick, MA). We used a search kernel of 40° that corresponds to a circular search window with a radius of approximately 7 cm. The correlation map was modified to up-weight the brain areas where the tract is represented on the surface. A weighting mask was derived by

multiplication of the intensities in the actual human tract map and the other species' predicted tract map. The values for the weighted correlation map are thus high in parts of the brain where both actual human and predicted tract show a termination, and where the spatial patterns of intensity values correlate. Weighted correlation maps were derived for each pair of 20 human subjects and five subjects of the other species and then averaged.

To quantify how well a tract is predicted, we computed Dice coefficients of similarity (Dice, 1945), which quantifies the amount of overlap of the tract maps. The metric was derived for each pair of 20 human subjects and five subjects of the other species. The Dice coefficient was computed for the binarized and thresholded actual human tract map and the tract map predicted by the other species. The threshold was chosen for each tract individually so that 40 % of surface vertices were covered by the human tract map. The same threshold was applied to the macaque and chimpanzee map. As a quantification of tract extension, we computed the ratio of the number of vertices covered by the thresholded human tract map and the number of vertices covered by both the human and the other tract map. To confirm that the pattern of values is robust, both Dice coefficients and tract extension ratios were computed also for percentages of surface coverage of 20 %, 30 % and 50 %.

The differences in tract extension ratios at a surface coverage of 40 % were assessed in a non-parametric permutation test implemented in PALM (Winkler et al., 2014) using 5000 permutations. We constructed a mixed-effects model matrix using R software (R Core Team and Foundation for Statistical Computing, 2017). As dependent variable we used the measure of tract extension ratio. As independent variable to model fixed effects we used species ('macaque', 'chimpanzee') and tract ('MDLF', 'ILF', 'SLF3', 'IFO', 'AF'). We also modelled the interaction effect of tract and species. As the 100 data points for each tract and species were

derived based on 20 human subjects and five subjects of the other species, we also modelled crossed-random effects for each subject as additional independent variable. The model was fitted separately for both hemispheres. The reported p -values were corrected for family-wise error over multiple contrasts.

4.3.9. Connectivity fingerprints

We characterized the effect of cortical expansion on brain connectivity using the concept of connectivity fingerprints (Passingham et al., 2002). In brain areas where cortical expansion can explain the human connectivity pattern, actual and predicted tract maps will have similar intensity values. In brain areas where the connectivity profile was further modified due to tract extensions, the intensity values of actual and predicted tract maps will show a discrepancy. By computing the intensity values of multiple tract maps in a brain area, we can derive a characteristic profile of values that can be understood as connectivity fingerprint of this area. In brain areas, where tract extension happened in addition to cortical expansion, we expect to observe a difference between actual human connectivity profile and the predicted connectivity profile. We manually selected two representative vertices and derived their actual and predicted connectivity profile: One vertex in the inferior parietal lobe, where we expect the intensity values of actual and predicted tract maps to be similar and one in the middle temporal gyrus, where we expect to find differences in actual and predicted tract maps. The whole set of tracts investigated (CST, MDLF, VOF, IFO, ILF, SLF3 and AF) was included to give a more detailed estimate of the connectivity fingerprint.

4.3.10. Correlation of tract extension with potential confounds

Since our analyses are based on comparing connectivity maps across species, it is important to show that the differences in connectivity profiles are not due to differences in our *ability* to estimate those connections but are instead genuine differences in connectivity. We identified three such sources of confounds: (i) biases in the cortical termination of connections due to differences in gyrification, (ii) noise in the estimate of local fiber orientations due to differences in dMRI acquisition, (iii) differences in the geometry of the white matter tracts affecting the correct estimation of their trajectories.

(i) Surface gyrification has been shown to influence tractography results, as streamlines are typically more likely to be detected on the crown of a gyrus than along its banks when tracking from deep white matter towards the cortex (Jbabdi and Johansen-Berg, 2011; Reveley et al., 2015). We quantified the mean curvature in the surface area covered by the thresholded tract map in all three species. Surface curvature maps were provided by the HCP-pipeline. We then derived the species ratios of curvature for human vs. chimpanzee and for human vs. macaque. Using the extended tract set, we derived a tract-wise correlation between the tract extension ratios (shown below in Figure 4.8) and the curvature ratios.

(ii) Another aspect that affects the performance of the tractography algorithm is the ability to estimate crossing fibers in FSL's bedpostX, which is influenced by differences in the dMRI sequence (Maier-Hein et al., 2017). This can be quantified by looking at the width of the posterior distribution of the orientation parameters, which in FSL is quantified by the dispersion of the fiber orientations (`dyads_dispersion`). To test if differences in acquisition parameters affected the dispersion in a way that biased our results, we performed a similar

analysis as described above for surface curvature. For each tract we masked the dyad dispersion vector images in the ROI covered by the volumetric tractogram thresholded at 30 % and computed the mean value across all three voxel-wise fiber orientations. We then derived a pair-wise species ratio between human vs. chimpanzee (and vs. macaque) and correlated this dispersion ratio with each tract's extension ratio.

(iii) The geometry of the tract itself can affect how well the algorithm can reconstruct the pathway. For example, it may be that tracts going through a narrow funnel are more likely to result in false positives/false negatives due to ambiguities between crossing and kissing fibers (Basser et al., 2000; Jones, 2010; Seunarine and Alexander, 2013). We quantify this aspect of the tracts' geometry by estimating the (cosine transformed) angle between crossing fibers in each voxel:

$$divergence_{i,j} = 1 - \cos(\angle(dyad_i, dyad_j))$$

This divergence measure was averaged across the three pairs of dyads (where i , and j indicate fiber direction) and then averaged within each tract's volumetric extent.

In addition to the factors described above, which quantify our *ability* to estimate the connectivity, we also evaluated if the quality of the myelin-driven registration biased the tract extension ratios. A spatial map of registration error was derived based on the inverse of the spatial correlation maps of actual human myelin and predicted myelin map (the maps shown below in Figure 4.4), i.e., 1 *minus* the intensity in the spatial correlation map. In other words, this measure indicated how badly the predicted myelin map based on surface registration correlated with the actual myelin map. For each tract, we then computed the mean of the error map in the area covered by the tract map and we then determined the tract-wise ratio in registration error between species. For the AF, and the MDLF we performed an additional

vertex-wise analysis on the relation of registration error and species difference. We calculated a correlation of the vertex-wise value in the registration error map and the absolute difference in the intensity of actual human and predicted tract maps. Finally, we assessed if spatial differences in the myelin signal affected the results. This was done by quantifying the mean myelin content in the area covered by the tract maps similar as described above for surface curvature.

4.4. Results

We set out to investigate different types of cortical reorganization affecting the temporal lobe across macaque, chimpanzee, and human brains. First, we investigated cortical relocation of brain areas by registering myelin maps of the cortical surface to one another and derived the local distortions required. Second, we used the resulting mesh deformations to transform maps of cortical projections of major white matter tracts that terminate in temporal and inferior parietal lobe. This allowed us to assess how well the myelin-based registration predicts actual projection maps across species or whether it cannot capture them, providing an index of tract extensions in the human brain.

4.4.1. Myelin-based surface registration

We developed a surface registration between species based on myelin maps using multi-modal surface matching (MSM, Robinson et al., 2018). Figure 4.3 shows the final results of chimpanzee-to-human, macaque-to-chimpanzee, and macaque-to-human brain registrations. The cross-species registration aligns the myelin maps well, with the predicted human maps showing most of the distinctive features of the actual human myelin map (Figure 4.3, top row). Posterior areas such as V1 are well aligned, with the highest myelin evident on the medial part

of the occipital cortex, having relocated quite substantially from a more lateral orientation in the macaque. The prominent myelin hot spot in the location of the MT+ complex is also noticeable. Areas where the myelin maps showed fewer distinctive features to guide the registration, such as in the prefrontal cortex, showed some differentiation between the predicted and actual human maps.

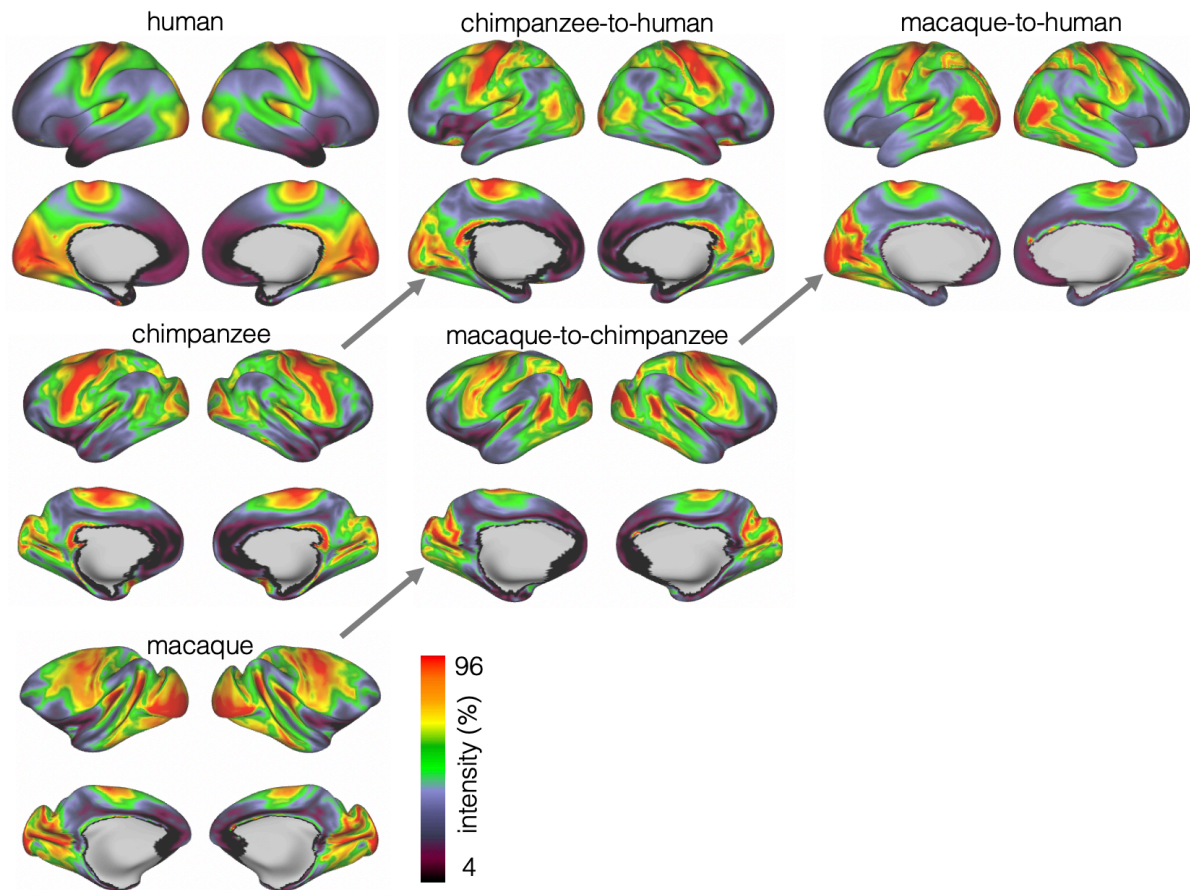


Figure 4.3 Myelin-based surface registration.

