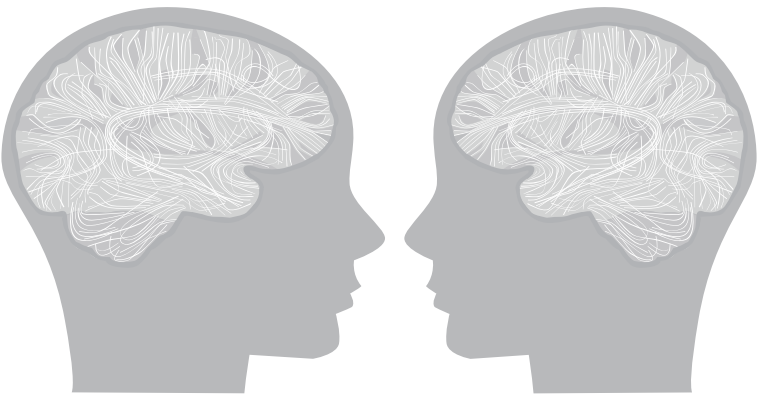
MICROSTRUCTURES OF LEARNING

Novel methods and approaches for assessing structural and functional changes underlying knowledge acquisition in the brain

May 23, 2014, Piratensalen, Grand Hotel, Lund, Sweden



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Organized by
The Pufendorf Institute for Advanced Studies and the *HuMeNS**-
interdisciplinary group at Lund University
with support from the Birgit Rausing Language Program, Lund University

*Merle Horne (Coordinator), Magnus Lindgren, Markus Nilsson, Mikael Roll,
Yury Shtyrov, Freddy Ståhlberg & Daniel Topgaard

Symposium proceedings

Microstructures of Learning

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Edited by
Merle Horne (editor in chief), Magnus Lindgren, Markus Nilsson,
Mikael Roll, Yury Shtyrov, Freddy Ståhlberg, Daniel Topgaard

ISBN: 978-2-88919-480-3
doi: 10.3389/978-2-88919-480-3

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*HuMeNS**-Advanced Study Group on the neuroscience of knowledge acquisition

***Humanities, Medicine, Natural Science, Social Science**
The *HuMeNS* Advanced Study Group has been formed as a result of the members' mutual interest in carrying out interdisciplinary studies on the microstructure of human cognition, in particular studies on changes in the brain's cellular structure during language acquisition. The research program involves collaboration by researchers at four different faculties at Lund University: Humanities, Medicine, Natural Science, Social Science, represented by the fields of linguistics, medical radiation physics, physical chemistry, and psychology, respectively. It is an outgrowth of a number of fruitful cross-disciplinary collaborations involving the Humanities and Medicine (*HuMe*) initiative, Lund University Bioimaging Center (LBIC), the Chemical Center, the Humanities Laboratory, as well as active international collaborative efforts at all departments involved. These environments have created a unique opportunity for neurolinguists and neuropsychologists to join forces with researchers in physical chemistry, medical physics and diagnostic radiation physics and pose research questions about neurocognition that were unapproachable even a few years ago.

The present symposium Microstructures of Learning: Novel Methods and Approaches for Assessing Structural and Functional Changes Underlying Knowledge Acquisition in the Brain was a major event during the *HuMeNS*'s Advanced Study Group period at the Pufendorf Institute, Lund University during 2013–2014.

The *HuMeNS*-group:

Merle Horne, Dept. of Linguistics, Center for Languages and Literature, Lund University

Magnus Lindgren, Dept. of Psychology, Lund University

Markus Nilsson, Lund University Bioimaging Center

Mikael Roll, Dept. of linguistics, Center for Languages and Literature, Lund University

Yury Shtyrov, Center for Functionally Integrative Neuroscience, Aarhus University

Freddy Ståhlberg, Lund University BioImaging Center

Daniel Topgaard, Div. of Physical Chemistry, Dept. of Chemistry, Lund University

The Pufendorf Institute for Advanced Studies

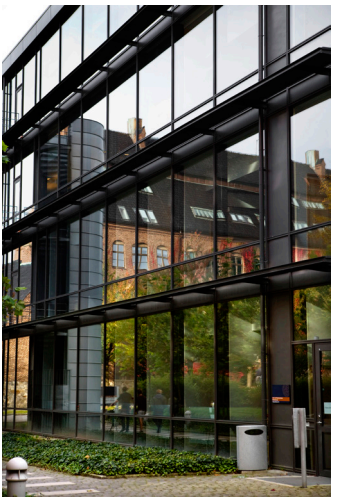
The Pufendorf Institute for Advanced Studies is a cross-disciplinary research institute, established in December 2008 by professor Göran Bexell, vice-chancellor of Lund University at that time. Professor Sture Forsén was appointed as acting head and Professor Sune Sunesson as director of the institute.

The Pufendorf Instutute aims to “promote collaboration within Lund University and to constitute an open and creative interdisciplinary environment where researchers can meet in free and open discussion”.

The Pufendorf Institute for Advanced Studies, Lund University

Pre-symposium program

Presentations by Lund University researchers on language processing and MRI methods, May 22, 2014



Faculty club, Center for Languages and Literature, Lund University

14.15–15.30:

Merle Horne: overview of HuMeNS interdisciplinary project on language learning

Sabine Gosselke: current ERP study on language learning

Mikael Roll: current EEG-fMRI study on Swedish tone processing

Pelle Söderström: planned EEG-fMRI studies on speech processing

15.30–15.45: Coffee break

15.45–17.30:

Markus Nilsson: Overview of work by the diffusion group at LBIC

Filip Szczepankiewicz: Measurements of microanisotropy using qMAS

Björn Lampinen: Measuring exchange using dPFG. Possibilities and challenges

Daniel Svärd: Effects of tissue lesions on DTI-measurements

Danielle van Westen: Confounding variables in comparisons with older control material

Jimmy Lätt: Overview of on-going studies, protocols and analyses

Symposium program (Symposium abstracts can be found on the Frontiers Event site: http://www.frontiersin.org/events/Microstructures_of_Learning_Novel_methods_and_approaches_for_assessing_structural_and_functional_ch/2304/abstracts)

9.15–9.45: Registration/Coffee
9.45–10.00: Welcoming remarks **(Merle Horne, Freddy Ståhlberg)**

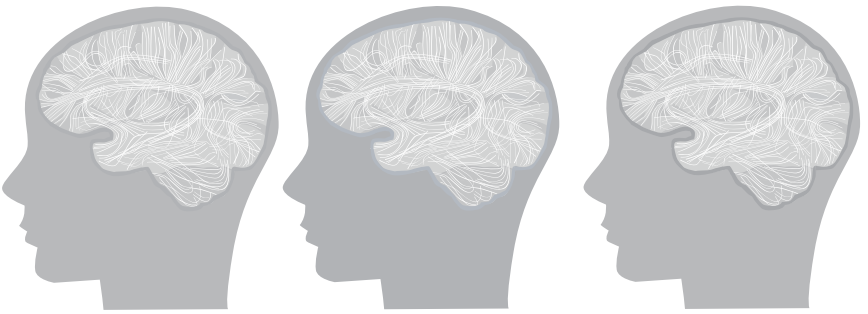
Session 1 (Chair: Yury Shtyrov)
10.00–11.00: **Ruth de Diego Balaguer** (Invited speaker): Cognition and Brain Plasticity Group, University of Barcelona, **"Brain structural and functional differences associated to language learning abilities"**
11.00–11.30: **Johan Mårtensson**: Dept. of Psychology, Lund University, **"Proficiency and brain structure during intense language learning"**
11.30–12.00: **Mikael Roll**: Dept. of Linguistics, SOL-Center, Lund University, **"ERP—exploring the temporal microstructure of cognitive functions in the brain"**
12.00–13.00: Lunch

Session 2 (Chair: Markus Nilsson)
13.00–14.00: **Derek K Jones** (Invited speaker): School of Psychology, Cardiff University, **"Tractometry and the search for the missing link"**
14.00–14.30: **Daniel Topgaard**: Dept. of Chemistry, Lund University, **"Multidimensional diffusion MRI: From colloid science to learning studies"**
14.30–15.00: Coffee

Session 3 (Chair: Magnus Lindgren)
15.00–16.00: **Teija Kujala** (Invited speaker): Cicero Learning, University of Helsinki, and Institute of Behavorial Science, University of Helsinki, **"Plasticity of early neural language processes"**
16.00–16.30: **Yury Shtyrov**: CFIN, Aarhus University, **"Electrophysiological and haemodynamic biomarkers of rapid acquisition of novel wordforms"**
16.30–17.00: **Markus Nilsson**: Lund University BioImaging Center, **"Quantification of diffusional anisotropy in regions of complex tissue microstructure using non-conventional diffusion MRI"**
17.00–17.15: BREAK

Session 4 (Chair: Daniel Topgaard)
17.15–18.15: **Yaniv Assaf** (Invited speaker): Dept. of Neurobiology, Tel Aviv University, **"New insights into neuroplasticity from micro-structural MRI"**
18.15–18.45: Discussion and closing remarks **(Freddy Ståhlberg, Merle Horne)**

Extended abstracts



Microstructures of Learning

Novel methods and approaches for assessing structural and functional changes underlying knowledge acquisition in the brain

Dorsal and ventral pathways in relation to language learning abilities

Ruth de Diego-Balaguer

ICREA, University of Barcelona, Barcelona, Spain; ruth.dediego@ub.edu

SUMMARY

Language-related areas within left frontal, parietal and temporal cortices are organized in dorsal and ventral segregated but highly interactive streams. Studying individual differences in functional and structural connectivity between those brain regions and how they change during language learning can clarify the function of each of these specific connections in learning dysfunction and inter-individual variability. While the dorsal stream has been related to articulation and production, the ventral stream has been associated with comprehension and semantic processing. To understand their role in the earliest stages of language learning, we have used artificial languages to study the acquisition of word forms from fluent speech without the influence of semantic information. The evidence commented on here indicates that the direct functional and structural connectivity between left frontal and temporal structures is relevant for audio-motor integration and critical for the acquisition of new word forms. Indeed, interference with this audio-motor component required for working memory maintenance of the phonological form disrupts language learning. Within the dorsal network as well, other studies highlight the importance of attention orienting associated with the left fronto-parietal network in the extraction of the embedded rules of words. Regarding the ventral network, the data indicate the relevance of the ventral connection between left frontal and temporal areas as a supporting pathway in the early acquisition process even when no semantic information is available.

Keywords: dorsal and ventral language pathways, word learning, audio-motor integration

INTRODUCTION

Speech is a complex auditory stimulation. Listeners confronted with this type of stimulation need to accomplish a series of steps in order to learn a new language from it and before word forms can be associated with specific meanings. Learners need to segment and locate word boundaries, create memory traces of the segmented words and extract the rule dependencies embedded in the words as well as between them.