Species average myelin maps (*left panel*) and myelin maps resampled across species after applying the MSM-derived registration.

4.4.2. Correlation of myelin maps and expansion maps

The quality of the myelin-based registration was assessed by computing spatial correlation maps of the actual human myelin maps and those predicted for chimpanzee and macaque. The

vast majority of the cortical myelin maps correlates well after applying the registration (Figure 4.4A). The correlation map shows that inferior frontal parts of the cortex and anterior temporal lobe remain most dissimilar. Despite the good alignment of critical posterior landmarks, such as V1 and MT+ complex, the correlation in posterior parietal areas is lower than in more central areas indicating some residual dissimilarity in the registered myelin maps.

Furthermore, we visualized the distortions underlying the myelin-based registration. The macaque-to-chimpanzee distortion map demonstrates that the largest expansion happened in frontal areas (Figure 4.4B). Other areas that show expansion are parietal cortex, posterior temporal and dorsal medial areas. The largest expansion from chimpanzee to human happened in superior temporal and prefrontal cortex, but the distortions are overall smaller and less extended than from macaque to chimpanzee. The macaque-to-human distortion map shows a similar pattern to chimpanzee-to-human, but it indicates a stronger distortion in frontal areas.

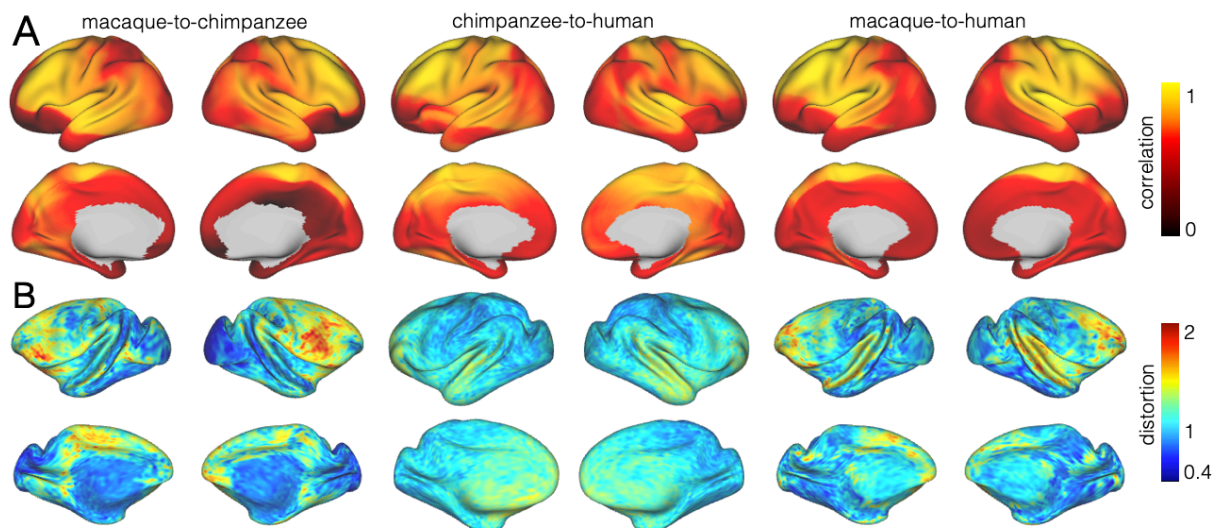


Figure 4.4 Correlation of myelin maps and mesh distortion.

A: Local spatial correlation of myelin maps after applying the MSM-derived registration. **B:** Relative distortion of the mesh underlying the registrations. The values indicate a relative expansion (> 1) or contraction (< 1) of the mesh.

4.4.3. Tract maps for temporal lobe tracts

We constructed the cortical projection maps of the following tracts in all three species: Middle longitudinal fasciculus (MDLF), inferior longitudinal fasciculus (ILF), the third branch of the superior longitudinal fasciculus (SLF3), the inferior fronto-occipital fasciculus (IFO), and the arcuate fasciculus (AF) (Figure 4.5A,C,E). The human and macaque tract maps resemble those obtained in previous studies (Schmahmann and Pandya, 2009; Mars et al., 2018c) and the chimpanzee SLF3 and AF are similar to previous reports (Rilling et al., 2008; Hecht et al., 2015). The other chimpanzee tracts are reported here for the first time, apart from a previous exploratory study (Mars et al., 2019).

We applied the myelin-based surface registration to assess whether the cortical relocation demonstrated in the myelin-based registration above fully explains the changes in tracts. Figure 4.5 shows actual and predicted tract maps. For visual assessment, a thresholded overlay of actual human and predicted tract maps is shown in Figure 4.6A. As described above, we focus on a description of temporo-parietal cortex given the multiple competing theories of its reorganization in different primate lineages. Result figures for the extended set of tracts including CST and VOF are discussed in the section below (chapter 4.4.4).

We assessed the success of the myelin-based registration in predicting the tract projections in a number of ways. First, weighted correlation maps provide a visualization of the local quality of the prediction (Figure 4.6B,C). A high value means that the myelin-based registration alone is sufficient to predict a tract's projection in this part of the brain. A low correlation value indicates that reorganization of a tract's connectivity pattern took place in addition to cortical relocation modelled by the myelin-based registration. Second, the Dice coefficient of similarity

provides a more general measure of similarity between the predicted and actual tract maps, where a Dice coefficient of '1' indicates perfect overlap and thus no tract extension into areas other than would be predicted by cortical relocation assessed using the myelin map registration (Figure 4.6D). Finally, we calculated a 'tract extension ratio' that indicates how much of the actual human tract projections extends into parts of the surface not predicted, where a value of > 1 indicates a tract extension into novel territory (Figure 4.6E). Both the Dice coefficients and tract extension ratios were computed for thresholded tract maps defined by the human tract map covering 40 % of the brain's surface, but the resulting pattern of values is robust across a range of thresholds (Figure 4.8D).

In general, it can be observed that the myelin-based registration can predict the tract maps well in both hemispheres, with the notable exception of AF and to a lesser extent ILF and SLF3 (Figure 4.6). AF in particular shows the lowest Dice coefficient and the highest extension ratio, indicating that this tract's differential projections in the human brain are not merely due to relocation of areas. The maps for macaque and chimpanzee are overall predicted to a similar degree, as can be seen in the overlay and correlation maps, with the exception of AF. The effect for AF is captured in the Dice coefficients and tract extension measures (Figure 4.6D, F). A statistical two-way analysis was performed in both hemispheres to assess the effect of species and tract on the extension ratios. In the left hemisphere, there was no significant main effect of species ($F(1, 179) = 1.38, p = 0.47$), but a highly significant main effect of tract ($F(4, 792) = 565.00, p < 0.001$) and a highly significant interaction effect of species and tract ($F(4, 792) = 207.73, p < 0.001$). In the right hemisphere, we found a significant main effect of species ($F(1, 179) = 16.76, p < 0.001$) as well as a highly significant effect of tract ($F(4, 792) = 261.94, p < 0.001$) and a highly significant interaction effect ($F(4, 792) = 225.70, p < 0.001$). We will discuss the various tracts in more detail below.

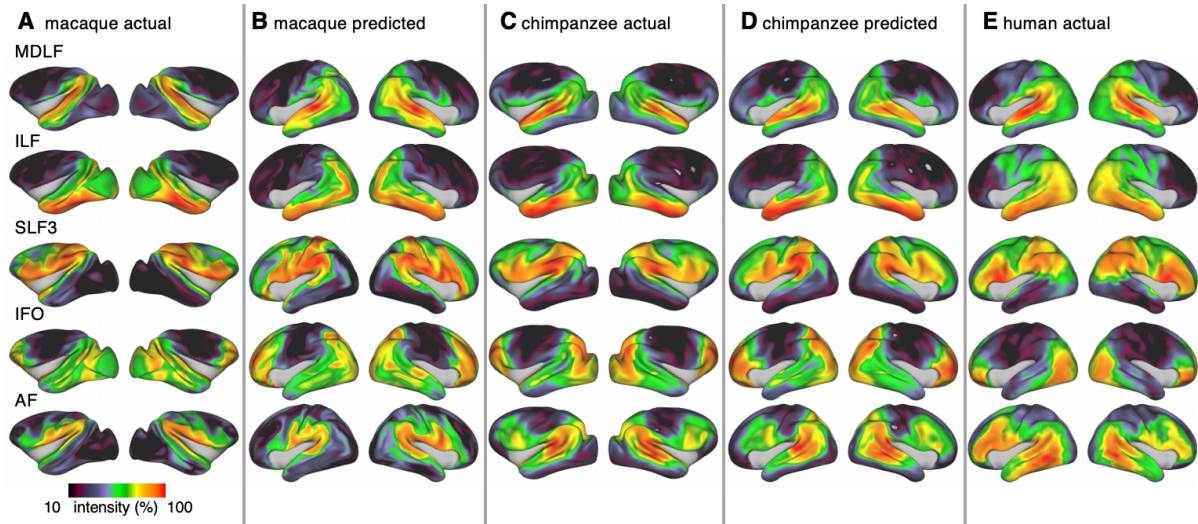
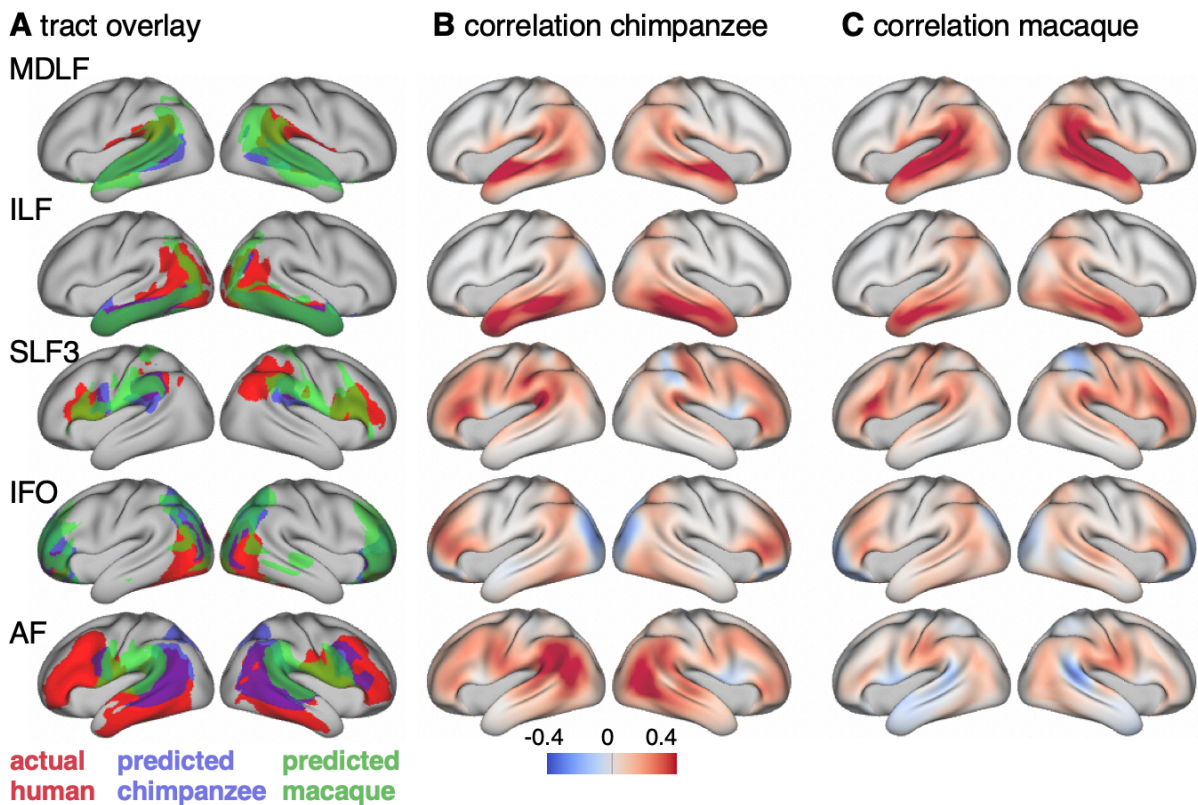


Figure 4.5 Actual and predicted tract maps.

The intensity in the tract maps reflect the probability of a tract's termination on the cortical surface as derived from tractography. Actual tract maps of macaque (**A**), chimpanzee (**C**) and human (**E**). **B** and **D** show the tract maps in human space, predicted by the myelin-based registration for macaque and chimpanzee.



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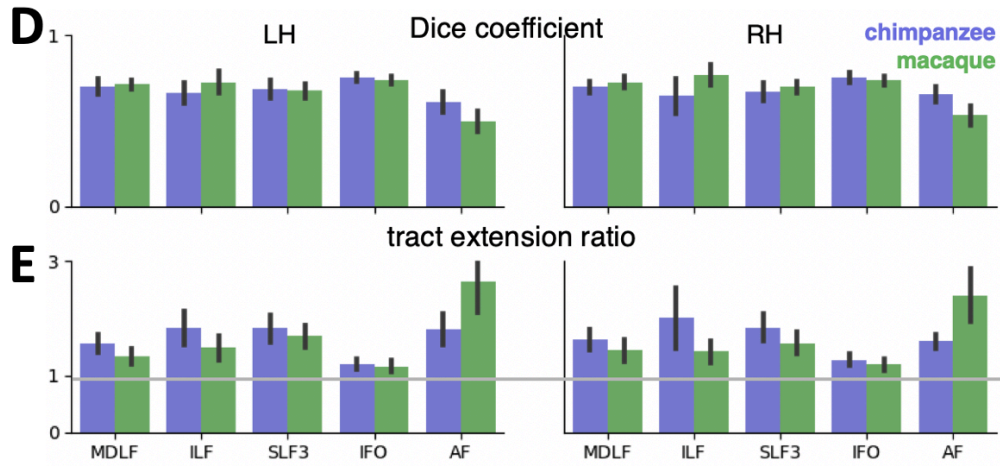


Figure 4.6 Cross-species comparison results.

A: Thresholded actual human tract maps (red) and tract maps predicted by the myelin-based registration for chimpanzee (blue) and macaque (green) (thresholds (t): MDLF: $t = 0.7$, ILF: $t = 0.7$, SLF3: $t = 0.85$, IFO: $t = 0.75$, AF: $t = 0.75$). **B, C:** Weighted correlation maps of actual human map and predicted chimpanzee and macaque map. **D:** Dice coefficients of overlap. **E:** tract extension ratios. Values in D and E were computed from actual thresholded human tract maps and tract maps predicted by the other species. Shown are mean and standard deviation derived from all pairs of human ($n = 20$) and macaque or chimpanzee ($n = 5$) subjects in left (LH) and right (RH) hemisphere.

The myelin-based registration results in good prediction for tract projections in the temporo-parietal cortex. The actual human tract terminations of MDLF span the superior temporal gyrus and reach the inferior parietal cortex (Figure 4.6A). In the macaque and chimpanzee, the actual MDLF terminates in superior temporal gyrus but reaches only to a small part of the inferior parietal cortex. When applying the myelin-based registration, macaque and chimpanzee MDLF are both predicted to reach a comparable portion of the human temporal lobe and parts of both angular and supramarginal gyri of the inferior parietal lobe (Makris et al., 2013). This overlap is captured in the weighted correlation maps, which have high values in the temporal lobe (Figure 4.6B,C). The Dice coefficients for the chimpanzee and macaque MDLF are high and the extension ratio is close to 1 indicating no tract extension in addition to cortical expansion (Figure 4.6D,E)

A similar observation can be made for the posterior terminations of SLF3 and IFO. The myelin-based registration can predict the parietal cortical projections to a large degree. The predicted cortical terminations of the tract show a strong overlap with the actual human tract terminations. In line with the overlay maps and the weighted correlation maps, the Dice coefficients are relatively high. Taken together, this suggests that expansion and relocation of brain areas is largely sufficient to model the posterior cortical terminations of SLF3 and IFO, while extension of the tract's connectivity pattern plays a minor role in explaining the species differences. Predicted ILF terminations show that the expected occipito-temporal connection can be modelled well (Catani and Thiebaut de Schotten, 2008). The extension ratio for ILF is elevated indicating that there is some remaining tract extension that has not been modelled by cortical relocation. This is also reflected in the tract overlay (Figure 4.6A), which shows that human ILF has more extended posterior projections than predicted by the myelin-based registration. Thus, although the overall architecture of the tracts is well predicted, this tract seems to have extended into new cortical territory in the human lineage.

The clearest case of a tract extension in the human brain was presented by AF. Human AF reaches anteriorly to inferior and dorsal frontal areas. The posterior projections of human AF reach into middle and inferior temporal cortex. The chimpanzee posterior terminations are in inferior parietal lobe and superior temporal cortex and in the macaque, the temporal projections reach superior temporal areas. For AF, the myelin-based registration does not provide a good prediction of the tract map across species, especially for the macaque. The correlation map for the chimpanzee shows low correlation along the temporal lobe and for the macaque, correlation values in temporal lobe are extremely low. AF has the lowest Dice coefficient, and the extension ratio is high, especially in the macaque, which is in line with overlay and correlation maps. The 'failure' of the myelin-based registration in the temporal lobe indicates that

extension and relocation of cortical areas is not sufficient to explain the posterior tract projections of AF, but that the tract extended into new cortical territory in the temporal lobe.

4.4.4. Tract maps of extended set of tracts

In addition to studying tracts in temporo-parietal cortex, we also assessed if the terminations of two further white matter tracts can be predicted by the myelin-based registration. We tracked cortico-spinal tract (CST) and vertical occipital fasciculus (VOF), which both terminate in areas of the cortex that are characteristically high in myelin (Dum and Strick, 1991; Schmahmann and Pandya, 2009; Glasser et al., 2014; Takemura et al., 2017). We included these tracts to demonstrate that the registration captures major relocations of highly myelinated areas across the whole cortex. Our proposed framework is thus not restricted to studying temporal lobe architecture, but it can be applied to comparative questions across the whole brain.