Language processing is ensured by a network of premotor and inferiofrontal areas delineated as Broca's territory and posterior superior and middle temporal areas labeled as Wernicke's territory. Direct connections between these language-related areas in the left frontal and temporal cortices are sustained dorsally through the arcuate fasciculus and ventrally by paths running through the extreme capsule (Saur et al., 2008; Brauer et al., 2011, 2013). The dorsal connection is also assured indirectly through the parietal lobe (Geschwind territory) with shorter segments (Catani et al., 2005): an anterior segment connecting Broca's and Geschwind territories, and a posterior segment connection between Geschwind and Wernicke's territories (Figure 1). Despite their close

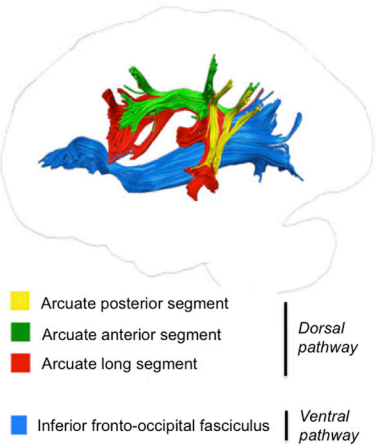


Figure 1: Reconstruction of the white matter tracts constituting the left dorsal and ventral pathways.

interaction, a division of labor has been proposed for dorsal and ventral streams. The dorsal stream sustains language articulation and production, mapping sounds onto articulatory-based representations. The ventral stream sustains language comprehension, mapping sound onto meaning (Hickok and Poeppel, 2007; Saur et al., 2008; Rauschecker and Scott, 2009).

Lesions in each of these segments produce different aphasic syndromes (Catani and Ffytche, 2005). In a similar way, each of these segments may have differential functions during the early stages of language learning.

AIM AND APPROACH

In a series of studies, we have therefore aimed to tease apart the roles of the different connections in the learning process. In addition, we have been interested in determining whether the ventral pathway is engaged during the learning process even in the earliest stages when no semantic information is available.

The approach adopted in these studies has been to use a simplified artificial language composed of nine trisyllabic word forms (e.g., *tupiro*, *tumaro*, *tufero*) with a set of 3 different embedded rules. All three rules consisted of a systematic dependency (e.g., *tu_ro*) such that the first syllable determined the last syllable of the word (as in *untreatable*, *unbearable*, *unsuitable*). This paradigm allowed us to manipulate the learning cues available in speech to study using the same material, word segmentation, memorization of the phonological word forms and rule generalization of the dependencies embedded in the words. Diffusion tensor images (DTI) and brain activations throughout the short continuous exposure to the language were measured. Participants passively listened to the artificial language knowing that after a few minutes of exposure they would be tested for their recognition/discrimination of the words in the artificial language. Importantly, the word forms had no semantic information associated with them. This approach allowed: (i) studying individual differences in functional and structural connectivity between different parts of the network to relate these with performance; (ii) studying the cognitive functions associated with the individual differences and therefore understanding the function of each of these networks in language learning.

DORSAL AND VENTRAL NETWORKS IN LANGUAGE LEARNING

In order to identify the different networks that are engaged during word learning from continuous speech, we performed an Independent Component Analysis on the pattern of brain activations throughout the learning process (Lopez-Barroso et al., 2015). Independent component analysis allows one to separate brain activity into a set of spatially independent networks with particular time courses. This analysis pinpointed a set of three dorsal networks: an Auditory-Premotor network, a Sensory-Motor network and a Fronto-Parietal network engaged when exposed to the new language. In addition, a ventral Fronto-Temporal network was also engaged. An analysis of the level of engagement of these networks through exposure was also performed. This analysis showed the dorsal Auditory-Premotor network (involving Broca's and Wernicke's regions) to be engaged throughout the whole language exposure period. Moreover, the degree of engagement of this latter network in the first two blocks was directly correlated with the word learning performance achieved after exposure to speech.

The specific importance of this left dorsal audio-motor network is reinforced by the results of the study of individual differences in the structural connectivity between the different regions involving the dorsal network. We extracted measures of structural connectivity related to microstructural properties of fiber organization of each of the dorsal direct and indirect segments and the ventral connection (i.e., fronto-occipital fasciculus). We observed that only the increased structural connectivity between Broca's and Wernicke's territories (reflected in decreased radial diffusivity) was related to greater word learning performance. Increased functional connectivity between these same regions was also the only structural connectivity showing a correlation with learning (López-Barroso et al., 2013).

In both studies, the direct connection between premotor and posterior superior temporal regions relates to individual differences in the acquisition of word forms. This relation appeared both as regards structural and functional connectivity. Because the connection between those regions is essential for audio-motor integration, i.e., the coupling between auditory and articulatory information, the results indicate the importance of auditory-motor integration in word learning from speech input. Indeed, articulatory rehearsal, a function that requires audio-motor integration, is necessary to maintain phonological information in working memory. Blocking its use with articulatory suppression (i.e., continuously uttering a nonsense syllable while exposed to the language) interferes both with word and rule learning (López-Barroso et al., 2011). Interestingly, during this interference, blocking the use of the dorsal network, word and rule learning are then related to the structural connectivity of the ventral pathway. This result highlights the relevance of the ventral pathway, even when semantic information is not available, as an alternative effective connection between frontal and temporal areas when the dorsal pathway is not available.

These different studies support the assumption of a critical role played by the left dorsal stream in word learning. Rule learning also appears to rely on this dorsal stream. Ongoing work is attempting to disentangle the specific contribution of the anterior and posterior connections involving the parietal lobe in word and rule learning and its underlying function. On the one hand, while the search for word boundaries needed for segmentation engages the inferior frontal gyrus, the parietal lobe seems to be more active when words are already segmented and their phonological forms can be maintained and their memory traces strengthened (Lopez-Barroso et al., in preparation). The role of the dorsal stream as a whole taking on the roles of the direct and indirect connections would be consistent with a template matching explanation (Warren et al., 2005). The ongoing auditory stimulation in the temporal lobe would be matched to the word candidate during learning. This word candidate would be handled in working memory through the engagement of the parietal lobe and maintained through the direct fronto-temporal connection allowing audio-motor integration and rehearsal. On the other hand, rule learning (De Diego-Balaguer et al., 2007, 2011) and the detection of prosodic cues predicting grammatical dependencies (Roll and Horne, 2011) has been reported to trigger attentional mechanisms. In language comprehension, focusing of attention on perceptual cues such as prosody to facilitate sentence comprehension has been associated with parietal activations overlapping with those of the attention orientating network (Kristensen et al., 2012). Therefore, indirect connections through the parietal lobe may also be relevant for different aspects of word and rule learning.

CONCLUSION

Different dorsal and ventral networks seem to be orchestrated during language learning. Within the dorsal pathway, the contribution of direct and indirect segments can be functionally dissociated. The ability to learn new words relies on an efficient and rapid communication between temporal and frontal areas through the direct long segment connection of the arcuate fasciculus. Its function appears to sustain the audio-motor interface necessary for articulatory rehearsal in working memory. Preliminary data indicate that the indirect segment involving the parietal lobe seems to have a greater role after word segmentation as well as for rule acquisition. Blocking the use of the dorsal network interferes with language learning. The ventral network can partially compensate for the dorsal interference and therefore seems to play an important role even when no semantic information is available. This result is consistent with developmental data (Brauer et al., 2011) and reports of recovery after lesions in the dorsal pathway (Yeatman and Feldman, 2013).

ACKNOWLEDGMENTS

This work has been supported by Grants from the MINECO PSI2011-23624 and ERC StG 313841 TuningLang.

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Plasticity of early neural language processes

Teija Kujala

Cicero Learning, University of Helsinki and Cognitive Brain Research Unit, Institute of Behavioural Sciences, University of Helsinki, Finland; teija.m.kujala@helsinki.fi

SUMMARY

We speak and understand speech with a remarkable speed, while we are concurrently able to think: we plan what we say next and analyze what we hear during a conversation. This is likely to be based on automatic neural speech processes which free capacity for these complex functions. There is ample neuroscientific evidence for such automatic analysis of speech. Speech memory traces, acquired for the native language in early childhood, are activated irrespective of whether or not we attend to the speech input. These memory traces are malleable. New memory representations emerge even after childhood through foreign language learning. The development of speech and sound memory traces can be influenced even before birth. It was recently shown that prenatal exposure to novel sound elements in word-like stimuli results in memory representations for these stimuli, which can be detected after birth by recording neural responses. Furthermore, it was found that memory traces formed before birth may persist for several months after birth. Accurate speech–sound traces are pertinent also for fluent reading. Their weakness and poor connections to print were proposed to be one of the primary causes of developmental dyslexia. This theory is supported by results showing that training including auditory/speech or audiovisual stimuli improves reading skills and facilitates neural processes in dyslexia. Evidently, the accuracy of automatically operating speech memory traces is vital for language functions, and by investigating these memory processes, we can illuminate even the neural plastic mechanisms of the brain in early infancy and language-related dysfunctions.

Keywords: plasticity, language, brain, dyslexia

INTRODUCTION

During a conversation, we think and use extensively our long-term and working memory systems, involving complex neural processes. It is plausible that some of the speech processes are automatized, which frees capacity for the more demanding thinking functions.

In order to investigate the distinct information processing stages and to disentangle different subprocesses, temporally accurate brain research methods are invaluable. With electroencephalography (EEG) and magnetoencephalography (MEG) one can detect neural responses on the millisecond scale, enabling one to determine temporal dynamics of stimulus-specific processing.

For illuminating pre-attentive low-level processes, a feasible approach is to record mismatch negativity (MMN) with EEG or MEG. The MMN reflects early steps of sound and speech discrimination accuracy, and is elicited by acoustic changes and violations of sound regularities irrespective of the direction of the individual's attention (Kujala and Näätänen, 2010). The MMN has partly separate neural substrates for speech and non-speech sounds. MMNs are relatively stronger in the right than left hemisphere for non-speech sounds, whereas the relative lateralization is reversed for speech sounds. These effects can be detected from participants not attending to the auditory stimuli, which suggests automatic activation of speech memory traces. The MMN (or its positive counterpart, mismatch response, MMR) is even elicited in infants and fetuses, being therefore a feasible tool for investigating the early neural development.

PLASTICITY OF LANGUAGE FUNCTIONS

The low-level neural auditory processes, as reflected by the MMN, are modulated by experience (Kujala and Näätänen, 2010). During infancy, the representations of the native language phonemes are established. However, exposure to a foreign language, for instance, modulates these representations. This was demonstrated by a study comparing MMNs for a Finnish vowel contrast not existing in Hungary (Budapest dialect) in Finns and Hungarians with or without a Finnish command (Winkler et al., 1999). As expected, Finns had an MMN for this contrast, whereas Hungarians without a Finnish command had no response. Hungarians with a Finnish command acquired while living in Finland, however, had a fairly similar MMN as the Finns. Results of a vowel identification test consistently showed that Hungarians without a Finnish command performed at a chance level, whereas the other two groups successfully identified the vowels. These results suggest that the acquisition of a foreign language modulates phonetic neural representations, promoting the development of phoneme representations of this language.

Being challenging to investigate, learning and memory mechanisms in infancy are still largely unexplored in human neuroscience. The MMN/MMR is an ideal tool for this purpose, since it can be recorded even at the fetal stage. It was recently shown that fetuses can form neural representations of novel speech sounds (Partanen et al., 2013a; Figure 1). A group of pregnant mothers played a CD containing pseudowords with frequency changes uncommon in their native language five to seven times a week since pregnancy week 29 until birth. Another group served as a control without such stimulation. After birth, the infants who had been exposed to novel speech material had significant MMRs for the frequency changes, whereas the control infants had no significant MMR for these changes. Evidently, sounds of the environment impose an effect on the developing brain at the fetal stage. A further study determined whether the auditory memory traces formed before birth are persistent (Partanen et al., 2013b). An infant group had been exposed to the melody "Twinkle twinkle little star" before birth, whereas another infant group received no such stimulation. It was found that the exposed group had a stronger neural response for this melody than the unexposed group after birth. Moreover, this group difference was present even after the 4-month follow up period included in this study. Thus, the fetal memory traces may persist at least for several months.

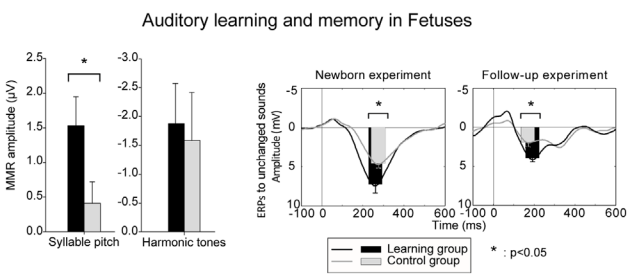

Figure 1: Left: infants who prenatally were exposed to pitch changes in pseudowords show enhanced neural responses to these changes (exposed group: black bar; control group: gray bar). These groups have similar responses for harmonic tones, indicating comparable basic auditory skills. Right: effects of fetal learning on neural responses to a melody persist until 4 months of age. Adapted from Partanen et al. (2013a,b).

DYSFUNCTION OF LOW-LEVEL LANGUAGE PROCESSES IN DYSLEXIA

A number of inherited or acquired disorders are associated with impairments in the low-level language functions (Näätänen et al., 2012). Developmental dyslexia is discussed here, since it is a major deficit causing learning impairments, its prevalence estimates being 5–17%. Dyslexia is also closely linked to dysfunctional language processes; it is primarily thought to result from impaired processing related to phonology (Ramus, 2004).

Studies employing MMN recordings have illuminated auditory-phonetic dysfunctions in dyslexia, suggesting low-level neural speech and non-speech discrimination deficits (Kujala, 2007). Furthermore, our very recent results suggest that dyslexic individuals have a fundamental problem in forming memory representations for novel speech input. Shtyrov et al. (2010) showed that the adult brain forms automatically and rapidly, in about 15 min, memory traces for novel words. We addressed this ability in 9- to 12-year-old children with or without dyslexia (Kimppa et al., in preparation). Our results showed that whereas in children without dyslexia a biphasic enhancement of a response for novel words developed toward the end of an ERP recording session, the early response did not emerge at all in dyslexic children. These results suggest inefficient memory formation for novel words in dyslexia.

The strong connection between reading skills and low-level auditory processing as reflected by the MMN was suggested by a study determining whether the MMN recorded in kindergarten is associated with reading skills at school (Maurer et al., 2009). It was found that children who became better readers had a stronger left-hemispheric lateralization of the late MMN (lMMN) for phoneme changes recorded in kindergarten than children with a poorer reading outcome at school. Furthermore, similar results were found when only a subgroup of dyslexic children was included in the analysis.

DOES INTERVENTION IMPROVE LOW-LEVEL AUDITORY PROCESSING IN DYSLEXIA?

Evidently, reading skills and dyslexia are associated with neural low-level auditory processing deficits. Moreover, the integrity of these processes before school age even predicts reading skills at school. However, what will happen to these processes if reading skills are improved with intervention in dyslexia?

This issue has been investigated by determining the effects of intervention on reading-related skills and MMN responses in children with or at risk for dyslexia. One of these studies (Kujala et al., 2001) included first graders with dyslexia and assessed the effects of an audiovisual Audilex program including no linguistic stimuli on reading skills. The intervention, requiring matching of visual and auditory patterns, was given for 7 weeks, twice a week

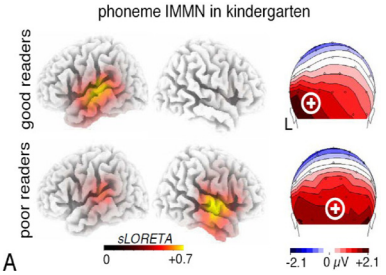

Figure 2: The lMMN to phoneme changes predicts future reading skills. lMMNs were recorded for phoneme changes in children at kindergarten and the amplitudes were correlated with reading-skill measures at school-age. It was found that children who at school became better readers had a more left-lateralized lMMN than children who became poor readers. Adapted from Maurer et al. (2009).

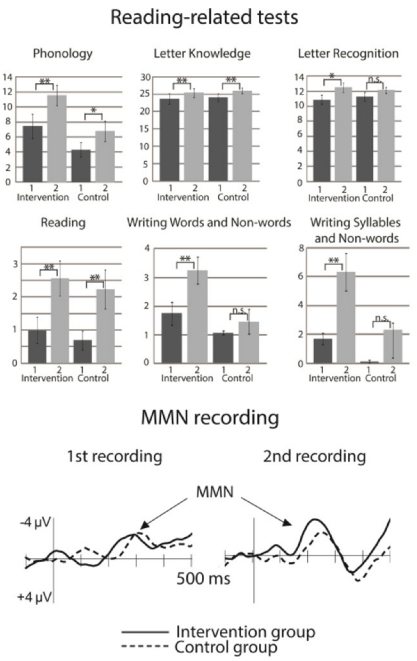

Figure 3: GraphoGame intervention effects on pre-reading skills and MMN. Top: results of the first assessment are with dark gray and the second assessment with light gray. Bottom: the MMNs for vowel changes before and after the training. Adapted from Lovio et al. (2014).

for 10–15 min for a group of these children, a matched dyslexic group serving as a control. It was found that this intervention improved reading accuracy and skills related to reading. Furthermore, an MMN, obtained for tone-order reversals, not differing between the groups in the baseline recording, was larger in the training than control group after the intervention. In addition, the training group became faster in detecting tone-order reversals in a separate behavioral test after the intervention. These results suggest that audiovisual intervention can improve reading skills of dyslexic children and this improvement is associated with neural plastic changes in auditory discrimination.

However, since slow progress in reading-skill learning may negatively influence motivation to learn at school, it stands to reason that children's reading skills should be supported even prior to school start. The effects of GraphoGame intervention (Lyytinen et al., 2009) on preschool children at risk for dyslexia were determined by assessing pre-reading skills and by recording the MMN for speech–sound changes (Lovio et al., 2014). GraphoGame audiovisually improves associations between graphemes and phonemes. It was applied to the training group for only 3 h altogether in smaller sessions, whereas the control group did calculation exercises in the same game environment.

The intervention group improved in all subtests on pre-reading skills, whereas the control group only improved in some of them (Figure 3). The intervention also improved neural processing of speech–sound changes: the MMN

amplitude for these changes, not differing between the groups in the baseline recording, grew more prominently in the training than control group (Figure 3). Moreover, the change in some pre-reading-skill scores and in the MMN amplitude for the vowel deviant correlated significantly. These results suggest that even a brief intervention with GraphoGame improves reading skills and neural speech discrimination accuracy in pre-schoolers.

SUMMARY

The analysis of speech occurs partially without our awareness, which may be the basis of our ability to think during conversation. This low-level speech processing, as reflected by the MMN, is influenced by learning and experience, even over some tens of minutes. Since these processes can be investigated even from inattentive participants, the neural basis of perception and its plasticity can be assessed even in the early infancy. Recent studies showed that even fetuses form auditory memory traces which may last for several months after birth.

Impairments of low-level auditory processing are associated with many speech-related disorders, such as dyslexia. Furthermore, abnormal lateralization of the MMN for vowel changes predicts poor reading skills at school. However, intervention improving reading-related skills in children with dyslexia or dyslexia risk also causes plastic neural changes, as reflected in the growth of the MMN. This further supports the idea that low-level neural processes are associated with, or even underlie, language-related functions such as reading.

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Proficiency and brain structure during intense language learning

Johan Mårtensson[1], Johan Eriksson[2], Timothy Brick[3], Nils Bodammer[4], Magnus Lindgren[5], Mikael Johansson[6], Lars Nyberg[7] and Martin Lövdén[8]

1. Department of Psychology, Lund University, Lund, Sweden; johan.martensson@psy.lu.se
2. Umeå University, Umeå, Sweden; johan.eriksson@umu.se
3. Max Planck Institute for Human Development, Berlin, Germany; brick@mpib-berlin.mpg.de
4. Max Planck Institute for Human Development, Berlin, Germany; bodammer@mpib-berlin.mpg.de
5. Department of Psychology, Lund University, Lund, Sweden; magnus.lindgren@psy.lu.se
6. Department of Psychology, Lund University, Lund, Sweden; mikael.johansson@psy.lu.se
7. Umeå University, Umeå, Sweden; lars.nyberg@umu.se
8. Aging Research Center (ARC), Stockholm, Sweden; martin.lovden@ki.se

SUMMARY

Foreign language acquisition can lead to changes in brain structure in young adults. Conscripts at the Swedish Armed Forces Interpreter Academy undergo highly intense foreign language training and approach near fluency over the course of a year.

Structural MRI before and after 3 months of training revealed gray matter increases in the left dorsal middle frontal gyrus, left inferior posterior frontal gyrus, left superior temporal gyrus and the right hippocampus. Gray matter increases were related to language performance, with higher increases in the right hippocampus and left superior temporal gyrus for interpreters with better performance at the end of the semester. In contrast, participants who had to work relatively harder to achieve the goals of the interpreter program exhibited the most widespread cortical change in the middle frontal gyrus.

A possible explanation is that participants with larger increases in the middle frontal gyrus put more strain on the articulatory network during acquisition. Preliminary findings from probabilistic tractography between the inferior frontal gyrus and middle frontal gyrus indicate higher increases in fractional anisotropy for individuals who had to work more intensively to achieve the goals of the academy. Change scores were at least partially dependent on starting values with larger increases and more work required for individuals with lower fractional anisotropy scores in part of the articulatory network prior to admission to the academy.

Keywords: diffusion tensor imaging, language acquisition, cortical thickness, hippocampus, plasticity

INTRODUCTION

Repeated practice can lead to structural brain change (Zatorre et al., 2012). Examples from motor training (Draganski et al., 2004), navigation (Woollett and Maguire, 2011) and meditation (Tang et al., 2010) illustrate the width of training forms that have been used to induce plasticity. Recent studies in the domain of language have shown that learning a new language can lead to changes in gray- (Mårtensson et al., 2012) and white-matter morphology (Schlegel et al., 2012).