In the case of CST, the actual tract projections in the three species show terminations in pre- and post-central gyrus (Figure 4.7C,E). For the chimpanzee and macaque, these terminations span a relatively large portion of the frontal cortex, while in the human the anterior part of the frontal lobe does not show any tract terminations. The frontal difference in the three actual tract maps is related to the relative increase in human prefrontal cortex, which is low in myelin content. After applying the myelin-based registration to the macaque and chimpanzee tract maps of CST, the predicted terminations in human space show strong overlap with the actual human tract map. The myelin-based registration thus can model the effect of an increased prefrontal cortex on CST tract terminations. This overlap is captured in the weighted correlation maps, which have a high value in areas surrounding the central gyrus (Figure 4.8A).

The Dice coefficients for chimpanzee and macaque CST are relatively high (Figure 4.8D). The extension ratio is close to 1 in the left hemisphere and slightly elevated for the macaque right hemisphere. The posterior terminations of VOF are also predicted well by the myelin-based registration. The predicted cortical terminations of the tracts show a strong overlap with the actual human tract terminations. The termination zones of VOF are found in occipital lobe in all three species. Primary visual cortex has moved from the lateral surface in the macaque to the medial surface in the human, which is modelled well in our myelin-based registration. When applying the registration, macaque and chimpanzee VOF is predicted to reach posterior and medial parts of the occipital lobe, overlapping well with the actual human tract map. Dice coefficients for VOF are high in both species and the extension ratio close to 1. This result suggests that expansion and relocation of brain areas is largely sufficient to model the posterior cortical terminations of VOF, similar as for MDLF, IFO, ILF and SLF3.

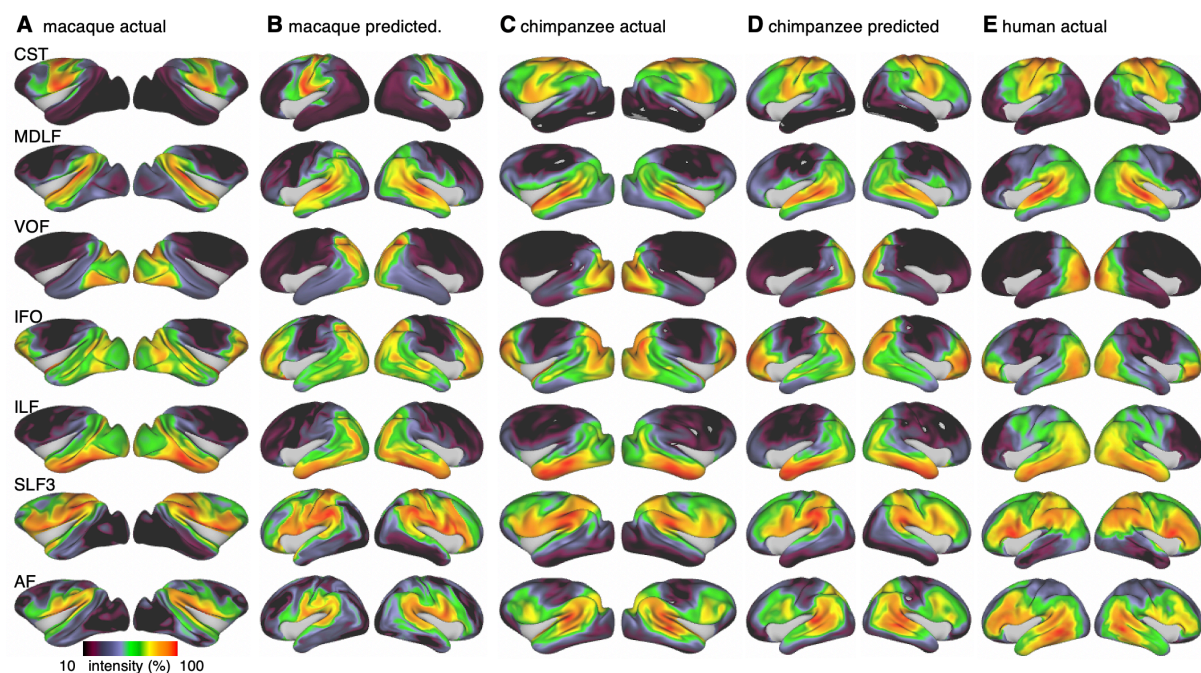
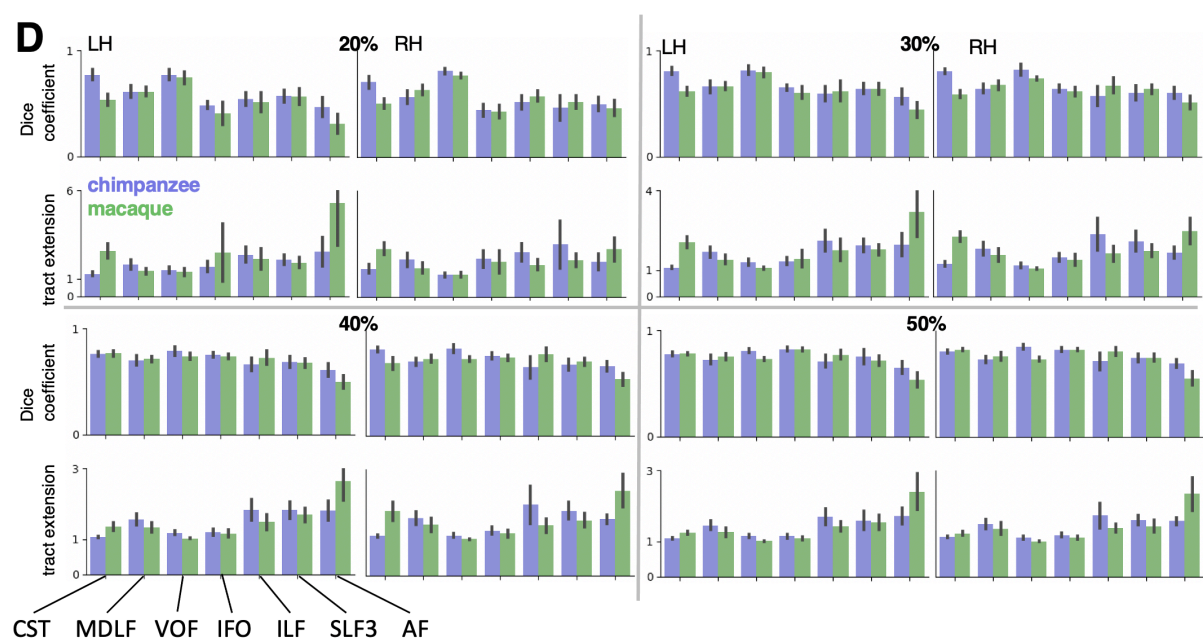
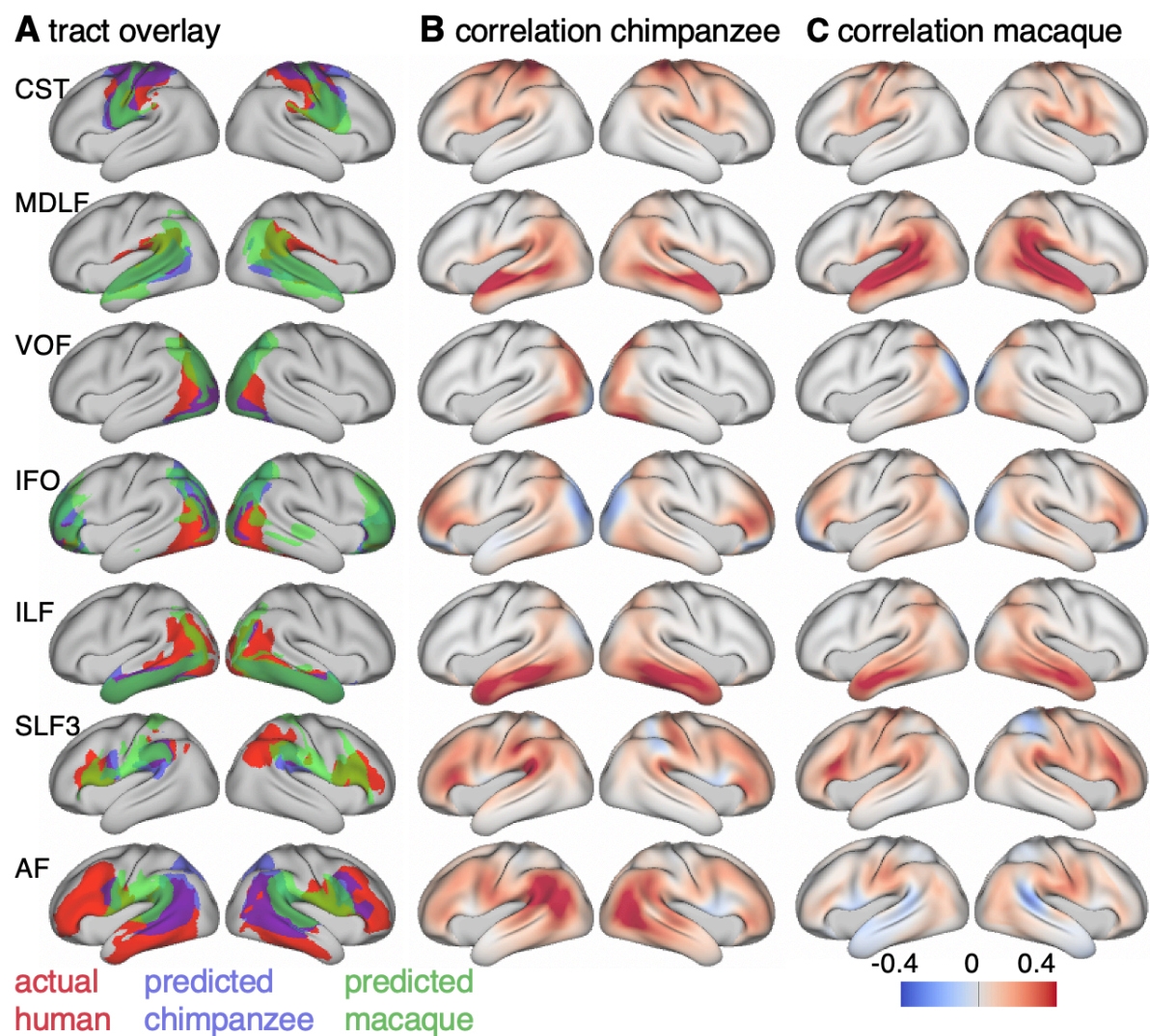


Figure 4.7 Tract maps of extended set of tracts.