Schlegel et al. (2012) followed students that participated in a 9-month intensive course in Modern Standard Chinese. Language learners showed reorganization of white matter in both left and right hemispheric regions, with the biggest areas of change located in the frontal lobe tracts near the crossing of the genu of the corpus callosum. Mårtensson et al. (2012) followed conscripts at the Swedish Armed Forces Interpreter Academy. Students are the academy are selected from applicants with strong language ability and are required to have knowledge of at least two foreign languages before entry. With no prior knowledge in their assigned language, the interpreters were taught a new language from scratch over the course of 10 months. This requires learning 300–500 new words each week, a pace that is unmatched in the Swedish education system. Structural MRI revealed cortical gray matter increases in the left dorsal middle frontal gyrus, left inferior posterior frontal gyrus, left superior temporal gyrus and subcortical changes in the right hippocampus. These changes were linked to later language performance, with larger increases in the superior temporal gyrus and hippocampus for participants who achieved higher proficiency in their studied language. Unexpectedly, the largest increases for the group appeared in the middle frontal gyrus, a motor region that overlaps with regions involved in perception of difficult speech (Meister et al., 2007). Participants with larger increases in this area had a harder time keeping up with the demands of the academy.

Could it be that participants with larger increases in the middle frontal gyrus put more strain on the articulatory network for difficult speech reproduction and processing? The languages learned consisted of Dari, Arabic or Russian, with quite different sound structures compared with the participants' native Swedish. Preliminary findings using diffusion

tensor imaging and probabilistic tractography between the inferior frontal gyrus and middle frontal gyrus point toward reorganization of white matter within parts of the articulatory network for participants who struggled to stay at the academy.

EARLIER GRAY MATTER FINDINGS

The cortical regions of interest reported by Mårtensson et al. (2012) hold key roles in sensorimotor aspects of language (Démonet et al., 2005; Hickok and Poeppel, 2007; Price, 2010). The inferior frontal gyrus is believed to be involved in the articulatory network (mainly in the left hemisphere), something which is also true for the left dorsal middle frontal gyrus, a region that overlaps with premotor language areas implicated in planning and top-down control of articulatory processes (Hickok and Poeppel, 2007; Meister et al., 2007). The superior temporal gyrus performs spectrotemporal analysis (bilaterally) and all of the regions belong to a frontal network of areas involved in speech processing (Hickok and Poeppel, 2007).

The findings of Mårtensson et al. (2012) provided evidence that learning a foreign language in adulthood could change the structure of language-related brain regions. Importantly, the right hippocampus and the left superior temporal gyrus were structurally more malleable in interpreters who acquired higher foreign language proficiency. To the authors' knowledge, this is the first time that hippocampal plasticity has been directly linked to learning outcomes in humans. The results also implicated that interpreters that had to work relatively more intensively to master their assigned language displayed larger gray matter increases in the left dorsal middle frontal gyrus, which was the cortical region that saw the largest increase (see Figure 1). This region overlaps with premotor language areas implicated in planning and top-down control of articulatory processes (Hickok and Poeppel, 2007; Meister et al., 2007) and belongs to the articulatory network alongside the inferior frontal gyrus.

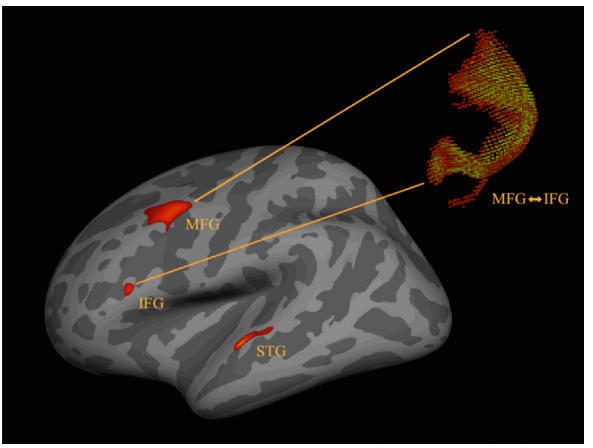

Figure 1: A reconstructed tract between the left dorsal middle frontal gyrus (MFG) and inferior posterior frontal gyrus (IFG) connects two of the areas outlined in Mårtensson et al. (2012). When compared to controls, conscript interpreters showed larger increases ($p < 0.001$; cluster size >100) in the MFG, IFG, and mid posterior superior temporal gyrus (STG). Increases in Fractional Anisotropy within the tract connecting the MFG and IFG were positively related to teacher ratings of study effort (struggle).

In essence, the major cortical change was driven by participants who had to work harder to stay in the academy program, and those individuals saw larger increases in premotor language areas that are part of the articulatory network.

We speculate that for interpreters struggling relatively more to master a new language, the demands on articulatory areas were elevated, which lead to increased cortical thickness in the middle frontal gyrus. If this is the case then it is plausible that these demands also affected white matter connectivity within the articulatory network. Based on the regions of interests in Mårtensson et al. (2012) this would include tracts connecting the inferior frontal gyrus and middle frontal gyrus in the left hemisphere.

WHITE MATTER ANALYSIS

Participants

Fourteen (six women) volunteers were recruited from the Swedish Armed Forces Interpreter Academy. Students are required to have top grades in at least two foreign languages (making all students multilingual). Eight of the

interpreters studied Dari, four studied Arabic, and two studied Russian. None of the interpreters had prior knowledge in his or her assigned language. For further details of the subject group please see Mårtensson et al. (2012).

Proficiency and struggle

Our proficiency measure consisted of grades from the mid-year exam at the interpreter academy, including one written and one oral test. The written language test involves translating sentences and texts and the oral tests consist of non-simultaneous interpreting. For our measure of subjective struggle, we asked the head teacher at the academy to rate the amount of effort needed to stay in the program at post-test. The question was: "Judge how large effort was needed for each participant to achieve the goals of the interpreter academy and to be allowed to stay in the program" and was rated on a Likert scale of 1 (little effort) to 9 (large effort).

MR acquisition and preprocessing

Images were acquired at the Umeå Center for Functional Brain Imaging on a GE Discovery MR 750, 3 T scanner using a 32-channel phased-array head coil. A Spin Echo refocused EPI sequence was used. Images had a slice thickness of 2 mm. Sequence parameters were: TR = 8000 ms, 64 slices with no gap, acquisition matrix 128 × 128 interpolated to 256 × 256 matrix with a FOV of 250 mm, TE = 84.4 ms, 4 repetitions of 24 independent directions, $b = 1000$ s/mm^2 and 4 $b = 0$ images, Dual Spin Echo switched on and ASSET acceleration factor 2.

Preprocessing

Diffusion-weighted images were pre-processed using the FSL software package (Jenkinson et al., 2012). Probabilistic tractography via Probtrackx was then performed to obtain a connectivity index along white matter pathways, a measure that reflects fiber organization. In essence, we recreated a connection between the left dorsal middle frontal gyrus and the left inferior frontal gyrus based on the regions of interest (ROI) from Mårtensson et al. (2012). The settings used were: curvature threshold of 0.2, 5000 samples, a maximum number of 2000 steps and a step-length of 0.5. The mean fractional anisotropy and mean diffusivity of non-zero voxels were then extracted from within the tracts. The resulting values were exported for each participant for each time point (pre, post) into the R statistical package where difference scores (post–pre) were regressed against measures of struggle and proficiency.

Results

Increases in fractional anisotropy between the middle frontal gyrus and inferior frontal gyrus were predictive of struggle [$R^2 = 743$, $t(300) = 2.48$, $p = 0.04$]. Additionally, an interaction between starting values of fractional anisotropy and change scores was found [$R^2 = 205$, $t(811) = -2.53$, $p = 0.04$]. No results were found for mean diffusivity or in relation to proficiency (all $p > 0.05$).

DISCUSSION AND CONCLUSION

Foreign language acquisition can lead to structural brain change. In Mårtensson et al. (2012) structural MRI was used before and after 3 months of training to reveal gray matter increases in the left dorsal middle frontal gyrus, left inferior posterior frontal gyrus, left superior temporal gyrus, and right hippocampus. The largest cortical increase occurred in the middle frontal gyrus, an effect that was relatively greater in participants who struggled to achieve the goals of the academy. We speculate that these interpreters placed higher demands on articulatory network areas which led to increased cortical thickness in the middle frontal gyrus, an area that overlaps with regions that are involved in processing of difficult speech (Meister et al., 2007). We found preliminary evidence supporting this hypothesis; changes in white matter connectivity within the articulatory network, consisting of the tracts connecting the inferior frontal gyrus and middle frontal gyrus in the left hemisphere, were related to measures of struggle. Participants who struggled relatively more in order to remain in the interpreter program exhibited greater increases in fractional anisotropy over time in these areas, an effect that was modulated by starting values, with larger increases for participants with low starting values of fractional anisotropy before entry admission into the academy and vice versa.

A possible explanation is that individuals with a well developed auditory network were more efficient in processing difficult speech and had less cause and/or less headroom for changes in fractional anisotropy in this area. Fractional anisotropy has been known to increase as an effect of language learning (Schlegel et al., 2012) and it is possible that there were differences in ability prior to entry at the academy. Short term word learning has also been known to be mediated by structural white matter differences in the arcuate fasciculus (López-Barroso et al., 2013).

In our relatively small sample of individuals ($n = 14$) and with effects that border on accepted levels of statistical significance ($p = 0.04$), we can conclude that participants with larger increases in the middle frontal gyrus probably put more strain on the articulatory network and that said effects were related to learning outcomes in the form of teacher ratings of the effort expended by students to stay in the academy's interpreter program.

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Electrophysiological and hemodynamic biomarkers of rapid acquisition of novel wordforms

Yury Shtyrov[1], Lilli Kimppa[2] and Max Garagnani[3]

1. Center of Functionally Integrative Neuroscience, Aarhus University, Aarhus, Denmark; yury.shtyrov@cfin.au.dk
2. Cognitive Brain Research Unit, Helsinki University, Helsinki, Finland; lilli.kimppa@helsinki.fi
3. Brain and Language Laboratory, Free University – Berlin, Berlin, Germany; m.garagnani@fu-berlin.de

SUMMARY

Humans are unique in developing large lexicons; to achieve this, they are able to learn new words rapidly. However, the neural bases of this rapid learning, which may be an expression of a more general mechanism rooted in plasticity at cellular and synaptic levels, are not yet understood. Here, we highlight a selection of recent EEG and fMRI studies that attempted to trace word learning in the human brain non-invasively. They show a rapid development of cortical memory traces for novel wordforms over a short session of auditory exposure to these items. Moreover, they demonstrate that this effect appears to be independent of attention, reflecting the largely automatic nature of word acquisition. At the same time, it seems to be limited to stimuli with native phonology, likely benefiting from pre-existing perception–articulation links in the brain, and thus suggesting different neural strategies for learning words in native and non-native languages. We also show a complex interplay between overnight consolidation, amount of exposure to novel vocabulary and attention to speech input, all of which influence learning outcomes. In sum, the available evidence suggests that the brain may effectively form new cortical circuits online, as it gets exposed to novel linguistic elements in the sensory input. A number of brain areas, most notably in the hippocampus and neocortex, appear to take part in word acquisition. Critically, the currently available data not only demonstrate a hippocampal role in rapid encoding followed by slow-rate consolidation of cortical memory traces, but also suggest immediate neocortical involvement in the word memory trace formation.

Keywords: brain, cortex, word learning, fast mapping

INTRODUCTION

Humans are unique in developing large lexicons as communication tools; to achieve this, they are able to learn new words rapidly. However, the neural bases of this rapid learning, which may be an expression of a more general cognitive mechanism likely rooted in plasticity at cellular and synaptic levels, are not yet understood. In this presentation, we highlight a selection of recent studies that attempted to trace word-learning in the human brain non-invasively (e.g., Breitenstein et al., 2005; De Diego Balaguer et al., 2007; Mestres-Misse et al., 2007). To explore this in more detail, we will present our own EEG and fMRI studies that demonstrate rapid development of cortical memory traces for novel word forms over a short session of auditory exposure to these items.

EEG EVIDENCE OF RAPID WORD LEARNING

In the first EEG studies (Shtyrov et al., 2010; Shtyrov, 2011), we exposed our subjects to familiar words and novel spoken stimuli in a short passive perceptual learning session in which the subjects were not instructed to pay attention to the stimuli or actively memorize them. We compared automatic ERP brain responses to these items throughout the learning exposure. Initially, we found enhanced early (~100 ms) electrophysiological activity for known words, indexing the ignition of their underlying memory traces. However, just after 14–20 min of learning exposure, the novel items exhibited a significant increase in response magnitude matching in size that of real words. This activation increase reflects rapid mapping of new word forms onto the lexicon. In a manner similar to familiar words, the neural activity subserving rapid learning of new word forms was generated in the left-perisylvian language cortex, especially anterior superior-temporal areas, as suggested by distributed source analysis of the ERP data. These phenomena were found in independent experiments using both English (Shtyrov et al., 2010) and Finnish (Shtyrov, 2011) subjects and stimuli, and were confirmed using both factorial and linear regression analyses. Furthermore, acoustically matched novel non-speech stimuli did not demonstrate a similar response increase, suggesting a neural specificity of this rapid-learning phenomenon for linguistic stimuli (Shtyrov, 2011).

AUTOMATICITY AND SPEECH SPECIFICITY

To investigate the role of attention and phonological properties in rapid-learning phenomena, we ran a further study, in which the subjects were repeatedly presented with (i) known words, (ii) phonotactically legal and phonologically native novel wordforms and (iii) novel pseudowords with non-native speech sounds. In a counterbalanced design, they were either asked to pay close attention to the stimuli and memorize them, which was assessed using recall tasks after exposure, or ignore the auditory signal and concentrate on a primary non-linguistic visual task. We found (Kimppa et al., in preparation) that, in both ignore and attend conditions, a negative-going ERP peak at ~50 ms after the disambiguation point was strongest for the real words and weakest for the non-native words. Further, while there was a habituation-related decline in this early response to known words over the short exposure session, ERPs to both pseudoword types did not show such attenuation. Crucially, the ERP response to pseudowords with native phonology increased during the recording session and, by the end of session, its amplitude resembled the initial response to words. At the same time, non-native stimuli did not show such rapid-learning dynamics. This activity increase for native novel wordforms, which we suggest to be a neural signature of rapid learning, was also evident in underlying cortical source activations in the posterior temporal and inferior frontal cortices. A later response phase at ~150 ms did not show incremental change for any of the word types; rather, all responses declined across conditions. The results suggest that rapid formation of novel memory traces for phonologically plausible stimuli takes place automatically in both passive and attentionally demanding conditions, whereas the role of attention appears to be crucial at the behavioral level of word retrieval, as established in behavioral assessment. This was further revealed by regression analysis linking the neural response enhancement for pseudowords with post-exposure recall performance of heard items. That is, attentive listening yielded superior recall and predicted the magnitude of change in ERP responses for pseudowords. Memory trace formation for words with non-native phonology does not seem to take place during this short exposure time, at least not with the amount of repetition used in this experiment. The results suggest phonologically restricted, attention-independent plastic changes and rapid learning in the brain, reflected in the very early phase of activation pattern changes for novel words. This implies that rapid mapping phenomena are restricted to words with native-language phonology, benefiting from pre-existing perception–articulation links in the brain, and suggests different neural strategies for learning new words from the native and non-native languages.

RAPID LEARNING VS. CONSOLIDATION: fMRI EVIDENCE

All of the above findings clearly indicate that many exposures to a novel word during a short time may lead to a fast formation of cortical memory traces. Yet, the mainstream view is that the newly learnt items acquire lexical status only after a period of sleep, during which cortical consolidation processes are believed to occur (Dumay and Gaskell, 2007; Davis and Gaskell, 2009; Davis et al., 2009). To investigate the effects of sleep and number of exposures on word acquisition, we ran a further fMRI study using stringent behavioral learning regimes, systematically modulating the level of exposure to new vocabulary. We presented our volunteers with spoken familiar words and novel pseudowords during two behavioral training sessions taking place on two consecutive days (day 1, day 2). The number of times each item was repeated (20 vs. 150) varied orthogonally to the day-of-training. After the training (on day 2), we used fMRI to measure hemodynamic brain responses to these trained and, as a further control, to previously unheard (untrained) items. We also modulated the amount of attention that volunteers paid to the auditory input during the fMRI acquisition, measuring all brain responses under two conditions: participants' attention was either directed toward the auditory stimuli, or they were instructed to ignore the sounds and pay attention to a silent video. We found (Garagnani et al., in preparation) that brain responses to words and pseudowords in the left STG were differentially modulated by day/training. While word responses were generally smaller and mostly unaffected by training, we found that untrained, day 2-trained, and day 1-trained pseudowords exhibited increasingly smaller responses (i.e., gradually becoming more and more like real word ones). This is in line with previous results, indicating that sleep leads to consolidation of newly acquired representations. When pulling apart data for 20- and 150-times-repeated pseudowords, however, the effects of sleep appear limited mostly to the latter items, suggesting a critical role for the number of repetitions required to set off word learning and consolidation processes. Nevertheless, training does affect brain responses to novel items, even on the same day; hence, sleep may not be necessary for changes to observed cortical responses. We also found that pseudoword and word responses are differentially affected by attention, with responses to pseudowords exhibiting larger effects of attention than words (though this effect was not changed by training or sleep) and that attention to stimuli leads to better subsequent memory for pseudowords.

CONCLUSION

Echoing early behavioral studies in ultra-rapid word learning, the current experiments can be taken to suggest that the brain may effectively form new cortical circuits online, as it gets exposed to novel linguistic patterns in the sensory input. Critically, the currently available data not only demonstrate a hippocampal role in rapid encoding followed by slow-rate consolidation of cortical word memory traces, but also suggest immediate neocortical involvement in the word memory trace formation (Shtyrov, 2012). Whilst the recent data reviewed here can in principle be

accommodated by existing conceptual frameworks of learning (such as the complementary learning systems approach), they do call for an update of these concepts that would be needed in order to account for a "cortical shortcut" in linguistic memory trace formation (Figure 1). Investigating neural mechanisms of word learning is both a scientifically fascinating endeavor that can shed light on brain underpinnings of language, neuronal plasticity and memory, and an important research avenue for understanding the nature of learning dysfunctions and language deficits linked to a range of hereditary and acquired conditions.

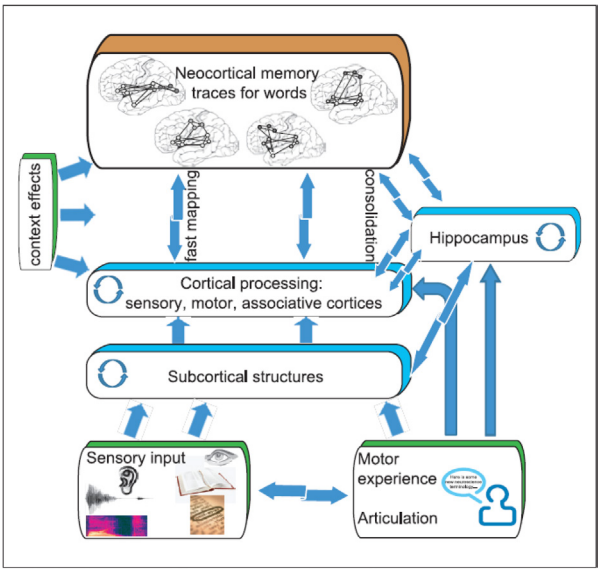

Figure 1: Conceptual sketch of neural bases for word learning [adapted from Shtyrov (2012)]. We stress here the putative existence of a fast neocortical route (complementary to the hippocampal route, associated with slower consolidation processes), which may underlie rapid learning and fast mapping phenomena.

ACKNOWLEDGMENTS

We specifically highlight that our key collaborators in the original studies and co-authors in the corresponding papers and manuscripts are Dr. Vadim Nikulin (Charite – Medical University Berlin), Prof. Friedemann Pulvermueller (Free University Berlin), Prof. Teija Kujala (Helsinki University), Dr. Alina Leminen (Aarhus University), Dr. Martti Vainio (Helsinki University) and Dr. Matthew H. Davis (MRC Cognition and Brain Sciences Unit, Cambridge). We are very grateful to these persons for their invaluable contribution to the original research summarized here.

We also wish to thank the Lundbeck Foundation (Denmark), the Medical Research Council (MRC, UK), the Academy of Finland and the Biotechnology and Biological Sciences Research Council (BBSRC, UK) for their financial support of our research.

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ERP—exploring the temporal microstructure of cognitive functions in the brain

Mikael Roll

Linguistics, Center for languages and literature, Lund University, Lund, Sweden; mikael.roll@ling.lu.se

SUMMARY

Current investigations are making it possible to analyze MRI data at a spatial microstructure level. However, temporal resolution is poor in MRI. Using electroencephalography (EEG) and the event-related potentials (ERP) technique, neurocognitive events can be recorded with a millisecond precision. However, this method provides limited information on the brain sources of the signal. The present contribution provides a short introduction to the use of EEG in neurocognitive research.

Keywords: EEG, ERP, brain

MRI, EEG, AND BRAIN IMAGE RESOLUTION

Whereas techniques based on magnetic resonance imaging (MRI) can reach good spatial resolution in the analysis of brain activity, their temporal resolution is poor. Methods involving recording of the electrical activity of neurons, conversely, have the potential of showing changes in the brain signal at the millisecond level. The most common methods are based on electroencephalography (EEG). EEG uses electrodes to measure voltage fluctuations over the scalp of a person. The electrodes are often attached to a cap for convenience and ease of application. The main advantage of using EEG in research is that it provides high temporal resolution of brain activity at a relatively low cost. The rationale for using EEG in neurocognitive research is that part of the electrical activity picked up at the scalp surface stems from brain activity.