Actual tract maps of macaque (A), chimpanzee (C) and human (E). B and D show the transformed tract maps in human space, predicted by the myelin-based registration for macaque and chimpanzee.



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Figure 4.8 Species comparison of extended set of tract maps.

A: Thresholded actual human tract maps (*red*) and tract maps predicted by the myelin-based registration for chimpanzee (*blue*) and macaque (*green*) (thresholds (*t*): CST: $t = 0.6$, MDLF: $t = 0.7$, VOF: $t = 0.6$, IFO: $t = 0.75$, ILF: $t = 0.7$, SLF3: $t = 0.85$, AF: $t = 0.75$). **B, C:** Weighted correlation maps of actual human map and predicted chimpanzee and macaque map. **D:** The values are shown for four different percentages of surface coverage (20%, 30%, 40%, 50%). Shown are mean $\pm$ 95 % confidence interval derived from all pairs of human ($n = 20$) and macaque or chimpanzee ($n = 5$) subjects in left (LH) and right (RH) hemisphere. The low dice coefficient and high extension ratio for AF in macaque and – to a lesser degree – in chimpanzee, is present for all percentages of surface coverage.

4.4.5. Connectivity fingerprints

To further characterize the effects in the predicted tract maps, we obtained connectivity fingerprints at two representative vertices on the left brain surface: One in inferior parietal lobe, where most tracts are predicted well and one in the middle temporal gyrus, where we observed strong species differences, in particular for AF. The connectivity fingerprints were derived based on the intensity values in the actual and predicted tract maps using an extended set of seven tracts to give a more detailed picture, i.e. the tracts shown in Figure 4.7. Figure 4.9 demonstrates that in the inferior parietal lobe, the connectivity fingerprint of actual human and predicted chimpanzee and macaque tract maps are highly similar, except for a small increase in the intensity for ILF indicating tract extension as discussed above. This indicates that the myelin-derived registration can predict this area's connectivity profile well, despite the local expansion of the cortical sheet. For the temporal vertex, however, there is a strong mismatch regarding the connectivity profile, in particular regarding the intensities for AF. This indicates that the connectional fingerprint of this temporal area is different in the human than would be predicted purely based on cortical relocation. Thus, the connectivity fingerprints of the two representative areas match the pattern of species differences that emerged from the results above.

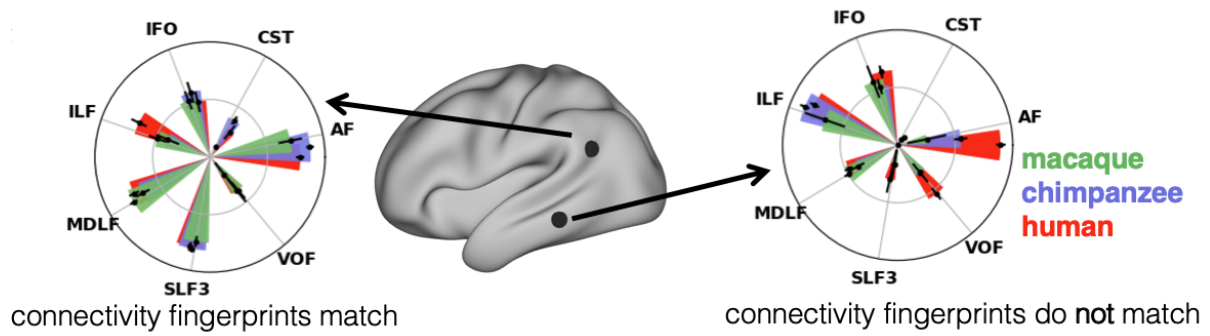


Figure 4.9 Connectivity fingerprints.

Connectivity fingerprints at two example vertices in inferior parietal and temporal lobe derived from the intensity values of an extended set of tract maps. Shown are mean and standard deviation (human: $n = 20$, chimpanzee and macaque: $n = 5$).

4.4.6. Correlation of tract extension with potential confounds

The tract-wise correlation analyses show that there is no linear relationship between any of the potential confounds and tract extension ($r < 0.2$, ns) (Figure 4.10A). It can also be observed that AF predicted by the macaque, which has the strongest tract extension ratio, is not an outlier within the distribution of tracts for any of the measures that we assessed. An exception is the ‘complexity’ measure, where AF is found at the lower end of the distribution. This indicates merely that AF differs in the complexity measure compared to the other tracts, but this does not indicate that the effect in tract extension is driven by tract complexity. Other tracts that have a high extension ratio (e.g., chimpanzee ILF in the right hemisphere) are found at the other end of the distribution.

In addition to the tract-wise analyses, we also performed a vertex-wise analysis on the whole-brain tract map of AF (Figure 4.10B). A correlation between registration error and absolute species difference in tract map intensities (actual human *minus* predicted) shows that there is no linear relationship for both hemispheres and both species ($r < 0.22$, $p < 0.001$). For visual

comparison, we plotted the 2D joint distribution of registration error and species difference for AF, where we observed the strongest tract expansion, and for MDLF, where we observed only small tract expansion (Figure 4.10B,C). The joint distribution plots of the two tracts show a similar pattern, which demonstrates that species differences in AF cannot be attributed to an unusual bias due to registration error. In sum, these control measure show that our observed effects of tract extension cannot be explained by potentially confounding factors, such as gyral bias and registration error.

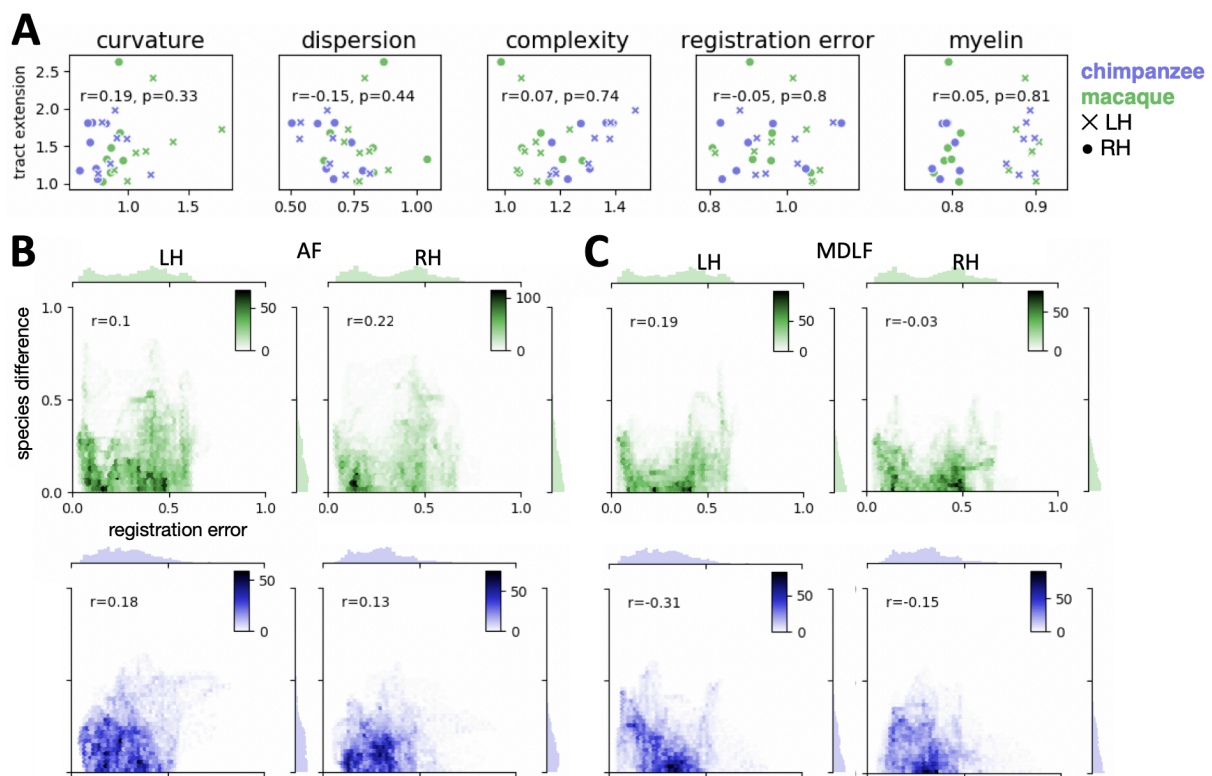


Figure 4.10 Analyses for potential confounds.

A: Tract-wise correlation of tract extension ratio and potentially confounding factors ($n = 28$). Labels for tracts are not provided for readability but can be inferred from Figure 4.8D. For details refer to the text. **B, C:** Whole-brain vertex-wise correlation of absolute species difference in actual and predicted tract map (B: AF; C: MDLF) and the registration error map (i.e., 1 minus the myelin correlation map shown in Figure 4.4A). *Marginal histograms:* distribution of vertices across 50 regular bins, *inset scale:* number of vertices within the bins of the joint distribution scatterplot (colour mapping as in A, $n = 20252$ vertices, $p < 0.001$ for all correlations in B and C).

4.5. Discussion

The goal of this study was to study brain specializations of the temporal lobe across multiple primate species in the context of two forms of cortical reorganization: cortical relocation due to local expansions and extensions of tracts into new cortical territory (Figure 4.1). For this reason, we developed a cross-species surface registration method based on cortical myelin content, which gives us an index of how cortical areas have relocated during evolution. In a subsequent step, we tested if cortical relocation can predict the connectivity patterns of a set of tracts across species. We showed that cortical expansion and resulting relocation of brain areas alone provide a good prediction of several tracts' terminations in posterior temporal and parietal cortex. In the case of AF in particular, we showed that an additional change in brain architecture was extension of the tract into new cortical areas independent of cortical expansion and resulting relocation.

As pointed out in the introduction, different lines of evidence on temporal reorganization across primate species have emphasized either the expansion and subsequent relocation of brain areas or changes in temporal lobe connectivity. Both approaches are valuable, and we here demonstrate that both are applicable to different parts of the temporal lobe. Previous work has suggested expansion and relocation of areas in posterior temporal cortex and adjacent parietal cortex (Mars et al., 2013; Patel et al., 2019), which is consistent with the ventral location of MT+ complex in humans compared to other primates. Similar reorganizations have been suggested by Haak and colleagues (2018), who proposed further major relocations of the myelin-rich primary visual cortex in the human. These major relocations were captured by our myelin-based registration and demonstrated to predict certain features of white matter tracts, such as the posterior projections of MDLF.

The aim of our research is not to find the ‘best’ species registration, but to shed light on brain evolution by studying where registrations based on different modalities disagree. All aspects of brain architecture can be modified during the course evolution and each individual aspect would provide us with a unique registration. The present study represents only one example of how cortical specializations can be studied by comparing cross-species registrations of different modalities. Testing the effect of a myelin-based registration on connectivity is not meant to imply pre-eminence of myelin over connectivity. In fact, the reversed approach, i.e., to derive a registration based on individual tract maps and then test their differential effect on transformed myelin maps, would be highly informative. We chose myelin here as the primary feature, because we wanted to test the specific hypothesis that some tracts expanded while others simply followed relocation of areas across the cortex. Thus, we used a measure that could index the relocation (myelin) and test it on our measure of interest (the tracts).

Separate from cortical relocation, changes in connectivity between humans and other species have been described for a variety of association tracts, including the temporal projections of AF into middle temporal gyrus (Rilling et al., 2008; Eichert et al., 2019b), the frontal projections of SLF3 (Hecht et al., 2015), and expansion of the ventral route consisting of MDLF and IFO (Makris et al., 2009, 2013; Forkel et al., 2014). Both of these latter tracts have been suggested to play a role in language functions in the human brain (Saur et al., 2008; Makris and Pandya, 2009; Makris et al., 2009; Catani and Bambini, 2014; Hagoort, 2016).

In the case of MDLF, the human tract projections can be predicted well by the cortical relocation model. This indicates that the pattern of cortical terminations changed according to the general expansion and relocation of brain areas, without additional extension into new regions of the brain. For other tracts reaching to temporo-parietal areas, such as ILF, IFO and

SLF3, the posterior tract projections can also be modelled well, despite the large distortions of target areas within the cortical sheet. These tracts seem to follow the evolutionary scenario described in the upper row in Figure 4.1B,D, where a tract's extension in the human brain can be explained by relocation of areas along the cortical sheet. This does not necessarily mean that the tracts have not been recruited for new functions, but the type of change is different from that of tracts such as AF.

AF showed the lowest consistency across species when applying the myelin-based registration. Dice ratio and extension ratio reflect the increased tract termination especially into the temporal lobes, which can be observed in the tract maps. This points to further evolutionary adaptations that specifically affected the connectivity pattern of AF independent of cortical relocation, the scenario described in the bottom figure in Figure 4.1B,D. Our result is consistent with previous accounts in the literature (Rilling et al., 2008; Ardesch et al., 2019; Eichert et al., 2019b), but the approach described here enabled us to formally test this hypothesis in the wider context of cortical reorganization across three primate species and to quantify the species differences. Importantly, extension of a tract into new cortical territories alters the unique connectivity fingerprint of the innervated areas, which profoundly changes the computational capabilities that area supports (Mars et al., 2018b). Being able to dissociate different modifications of brain architecture can inform us about how temporal lobe specializations link to uniquely human higher cognition (Roelofs, 2014; Schomers et al., 2017; Qi et al., 2019).

Apart from modifications of AF, we also noted some minor extensions of ILF into temporo-parietal cortex. ILF's extension in the human brain is consistent with reports that showed a split of this tract into multiple subtracts due to the expansion of parts of the temporal cortex, including the fusiform gyrus (Latini et al., 2017; Roumazeilles et al., 2020). This extensions

could be related to the increase of cortical territory related to processing social information, such as social networks and faces (Sallet et al., 2011; Noonan et al., 2018). Previously, it has been shown that parietal SLF3 projections are most prominent in the human brain, which has been linked to our unique capacity of social learning (Hecht et al., 2013). We show that species differences in the posterior projections of SLF3 can be mostly explained by local expansion of the posterior temporal and parietal cortex. Similarly, we show that cortical expansion can model the terminations of MDLF, a tract which has been linked to visuospatial and integrative audio-visual function (Makris et al., 2013). Our results thus suggest that SLF3 and MDLF didn't undergo additional evolutionary modifications that affected their posterior terminations.

The MSM framework we adopted is ideally suited to work with multi-modal descriptors of the cerebral cortex. It has become a vital tool for human surface registration (Abdollahi et al., 2014; Glasser et al., 2016; Garcia et al., 2018) and here we demonstrated its utility for cross-species research. With the presented surface matching method, we showed that a registration based on T1w/T2w MRI data can match critical landmarks across species. We have referred to these maps as 'myelin maps' in accordance with other studies in the literature (Glasser et al., 2014; Large et al., 2016) but it should be noted that this is a heuristic. T1w/T2w maps are sensitive to other features than myelin and other sequences are sensitive to aspects of cortical myelin (Lutti et al., 2014). The crucial point is that the maps we employed here are similar across species, allowing us to compare like with like (Glasser et al. 2014). Projecting data of different modalities to a surface representation is a useful tool for comparative neuroscience. It allows us to visualize the different modalities within the same cortical sheet and to compare topologies on this 2D surface, which opens a wide array of mathematical tools. A similar approach has been taken to investigate the relationship between gene expression and myelin content of the

cortex (Burt et al., 2018) and gradients of change across multiple modalities of brain organization (Huntenburg et al., 2018; Blazquez Freches et al., 2020).

The presented approach can be flexibly modified to include a variety of cortical features, which can be compared across species. Myelin does not provide high contrast in the large human frontal cortex and, as such, it is difficult to provide a good registration in frontal areas. Furthermore, the effects we report can only be reliably interpreted within the spatial resolution of brain areas. More fine scale species differences and homology assignments are not possible with the data shown here. However, the current method can be generalized to any modality of cortical organization, so future studies can incorporate modalities that have greater contrast in this part of the brain such as neurite orientation dispersion and density imaging (NODDI) measures (Zhang et al., 2012) and resting state fMRI networks (Vincent et al., 2007).

In sum, here we present a framework for analysing structural reorganization of the temporo-parietal cortex across different primate brains. We dissociated cortical relocation of areas due to local expansion and modifications of white matter tract connectivity. Future work will expand this approach not only to different modalities, but also to a much wider range of species, which is now becoming increasingly possible due to the availability of multi-species datasets (Milham et al., 2018; Heuer et al., 2019). This provides a crucial step towards the understanding of phylogenetic diversity across the primate brain.

5

General Discussions

5.1. Summary of Findings

The overarching aim of this thesis was to further our understanding of the brain specialisations underlying human vocal motor control. To do this, I used a multi-modal neuroimaging approach including functional MRI during task, quantitative MRI and comparative neuroanatomy across the primate lineage. These methods allowed me to characterize human laryngeal motor cortex and to study the evolutionary principles governing changes in brain organization.

5.1.1. Mapping human laryngeal motor cortex during vocalization

First, I conducted an fMRI study to map the cortical areas involved in human laryngeal motor control during vocalization. I employed a novel task paradigm using a factorial design to isolate laryngeal activity from articulation and to control for breathing, a confound that many previous studies had not accounted for. Furthermore, I mapped the cortical representations of the right hand, the lips and the tongue, and basic vowel production using separate tasks. I found that vocalization evokes brain activity in a dorsal and in a ventral region within the central somatomotor region. There was a consistent somatotopic arrangement of cortical representations in individuals running dorsal-to-ventral: hand – larynx (dorsal) – lips – tongue – larynx (ventral).

Using structural and quantitative MRI, I characterized the microstructure underlying these activations in individuals. I found that cortex underlying the dorsal larynx representation is similar to that underlying the primary motor representation of the hand, lip and tongue. The microstructural profile underlying the ventral larynx representation was different as indexed by lower myelin content and lower cortical thickness. I concluded that the dorsal larynx

representation is located in primary motor cortex, while the ventral larynx area is not. I speculated that the ventral larynx representation is located in premotor cortex, as in other primates. This study is the first to characterize the cortical microstructure underlying the larynx representations and I provided novel evidence to understand the evolution of laryngeal motor cortex.

5.1.2. Morphological and functional variability in central and subcentral motor cortex

Next, I investigated the sulcal morphology underlying these brain activations to probe the relationship between structural and functional variability. I used a manual labelling approach to identify five segments of the central sulcus and the subcentral sulci in individual hemispheres. My assessment revealed a complex sulcal morphology in the subcentral region and high inter-individual variability. I found that the anterior subcentral sulcus (*ascs*) typically consists of two distinct segments, a lateral and an opercular one, as opposed to being one continuous sulcus. Based on the position of the lateral segment of the anterior subcentral sulcus (*ascs_lat*), I classified individual hemispheres into five morphological types.

Furthermore, I demonstrated a tight and consistent link between sulcal segments and the location of fMRI peaks in individuals. The ventral larynx representation is located in the subcentral region and often co-localized with *ascs_lat*. I concluded that the high morphological variability in the subcentral region might have constituted a confound in previous functional localizers of the larynx contributing to controversies surrounding the ventral larynx representation. This study demonstrated that individualized mapping of structure and function is critical for understanding the functional neuroanatomy of the human brain.