SOURCE OF EEG SIGNALS

The EEG signal picked up at the surface of the head stems from a mixture of neural activity. It might be thought that the largest contributing neural event would be the "action potential," i.e., when neurons send out signals through their axons. However, action potentials are brief and tend to cancel out each other. Rather, the major contributors to the EEG signal are "postsynaptic potentials," i.e., depolarization or hyperpolarization of the cell membrane of a neuron receiving chemical transmission from the axon of another, firing neuron. Postsynaptic potentials from groups of neurons aligned together – especially in a favorable direction and in the more superficial layers of the brain – can influence the summed EEG signal enough to reveal important brain responses to different kinds of stimulation.

CAPTURING EEG SIGNALS

To record brain responses to a certain type of event, the relevant signal needs to be separated from noise. Muscular activity produces potential fluctuations that can be hundreds of times larger than those produced by neural activity. To a certain extent, modeling stereotypical artifacts such as eye blinks and saccades using different algorithms and removing the part of the EEG that is thought to correspond to the artifacts can attenuate the noise. Muscular tension often causes high frequency noise that can be reduced by low-pass filtering. Correspondingly, skin potentials due mostly to sweating produce slow waves in the EEG, which can be attenuated through high-pass filtering. Eventually, pieces of EEG that are still too contaminated by noise even after trying to attenuate them by different methods must be removed.

Even after removing as much noise as possible, the response to a single stimulation still cannot be fully appreciated due to remaining noise as well as signal changes due to unrelated brain activity. To crystallize the EEG trace of a certain type of stimulus, it has to be presented repeatedly, and an average signal needs to be calculated.

EVENT-RELATED POTENTIALS (ERP)

The resulting average, time-locked to a stimulus, is called "event-related potential" (ERP). In neurocognitive research, the practice is to have at least 30 repetitions per participant and experimental condition (often much more to ensure good signal-to-noise ratio). Hence, grand averages over various participants can correspond to thousands of repetitions. Figure 1 shows three stages of averaging of auditory ERPs for high and low word tones in Swedish ("Accent 1" and "Accent 2"): single trial, average of around 100 trials for one participant, and average of around 1800 trials from 18 participants (Roll et al., submitted).

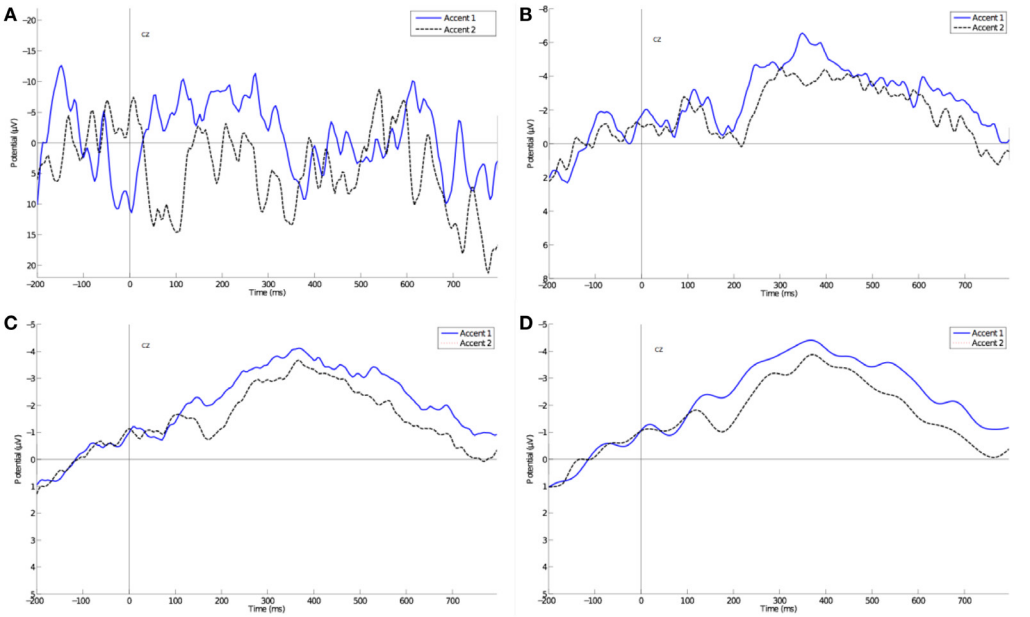

Figure 1: Event-related potentials (ERPs) of 1 trial per condition **(A)**, ~100 trials per condition **(B)**, and ~1800 trials per condition **(C)**, as well as following 20 Hz low-pass filter **(D)**. Blue lines show ERPs following a Swedish Accent 1 tone, and black, broken lines show ERPs following an Accent 2 tone.

After averaging, ERP components emerge. Figure 2 shows the N100 and P200 components found for auditory stimuli, in this case a main clause-initial rise in the intonation of Swedish. The N100 and P200 components are thought to originate mainly in the superior temporal cortex, involving primary and secondary auditory cortices (Roll et al., submitted). The intonation rise is unexpected in one of the conditions in Figure 2, due to the lack of a phrase boundary.

The unexpected sound change increases the N100 (Roll and Horne, 2011).

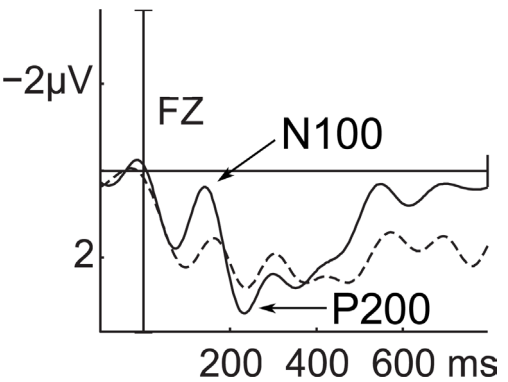

Figure 2: ERPs for an unexpected clause-initial rise in a Swedish sentence (solid line). There is an increase in the auditory N100 component (Roll and Horne, 2011).

In this way, ERPs of different experimental conditions can be compared, even ERPs associated with more abstract cognitive functions. As an example, Figure 3 shows a left anterior negativity (LAN) found around 300–450 ms following words violating grammatical agreement, such as **They sings* (Roll et al., 2013).

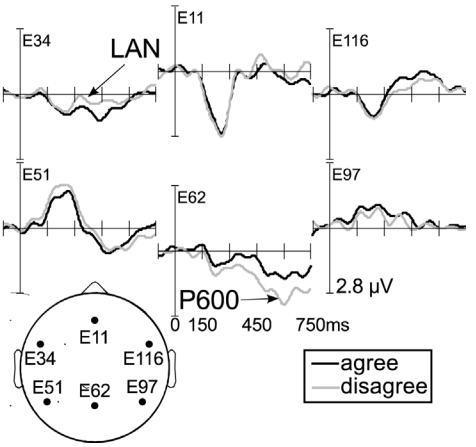

Figure 3: ERPs showing left anterior negativity (LAN) and late posterior positivity (P600) for agreement violations in Swedish.

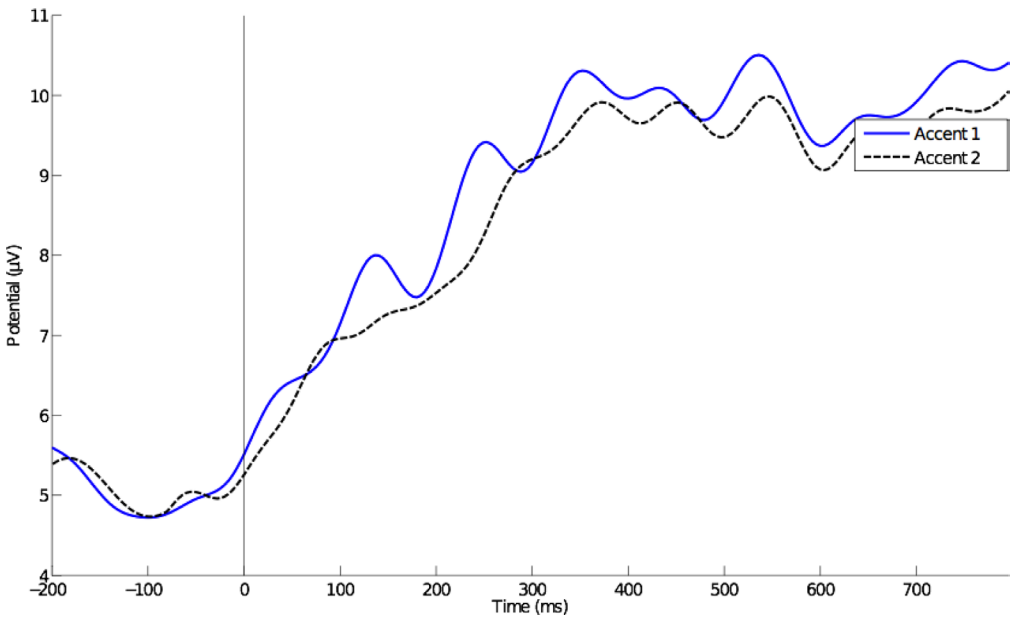

Figure 4: Global root mean squares for Swedish Accent 1 (blue, solid line) and Accent 2 tones (black, broken line). Accent 1 shows increased neural activity in comparison with Accent 2.

ERPS AND NEURAL ACTIVITY MEASURES

ERPs can show differences in the brain response to different experimental conditions at certain locations on the head. There is, however, no information in the ERP signal as to whether one condition involves increased brain activity as compared to another. In Figure 2 for example, there is no way of telling whether Accent 1 or Accent 2 gives rise to an increase in brain activation, only that the ERPs for Accent 1 are relatively more negatively charged and those for Accent 2, more positively charged. Negativity or positivity does not mean anything *per se*. In cases where this is unclear, a way of obtaining a measure of which condition causes a change in brain activity is to sum (root) mean squares of all values from the individual electrodes. This will add increases in positivity and negativity together, and will give an indication of where there are important changes in overall brain activity, and which condition causes such changes. Methods building on this principle are global field power and global RMS. Figure 4 shows the global RMS for the word accent comparison in Figure 2.

CONCLUDING REMARKS

Finally, there are various methods for localizing the source of EEG activity, some very sophisticated, taking into account the structure of the brain. Whereas these methods can give a good idea of the brain areas involved, the strength of EEG-based methods is still their temporal resolution.

ACKNOWLEDGMENT

This work was supported by grant no. 421-2010-1385 from the Swedish Research Council.

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Multidimensional diffusion MRI: a new tool for studying brain microstructure and the effects of learning

Daniel Topgaard

Department of Chemistry, Division of Physical Chemistry, Lund University, Lund, Sweden; daniel.topgaard@fkem1.lu.se

SUMMARY

Multidimensional diffusion MRI is a promising new MRI approach that has the potential to non-invasively give information about brain microstructure at an unprecedented level of detail. The present contribution briefly reviews the salient features of the novel method, its historical background and differences with conventional methods, as well as the remaining challenges that have to be overcome in order to make it applicable in learning studies.