5.1.3. Cross-species cortical alignment identifies different types of anatomical reorganization in the primate temporal lobe

Lastly, I used cross-species cortical alignment to study neuroanatomical reorganization within the primate lineage. I wanted to test if species differences in one modality (e.g. connectivity) changed together or independent with another feature (e.g. local expansions of the cortical sheet). To model relocation and expansion of the cerebral cortex, I implemented a surface-based registration that matches myelin landmarks in macaques, chimpanzees and humans. Then, I showed that this registration predicts the terminations of several white matter tracts across species. This indicates that cortical relocation and expansion are sufficient to explain the species differences observed in these tracts.

For the arcuate fasciculus (AF), however, I found that the myelin-based registration is not sufficient to explain the tract terminations in the temporal lobe. After applying the registration, the predicted terminations of the chimpanzee AF did not extend as far in the temporal lobe as for the human and even less so for the macaque. This finding suggests that the AF underwent additional evolutionary modifications that affected its termination zones in the temporal lobe. The registration framework that I developed can be flexibly adapted to integrate multiple descriptors of the cortex and multiple species, which allows us to test evolutionary theories of cortical reorganization.

5.2. Considerations and Future Work

5.2.1. Two larynx representations in the primate brain?

Chapter 2 revealed distinct dorsal and ventral larynx representations within the human central somatomotor region using fMRI. It is noteworthy that the larynx is the only effector, where basic movement activates two distinct loci in the central somatomotor region. Movement of other effectors, such as the hand, can also activate multiple loci in primary motor cortex, but these are represented in close proximity and typically represent subdivisions at a finer scale (Kolasinski et al., 2016; Schellekens et al., 2018). The observed duplication may thus, indeed, represent a unique functional specialization of the human larynx, but such a striking peculiarity also calls for further validations and examinations. Our finding that only the dorsal larynx representation is located in primary motor cortex, and not the ventral one, provides an important piece of evidence to inform evolutionary theories and to understand the functional implications of both larynx representations.

Our results are in line with a previous account of the motor system suggesting that the central somatotopic motor map extends outside of the primary motor cortex into premotor cortex (Woolsey et al., 1952; Graziano et al., 2002). Within this somatotopic map, a single muscle or effector can be represented multiple times. These representations activate depending on the behavioural context, the complexity of the movement and the timing of the movement sequence (Preuss et al., 1996; Rizzolatti and Luppino, 2001; Raos et al., 2003; Kambi et al., 2011; Baldwin et al., 2018). Primary motor cortex likely emerged after premotor and primary sensory cortex during the evolution of mammals (Kaas, 2011). Activations in primary motor cortex are thought to specifically support highly skilled movements and complex actions that require the coordination of multiple muscles (Georgopoulos et al., 1988; Kakei et al., 1999; Chouinard

and Paus, 2006; Kaas, 2012). This role is mediated by the direct cortico-spinal or cortico-bulbar projections. Premotor cortex, however, is involved in spatial guidance of movements, action selection and planning (Hoshi and Tanji, 2000; Kakei et al., 2001; Ohbayashi et al., 2003; Cisek and Kalaska, 2005) and less so in the integration of multiple muscles to form complex actions. The differential roles of premotor and primary motor cortex, however, are a matter of debate and comparative functional mapping of the speech motor domain in primates is rather limited, when compared to the large body of literature on the manual domain (Arbib, 2006).

I suggest the following account would allow us to reconcile findings from the human and the non-human primate literature as follows: Each effector is in principle represented multiple times within one unified map in the premotor and primary motor cortex of primates (Baldwin et al., 2018). However, the extent to which these representations activate during movement execution depends on the effector and the behavioural context, which naturally differs across species (Rizzolatti and Luppino, 2001). Movement of human articulators is most strongly correlated with activity located in primary motor cortex, while premotor activations are sub-threshold and appear less focal at the group level (Chang et al., 2009). Movement of the larynx, however, correlates with activity in both the premotor and the primary motor representation to a comparable level, which is generally low (note, that in several subjects larynx activations in chapter 2 failed to reach the significance level corrected for whole-brain comparisons). These differences among effectors are related to the need for fine control and movement repertoire, which is, after all, much lower for the larynx, compared with the articulators (Lameira, 2017b). The proximity of the dorsal larynx representation to lip and hand representations might be behaviourally relevant in the context of complex communicative actions in human language (Graziano et al., 2002; Fitch, 2011).

In the non-human primate brain, larynx movement mainly correlates with activity located in premotor cortex. A separate focal representation in primary motor cortex, which supports integration of multiple muscles necessary to produce a vocalization, is either underdeveloped or has been failed to be detected so far. In fact, Hines (1940) observed that electrical stimulation of the cortex dorsal to the tongue representation elicited vocalization in one single chimpanzee; a finding which received little attention in the literature so far and has not been replicated. Recently, a macaque fMRI study indicated that a dorsal central region is related to perception of self-generated auditory sequences. This activation could represent a non-human primate homologue of the dorsal larynx representation (Archakov et al., 2020).

My hypothesis is speculative but it is in line with established principles of brain evolution, rather than assuming an entirely new neural phenotype (Belyk and Brown, 2017). The account described above suggests a functional dissociation between the two larynx representations that can be directly tested in the human brain, as described in the section below.

5.2.2. Functional implications of the two larynx representations

In chapter 2 we discussed the hypothesis that the dorsal larynx representation facilitates pitch modulations and fine muscular coordination, which is essential during human speech production. The ventral larynx area, however, might support basic abduction and adduction of the vocal folds required to produce a vocalization. Strong empirical evidence for this functional hypothesis comes from a recent ECoG study showing that neural firing in the dorsal larynx area encodes pitch levels and that the micro-stimulation magnitude correlates with laryngeal muscle activity (Dichter et al., 2018). An fMRI study in macaques showed activation in the mid-portion of the anterior bank of the central sulcus related to the perception of self-generated

melodies, indicating that this functional dissociation might be valid throughout the primate lineage (Archakov et al., 2020). In contrast, however, previous human fMRI studies consistently failed to find an effect of pitch variations in the motor cortex (Peck et al., 2009; Kryshchuk et al., 2017; Belyk et al., 2018a). To study the functional roles of the human larynx representations in more depth, we will therefore require a methodology that is safe to apply to large study cohorts and that allows us to infer causal relationships, rather than purely correlational ones as in the case of fMRI.

Non-invasive brain stimulation, for example transcranial magnetic stimulation (TMS), is highly suitable for this challenge. Using brain stimulation, we can interfere with ongoing brain activity to assess the effects of stimulation on behavioural variables. The somatotopic relationships that we described in the speech motor system (chapter 2) could facilitate the functional localization of the stimulation areas, without requiring an fMRI localizer scan. The motor hot-spots for articulators, such as lip and tongue, can be identified in individuals using motor evoked potentials and the larynx areas could then be localized relative to these landmarks. TMS could be used in a ‘virtual lesion approach’ to test the contribution of the dorsal and ventral larynx representation to different aspects of speech production and perception (Chen et al., 1997; Lévêque et al., 2013; Finkel et al., 2019). TMS could also be used to test the precise timings of their functional involvement in vocal production and planning, when using an on-line paradigm where stimulation happens at different time points relative to vocalization onset (D’Ausilio et al., 2011).

In addition to examining the functional contributions of the two larynx areas, it is still unclear, to which extent the multiple representations of the larynx, and of other effectors, are reflected throughout the broader motor network. The brain contains several motor regions, where the

effector representations are typically arranged following a somatotopic patterning (Penfield and Boldrey, 1937; Glickstein et al., 2011; Nambu, 2011; Amiez and Petrides, 2014). Our study focussed on the central somatomotor region, but we provided initial evidence for two distinct larynx representation in the cerebellum. We interpreted these results cautiously, however, given that the MRI scanning sequence was not optimized for imaging the cerebellum. For the supplementary motor area we only found a single activation related to larynx activity. Future studies should systematically assess whether each motor region contains two separate representations of the larynx and if the duplication is maintained in the supporting white-matter network.

5.2.3. Understanding the cortical patterning of the brain

The study described in chapter 2 was the first to characterize the cortical microstructure underlying the larynx activations. We exploited recent advances in MRI sequence development that make such a quantitative approach to neuroanatomy on an individual subject level possible (Weiskopf et al., 2013; Lerch et al., 2017). Increasingly rich detail about anatomical descriptors is becoming available, which can be related to microscale cortical organization (Seidlitz et al., 2018). Despite these continuous technical advances, however, the spatial resolution of MRI is not likely to reach the micron resolution required for histological investigations.

Recent research initiatives, however, make it possible to link MRI-based measures directly to microscopic anatomical details. The BigBrain project, for example, is a three-dimensional ultra-high resolution reconstruction of a post-mortem human brain that allows for microstructural and gold-standard histological investigation (Amunts et al., 2013; Paquola et al., 2019). The derived histological atlases have been co-registered with the standard MNI

stereotaxic space, which allows bridging the insights at macro- and microscopic levels (Paquola et al., 2020; Wagstyl et al., 2020). Combining our functional dataset with such a resource, would allow us to understand the microstructural properties of the cortex underlying the task activations in much greater detail. It would be possible to approximate the cytoarchitectonic area underlying the ventral larynx area and to clarify if it is located in premotor cortex (BA6), in secondary sensory cortex (BA40) or in BA43.

This histological approach could be used further to investigate the relevance of the sulcal segments that we identified in the central and subcentral region. Based on the results from chapter 3 one might speculate that the lateral segment of the ascending subcentral sulcus demarcates the boundary of a cortical area. In a similar fashion, a range of studies reported relationships between cortical folding and the functional architecture of the brain (Amiez et al., 2006; Mangin et al., 2015; Sun et al., 2016). These accounts, however, are not without criticism and others argue that the link between morphological details and functional territories outside of primary brain areas is highly questionable (Brodmann, 1909; Zilles et al., 1997). By employing a multi-modal approach combining functional MRI data, sulcal labelling and histological information, we will be able to re-visit the role of cortical folds for brain mapping and to understand the implications of differential sulcal patterning.