Keywords: magnetic resonance, water, translational motion, biological tissues

INTRODUCTION

Just as physical exercise gives rise to growth of muscles, acquisition of new knowledge causes structural changes in brain tissue (Zatorre et al., 2012). Since the skull is neither transparent nor flexible, it is methodologically challenging to observe such changes without harming the studied person. Although visible light is stopped by the skin and skull, radio waves and magnetic fields readily penetrate both bone and soft tissue while weakly interacting with the magnetic moments, the so-called "spins," of the atomic nuclei in elements such as hydrogen. These facts form the physical basis for magnetic resonance imaging (MRI), a method which is unrivaled in its ability to provide information about brain structure by means of measurements that can be completely non-invasive.

The MRI signal mainly originates from hydrogen nuclei located on water molecules, and the translational diffusion of these molecules during the acquisition of the MRI signal determines the maximum resolution of resulting images. With currently available MRI equipment, the diffusion limit to the resolution is about 10–100 micrometers (Callaghan and Eccles, 1988), which is unfortunately not sufficient to directly observe the cellular structures of the brain. Although smaller structures will be blurred in the actual images, information about the microscopic structures can be inferred from the pattern of molecular motion, which can be monitored with diffusion MRI (dMRI) (Callaghan, 2011). The tissue geometry is imprinted on the micrometer-scale translational motion of the water molecules residing within and between its cells. Consequently, information about the tissue structure can be extracted by following the diffusion of the tissue water.

The idea of using nuclear magnetic resonance (NMR) to monitor molecular displacements in liquids was published already in 1950 (Hahn, 1950), and the first applications for studies of porous materials, such as rocks, plant tissues, and emulsions, appear in the 60s (Woessner, 1963; Tanner and Stejskal, 1968). The combination of information about translational motion from diffusion NMR and about spatial resolution from MRI was first described in the 80s (Taylor and Bushell, 1985; Le Bihan et al., 1986), but it was only after the discovery that ischemic stroke is readily detected in diffusion-weighted images that dMRI found more widespread clinical use (Moseley et al., 1990).

The parameters that are measured with conventional dMRI techniques are often loosely interpreted in terms of tissue properties such as cell density and the average orientation of the nerve fibers, but in recent literature, it has become apparent that there are many different changes in brain microstructure that give identical results in dMRI measurements (Jones et al., 2013). Consequently, conventional dMRI lacks the precision to critically test recent hypotheses on microstructural changes in the brain during learning (Zatorre et al., 2012).

We are currently developing a family of novel dMRI techniques that have the potential to provide quantitative information at an unprecedented level of detail: cell sizes, shapes, orientations, and membrane permeabilities, as well as blood capillary volumes, flow rates, and orientations; i.e., tissue properties that are directly related to the proposed changes of brain structure. Since the new techniques build on ideas derived from multidimensional NMR spectroscopy (Ernst et al., 1987), we have suggested the name "Multidimensional dMRI." In comparison to conventional dMRI, the potential increase in the amount of available information is analogous to the change from simple one-dimensional NMR spectroscopy of small molecules in the 60s to the sophisticated multidimensional NMR approaches for determining the three-dimensional structure of proteins in the 80s (Wüthrich, 1986). In the following text, the salient features of the new approach will be described, as well as its physical and biological limits, and the challenges that have to be overcome in order to make it practically feasible in learning studies.

CONVENTIONAL AND MULTIDIMENSIONAL DIFFUSION MRI

MR images are acquired by exposing the studied object to a sequence of carefully designed time-varying magnetic fields while recording the sum of the signals sent out by the individual spins. The resulting signal as a function of time is subsequently converted to an image through advanced computer processing. The frequency and lifetime of the signal is strongly dependent on the type of atomic nucleus and the molecule in which it is located, having the consequence that the MR images can be made selective to, e.g., liquid water.

In conventional dMRI, each image read-out is preceded by a sequence of magnetic field gradient pulses – a "motion-encoding block" – that attenuates the signal with an amount that depends on the molecular motion in a certain direction. Information about the rate and the directional dependence of the motion is extracted by analyzing the signal intensities of a series of images acquired with different strengths and directions of the motion-encoding. Pictorially, one can consider the motion-encoding block as a means of asking the water molecules how fast they are moving in a certain direction. The defining feature of multidimensional dMRI is that the motion-encoding block interrogates the molecules about their motion in several directions or at multiple points in time. The advantage of such a "multiple question" approach can be illustrated with following analogy. Assume that a university teacher wants to find out if there is any correlation between the students' lecture attendance and their scores on a written exam. Designing the investigation in the conventional dMRI way would correspond to asking the students about their attendance, converting the answers to a one-dimensional histogram, and then independently asking about the scores, yielding a second one-dimensional histogram. From these two independent questions and one-dimensional histograms it is of course impossible to figure out if there is any correlation. The more sensible approach, corresponding to multidimensional dMRI, would be to ask the questions simultaneously and store the answers as attendance-score pairs from which a two-dimensional histogram can be constructed and the correlation, if any, easily extracted. This "multiple question" approach for diffusion NMR was suggested already in the early 90s (Cory et al., 1990; Mitra, 1995), and the conceptual analogy with multidimensional NMR spectroscopy was noted by Callaghan and Furó (2004), but it is only in the last few years that a handful of dMRI groups have managed to adapt it for *in vivo* human imaging (Avram et al., 2012; Lawrenz and Finsterbusch, 2013; Nilsson et al., 2013).

LIMITS AND CHALLENGES

Physical limits

The fundamental limit to the amount of available information is determined by the nuclear relaxation times, which can be considered as the "attention span" of the spins, i.e., the time duration over which the spins can remember and reply to previously asked questions. The relaxation times are set by the background magnetic noise originating from nearby nuclear spins. For hydrogen nuclei on water molecules in tissue, the relaxation times are some hundred milliseconds, and all motion-encoding and signal read-out has to take place during this time. Typically, this process is repeated from a few up to a thousand times for different combinations of motion-encoding, giving a set of MR images where the signal is subsequently analyzed to extract the information about molecular motion and, by inference, tissue microstructure. The main challenge when designing multidimensional dMRI experiments is to squeeze in the desired amount of multiple motion-encoding blocks during the time allowed by the nuclear relaxation, while still leaving sufficient time for image read-out.

Biological limits

Multidimensional dMRI relies on measuring the micrometer-scale molecular motion resulting from diffusion and blood flow. Bulk tissue motion taking place on the same length- and time-scales would mask the desired information. Such motion results from, e.g., the beating heart, involuntary head movements, and vibrations from the MRI hardware. A serious challenge for microstructural interpretations of the observed diffusion patterns is insufficient knowledge about the spaces and tissue compartments that the water molecules have time to explore during the encoding times of up to a few hundred milliseconds. From soft matter science (Ferreira et al., 2013) it is well known that water will move around and through any object as long as there are continuous channels with sufficient space to accommodate a cluster of at least a few water molecules, i.e., about one nanometer. This means that water is only slightly impeded by macromolecules such as proteins and DNA, but is efficiently stopped by lipid membrane structures. From experiments on simple eukaryotic cells (Åslund and Topgaard, 2009; Åslund et al., 2009), we know that a water molecule samples the entire intracellular space on the time-scale of a few milliseconds, while molecular exchange over the cell membrane is strongly dependent on the physical state of the membrane lipids and the presence of aquaporins; the typical intracellular lifetime could vary from a few milliseconds for red blood cells to over a second for myelinated axons. Still, many basic questions remain about how the nano- and micrometer-scale cell and tissue structure affect the water motion.

Hardware limits

Multidimensional dMRI is critically dependent on the amplitude, duration, and precision of the time-varying magnetic field gradients produced by the MRI hardware. In contrast to conventional dMRI, the main limitation is

not the maximum amplitude as such, but rather the duration over which the gradient amplifiers can be driven to the maximum. At the top of our wish list for future improvements in clinical MRI hardware is more efficient cooling of the gradient coils and larger capacitors in the gradient amplifiers. In common with all MRI methods, any hardware improvements giving higher signal-to-noise ratio would be welcome.

Statistical/algorithmic challenges

The main drawback of conventional MRI is that the obtained parameters are influenced by a multitude of tissue properties, making it difficult to interpret the observed values in terms of microstructure. Multidimensional dMRI has the capability to separate the various properties, e.g., cell size and membrane permeability, the main challenge being to design time-efficient protocols for asking the spins the multiple questions about their motion, and robust computer algorithms for converting the obtained answers to easily interpretable parameters that have a simple intuitive relation to the desired tissue properties.

CONCLUSION

The multidimensional dMRI approach theoretically has the capability to provide information required for critically testing current hypotheses about microstructural brain changes underpinning the acquisition of new knowledge. While the physical and biological limits to the amount of available information is reasonably well understood, further improvement of MRI hardware and, more importantly, development and optimization of acquisition protocols and data analysis algorithms are required for realizing the full potential of multidimensional dMRI.

ACKNOWLEDGMENTS

The Swedish Foundation for Strategic Research is acknowledged for financial support (grant no. AM13-0090) of the “Multidimensional diffusion MRI” research program with participants at Linköping University (Hans Knutsson, Magnus Herberthsson), Lund University (Markus Nilsson, Daniel Topgaard), and Harvard Medical School (Carl-Fredrik Westin).

CONFLICT OF INTEREST STATEMENT

Patents related to multidimensional dMRI are held by CR Development AB (Lund, Sweden), where Daniel Topgaard is co-owner.

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Quantification of microscopic anisotropy with diffusion MRI

Markus Nilsson[1], Filip Szczepankeiwicz[2] and Daniel Topgaard[3]

1. Lund University Bioimaging Center, Lund University, Lund, Sweden; markus.nilsson@med.lu.se
2. Department of Medical Radiation Physics, Lund University, Lund, Sweden; filip.szczepankiewicz@med.lu.se
3. Department of Chemistry, Lund University, Lund, Sweden; daniel.topgaard@fkem1.lu.se

SUMMARY

The anisotropy of water diffusion in brain tissue can be quantified by means of the fractional anisotropy (FA) using diffusion tensor imaging (DTI). Studies of brain plasticity have reported elevated FA values in white matter as a response to learning. Such observations indicate that FA reflects properties of axons, in contexts where the diffusion is anisotropic. However, FA is also sensitive to the orientation dispersion of axons. Here we report on efforts to disentangle these effects by using a combination of isotropic and anisotropic diffusion encoding. The result is a parameter denoted "microscopic fractional anisotropy" (μFA). In white matter, values of μFA are uniform and high, but in gray matter, the values are low. In regions of crossing fibers, μFA has been observed to be high and FA low. Using simulations, we demonstrate the response of FA and μFA to changes in micro-geometry, such as fiber crossing angle. We conclude that FA from DTI reflects both the diffusion anisotropy and orientation dispersion. However, microscopic anisotropy and orientation dispersion can be quantified separately, which allows for more specific analyses of microstructural change.