When a similar whole-brain histological resources become available for the macaque brain, this will have great implications for comparative neuroscience (Howard et al., 2019). It will be finally possible to determine whether the ventral larynx representation in humans is located in a homologous cortical area compared to that identified in macaque monkeys. Furthermore, such a research programme could enable us to identify which sulci are homologous, i.e. conserved, between humans and macaques. This would allow us to integrate cortical folding

as modality for a cross-species registration framework (Leprince et al., 2020) to understand neocortical evolution and the phylogenetic relationships in more depth (Heuer et al., 2019).

5.2.4. Structural connectivity to study language evolution

The results of chapter 4 suggest that the arcuate fasciculus (AF) underwent critical changes during primate evolution and that it extends into new cortical territory in the human temporal lobe. While our study was not the first one to demonstrate the extension of the arcuate fasciculus (Rilling et al., 2008, 2012; Ardesch et al., 2019), we employed a multi-modal neuroimaging approach to dissociate different principles of cortical reorganization. Importantly, we used a set of seven homologous white matter tracts to demonstrate that the effect of tract extension is specific to the AF.

We did not link the species differences in the AF to functional brain activations, to directly probe the functional implications for language function. While the role of the human arcuate fasciculus in language and speech is long established (Friederici and Gierhan, 2013; Hagoort, 2019), it is unclear what role the homologous pathway plays in non-human primates. A recent comparative study combined tractography and fMRI across species to study the phylogenetic origin of the AF (Balezeau et al., 2020). The authors showed that the non-human primate AF connects to auditory fields in the superior temporal lobe suggesting that it represents an auditory prototype for the more extended and left-lateralized human AF.

Empirical studies that characterize the structural network supporting the laryngeal motor system are currently scarce (Simonyan et al., 2009; Kumar et al., 2016; Neef et al., 2020). Key insights stem from tract-tracing studies in non-human primates and from ablation-degeneration

studies in humans (Kuypers, 1958a, 1958c; Simonyan and Jürgens, 2003a, 2005) - both techniques with limited scalability and reproducibility due to technical challenges. MRI-based multi-modal neuroimaging and in particular diffusion-based tractography therefore provides a highly powerful tool to study the laryngeal motor network in a phylogenetic context (Mars et al., 2018a; Thiebaut de Schotten et al., 2019). One priority for future comparative studies on the language system will be to provide a model that accounts both for an expansion of the dorsal language stream and for species differences in the laryngeal motor network. Recent data sharing efforts for human and non-human primate datasets that feature both high-resolution structural and functional data will allow us build up our understanding of how adaptive changes in structural neuroanatomy enabled human language and speech (Milham et al., 2018; Thiebaut de Schotten et al., 2019; Autio et al., 2020).

5.2.5. A future framework to study speech evolution

This thesis successfully demonstrated how we can advance our understanding of language evolution by leveraging recent developments from systems-level neuroimaging and from comparative primate anatomy. We can take on board several critical lessons from my studies to inform future research.

The fMRI study in chapter 2 highlighted the importance of well-controlled human functional experiments in particular for the speech system. Special care needs to be taken when mapping speech effectors in a comparable way to other effectors. It is essential to study all effectors in a unified framework to work out the governing principles of the motor system and to link this to the behavioural relevance of muscle activity. Eventually, will then be able to infer, how the

speech motor system as a whole has specialized in the brain and how this relates to the needs of our ecological niche.

Furthermore, I demonstrated how we can employ multi-modal and quantitative neuroimaging to understand the structural basis of brain function. The merits of this approach extend far from human brain mapping to evolutionary questions, such as the one of language emergence. Neuroimaging is currently the only non-invasive method that allows us to study both human and non-human primate brains comparatively using the same technique (Friedrich et al., 2020). Comparable structural and quantitative maps, provide a data-driven way to establish homologous mappings across species (Glasser et al., 2014). Linking multivariate descriptors of brain anatomy to functional activation maps will help us to identify the critical species differences that support human speech.

In chapter 3, I demonstrated the importance of an individualized analysis, in contrast to prevailing approaches that average at the group level. A nuanced investigation of spatial anatomical patterns, such as sulcal segments, and functional patterns during rest and task will help us to identify systematic architectural clues that are generalizable across humans and to characterize their variability. Leveraging large-scale non-human primate MRI databases will allow us to perform comparable analyses across the primate lineage.

In chapter 4, I showed how we can link up our multi-modal and multi-species analyses in a comparative framework that allows us to dissociate different scenarios of brain reorganization. We applied this concept to study how changes in two structural descriptors relate to each other, namely structural connectivity and areal expansion. The framework is by no means limited to structural indices and can be readily applied to functional activation maps. Such a research plan

is ideally suited to study the evolutionary principles underlying a system as complex as language, where many different types of evolutionary change interacted across the brain.

5.3. Conclusions

The studies in this thesis addressed the brain specializations underlying human vocal control using multi-modal neuroimaging and comparative primate anatomy. By combining functional and quantitative MRI in humans, chapter 2 provided a neuroanatomical and functional characterization of human laryngeal motor cortex. The morphological study in chapter 3 is the first systematic description of the sulcal patterning in the subcentral region. I demonstrated the importance for a detailed assessment of both anatomy and function on an individual subject level. Finally, in chapter 4 I developed a cross-species registration framework that can account for both shared and divergent features within the primate lineage.

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Appendices

Appendix 1: MSM configuration parameters for individual myelin maps

MSM configuration parameters for the registration of individual subject myelin maps prior to averaging to create a species myelin map. The parameters were kept constant for the three species and for both hemispheres.

sulcal depth	myelin map
--simval=1,2,2,2 --sigma_in=4,4,2,1 --sigma_ref=2,2,1,1 --lambda=0,0.1,0.2,0.3 --it=50,3,3,3 --opt=AFFINE,DISCRETE,DISCRETE, DISCRETE --CPgrid=0,2,3,4 --SGgrid=0,4,5,6 --datagrid=4,4,5,6 --IN levels=4	--simval=2,2,2 --sigma_in=10,5,3 --sigma_ref=10,5,3 --lambda=0.1,0.1,0.3 --it=10,10,10 --opt=DISCRETE,DISCRETE,DISCRETE --CPgrid=2,3,4 --SGgrid=4,5,6 --datagrid=4,5,6 levels=3 (--trans: sulcal depth registration)

Appendix 2: MSM configuration parameters for cross-species registration

Settings for the MSM command for registrations using the macaque data as input (left panel) and chimpanzee data as input (right panel). The described ‘step’ refers to the numbering in chapter 4, Figure 4.2. Parameters were identical for the left and right hemisphere.

macaque mesh as input	chimpanzee mesh as input
step: 4→5 macaque-to-chimpanzee: MT+ ROI --sigma_in=25 --lambda=0.1 --it=10 --opt=DISCRETE --CPgrid=1 --SGgrid=3 --regoption=3 --regexp=2 --dopt=HO�CR --VN --triclique --k_exponent=2 --bulkmod=1 --shearmod=0.2 levels=1	step: 4→5 chimpanzee-to-human: MT+ ROI --sigma_in=20,15,5 --lambda=0.05,0.1,0.1 --it=10,10,10 --opt=DISCRETE,DISCRETE,DISCRETE --CPgrid=2,3,4 --SGgrid=4,5,6 --regoption=3 --regexp=2 --dopt=HO�CR --VN --triclique --k_exponent=2 --bulkmod=1 --shearmod=0.2 levels=3
step: 7→8 macaque-to-chimpanzee: three ROIs --sigma_in=25,15,5 --lambda=0.01,0.01,0.1 --it=10,10,10 --opt=DISCRETE,DISCRETE,DISCRETE --CPgrid=1,2,3 --SGgrid=3,4,5 --regoption=3 --regexp=2 --dopt=HO�CR --VN --triclique --k_exponent=2 --bulkmod=1 --shearmod=0.1 levels=3 (--trans: single ROI registration from step above)	step: 7→8 chimpanzee-to-human: three ROIs --sigma_in=25 --lambda=0.001 --it=10 --opt=DISCRETE --CPgrid=2 --SGgrid=4 --regoption=3 --regexp=2 --dopt=HO�CR --VN --triclique --k_exponent=2 --bulkmod=1 --shearmod=0.2 levels=1 (--trans: single ROI registration from step above)

step: 9→10 macaque-to-chimpanzee: myelin map <pre>--sigma_in=10,5,3 --lambda=0.1,0.1,0.1 --it=10,10,20 --opt=DISCRETE,DISCRETE,DISCRETE --CPgrid=2,3,4 --SGgrid=4,5,6 --IN --excl --regoption=3 --regexp=2 --dopt=HO�R --triclique --k_exponent=2 --bulkmod=1 --shearmod=0.2</pre> levels=3 (--trans: three ROI registration)	step: 9→10 chimpanzee-to-human: myelin map <pre>--sigma_in=25,10,5 --lambda=0.2,0.5,0.5 --it=10,10,10 --opt=DISCRETE,DISCRETE,DISCRETE --CPgrid=2,3,4 --SGgrid=4,5,6 --regoption=3 --regexp=2 --dopt=HO�R --triclique --k_exponent=2 --bulkmod=1.6 --shearmod=0.1</pre> levels=3 (--trans: three ROI registration)
step: 9→10 macaque-to-human (input: macaque myelin map transformed by applying the macaque-to-chimpanzee registration) <pre>--sigma_in=25,10,5 --sigma_ref=25,10,5 --lambda=0.1,0.1,0.1 --it=10,10,3 --opt=DISCRETE,DISCRETE,DISCRETE --CPgrid=2,3,4 --SGgrid=4,5,6 --datagrid=4,5,6 --IN --regoption=3 --regexp=2 --dopt=HO�R --triclique --k_exponent=2 --bulkmod=1.6 --shearmod=0.1</pre> levels=3 (--trans: chimpanzee-to-human myelin map registration)	