Keywords: diffusion MRI, anisotropy, plasticity

INTRODUCTION

Diffusion tensor imaging (DTI) is a neuroimaging technique that has been extensively used to study the white matter (WM) of the brain in terms of the so-called "fractional anisotropy" (FA). In general, FA in WM is reduced in neurodegenerative diseases (Assaf and Pasternak, 2008), but increases, e.g., during maturation (Lebel et al., 2008). The mechanism proposed to explain these changes is FA sensitivity to the density, myelination and other properties of axons (Tournier et al., 2011). In the context of learning, FA is a valuable candidate for investigating brain plasticity. For example, elevated FA has been reported after specific forms of training, such as juggling (Zatorre et al., 2012). However, FA exhibits poor specificity since it is sensitive to factors on both the microscopic and macroscopic levels (Tournier et al., 2011). In learning studies, the properties of interest, such as axon diameter, myelin thickness and axon packing density all contribute to FA, but it is also very sensitive to the axonal orientation dispersion (Alexander et al., 2001; Nilsson et al., 2012). This effect is illustrated in Figure 1, and can be conceptually understood by imagining a voxel containing multiple highly anisotropic microscopic environments in which the diffusion process can be described by a diffusion tensor. If these tensors are randomly oriented, diffusion on the voxel scale will appear isotropic. Thus, the observed FA will be zero, despite the presence of anisotropic diffusion on the microscopic level. This is an effect of tensor averaging, which always reduces the FA (Westin et al., 2002). The use of FA as a biomarker in regions of crossing, kissing, or fanning fibers is therefore limited because effects of axon architecture are entangled with effects of orientation dispersion. Adequate use of FA may thus be limited to regions of coherent white matter, which account for <10% of the WM in the brain (Vos et al., 2012). The use of FA is also dubious in gray matter (GM) due to the large orientation dispersion of dendrites (Shemesh and Cohen, 2011).

Effects of orientation dispersion and microscopic anisotropy can be disentangled by extending the single pulsed-field-gradient (PFG) acquisition used in DTI to include double PFGs (Mitra, 1995). Several methods have been

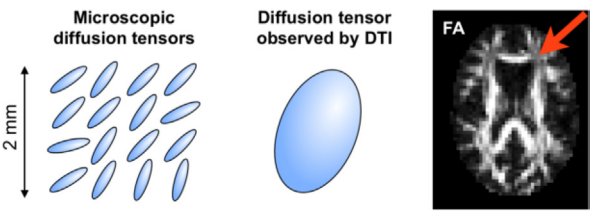

Figure 1: Effects of orientation dispersion. The left panel depicts microscopic diffusion tensors that are anisotropic but having varying directions. DTI yields the average tensor (middle panel), which is less anisotropic than the microscopic tensors. This effect results in low FA in regions of crossing fibers (right panel, red arrow).

proposed for quantification of microscopic anisotropy from double PFG experiments (Lawrenz et al., 2010; Jespersen et al., 2013). Such methods compare the MR signal acquired with the two blocks either in a parallel or perpendicular orientation, resulting in anisotropic or partially isotropic diffusion encoding, respectively. Lasič et al. (2014) proposed an alternative that involved complete instead of partial isotropic encoding, based on magic angle spinning of the q-vector (qMAS; Eriksson et al., 2013). Here, we demonstrate quantification of microscopic anisotropy (μFA) in a healthy volunteer using the qMAS technique, and we elucidate how the measures of anisotropy (μFA and FA) respond to various changes in micro-architecture. This novel method is applicable in research using clinical MRI scanners, and yields information that is unavailable when using conventional DTI.

THEORY

The MR signal S from a diffusion experiment can be regarded as the Laplace transform of a distribution of diffusion coefficients $P(D)$, according to

$$S(b) = \int_0^\infty P(D)\exp(-bD)\,dD \tag{1}$$

This relationship can be approximated according to

$$S(b) \approx \exp\left(-bm_1 + \frac{1}{2}b^2\mu_2\right) \tag{2}$$

where m_1 and μ_2 are the first moment and second central moment of $P(D)$, i.e., the expected value and variance of $P(D)$. The parameter m_1 is also referred to as the apparent diffusion coefficient (ADC), and μ_2 is related to the apparent diffusional kurtosis (ADK) acquired in diffusional kurtosis imaging (DKI) (Jensen et al., 2005) according to

$$\mu_2 \approx \frac{1}{3}m_1^2\mathrm{ADK} \tag{3}$$

The microscopic anisotropy can be estimated by considering the difference in μ_2 when estimated from data acquired with conventional and partial or complete isotropic diffusion encoding (Lasič et al., 2014; Westin et al., 2014). In the following analysis, we will assume that $S(b)$ is independent of the orientation of the sample, i.e., that $P(D)$ is independent of the direction of the diffusion encoding gradients. If this is not the case, as in white matter, we can construct the powder averaged signal by averaging the MR signal across multiple rotations of the diffusion encoding gradients. Thus, the MR signal will appear to originate from an object with isotropic diffusion on the macroscopic scale.

The key to estimating the microscopic anisotropy from an object with macroscopically isotropic diffusion is to analyze how the variance of $P(D)$ depends on the configuration of encoding blocks. The encoding can be anisotropic, partially isotropic, or completely isotropic, which we refer to as the shape of the diffusion encoding tensor (Westin et al., 2014). For isotropic encoding, where we denote the encoding tensor as $\mathbf{I}$, we can define $\mu_2(\mathbf{I}) = \mathrm{V}[\mathrm{P}(D|\mathbf{I})]$. This quantity represents the variation in mean diffusivities in the sample, since an experiment with isotropic weighting encodes the mean diffusivity of each microenvironment. A high value of $\mu_2(\mathbf{I})$ can occur, for example, in voxels that contain both tissue and cerebrospinal fluid (CSF), which have distinctly different mean diffusivities. In the analysis of the case with an anisotropic encoding tensor, denoted $\mathbf{N}$, we assume that

$$P(D\,|\,\mathbf{N}) = P(D\,|\,\mathbf{I}) * \mathrm{ARF}(D) \tag{4}$$

where * denotes a convolution and ARF(D) is the so-called anisotropy response function (Szczepankiewicz et al., 2015). From probability theory, it follows that the variance of the ARF, defined by $\Delta\mu_2 = V[\mathrm{ARF}(D)]$, is given by

$$\Delta\mu_2 = \mu_2(\mathbf{N}) - \mu_2(\mathbf{I}) \tag{5}$$

In case all microenvironments exhibit isotropic diffusion, the variance of $P(D)$ is independent of the shape of the diffusion encoding tensor, i.e., $\mathrm{ARF}(D) = \delta(D)$ and $\Delta\mu_2 = 0$. However, the situation is different when the diffusion is anisotropic in the microenvironments. Imagine a measurement performed on an ensemble of microscopic environments where the diffusion is well described by cylinder-symmetric diffusion tensors that share the same set of eigenvalues (i.e., identical axial and radial diffusivity, denoted AD and RD). The goal is then to estimate the fractional anisotropy of these microscopic tensors, given by

$$\mathrm{FA} = \left(\frac{\mathrm{AD} - \mathrm{RD}^2}{\mathrm{AD}^2 + 2\mathrm{RD}^2}\right)^{1/2} \tag{6}$$

If the microscopic tensors are randomly oriented, AD and RD cannot be assessed directly. However, their weighted sum and difference is reflected in the ADC and the variance of the anisotropy response function determined according to

$$m_1 = \frac{1}{3}\mathrm{AD} + \frac{2}{3}\mathrm{RD} \tag{7}$$

and

$$\Delta\mu_2 = \frac{4}{45}\left(\mathrm{AD} - \mathrm{RD}\right)^2 \tag{8}$$

Combining Eqs 6–8 yields

$$\mu\mathrm{FA} = \sqrt{\frac{3}{2}}\left(1 + \frac{m_1^2}{\frac{5}{2}\Delta\mu_2}\right)^{-1/2} \tag{9}$$

At this point, we denote the derived quantity μFA, since it will be dependent on microscopic anisotropy, but not on orientation dispersion. In fact, complete orientation dispersion, induced by powder averaging if required, is a prerequisite for this analysis. However, a tensor-based approach may permit a similar analysis without this constraint (Westin et al., 2014).

METHOD

The qMAS technique was implemented on a Philips Achieva 3 T system. Diffusion MRI with both anisotropic and isotropic encoding was performed, employing ten equidistant *b*-values between 100 and 2800 s/mm^2. Isotropic encoding was achieved with a numerically optimized qMAS approach (Topgaard, 2013). The echo time was 160 ms, repetition time 2000 ms, acquisition matrix 96 × 96, spatial resolution 3 mm × 3 mm × 3 mm, partial Fourier factor 0.8, and SENSE factor 2. Anisotropic encoding was performed in 15 directions for each *b*-value, using harmonically modulated gradients according to Lasič et al. (2014). The isotropic encoding was repeated 15 times per *b*-value in order to acquire an equal number of images for both types of encoding tensors. The total scan time was 10:12 min. DTI was performed on the data acquired with anisotropic encoding to obtain maps of FA, while the full data was used to estimate μFA using the methods described by Lasič et al. (2014). The analysis was also applied on data simulated by calculating the MR signal from a collection of diffusion tensors in order to demonstrate the theoretical response of FA and μFA when increasing RD with fixed AD, increasing orientation dispersion, and varying fiber crossing angle.

RESULTS

Maps of FA and μFA are shown in Figure 2. In WM, the μFA is uniform and high. In GM, it is lower but above zero, which indicates lower but still detectable levels of microscopic anisotropy. Qualitatively, the FA and μFA maps differ where high orientation dispersion is expected, for example, in regions of crossing fibers. The voxel-wise correlation between μFA and FA (Figure 2, right panel) shows that high FA is associated with high μFA, but not vice versa. Values of μFA above 0.8 were found exclusively in the WM.

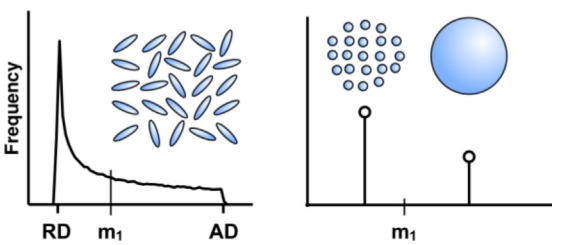

Figure 2: Illustration of two cases with different microscopic anisotropy, but identical macroscopic anisotropy. The cases have equal m_1 and $\mu_2(\mathbf{N})$, and would be indistinguishable by DTI and DKI. However, $\mu_2(\mathbf{I})$ is zero in the left case, since isotropic encoding would change the distribution into a delta-function at m_1. In the right case, $\mu_2(\mathbf{I}) = \mu_2(\mathbf{N})$, which would yield μFA = 0.

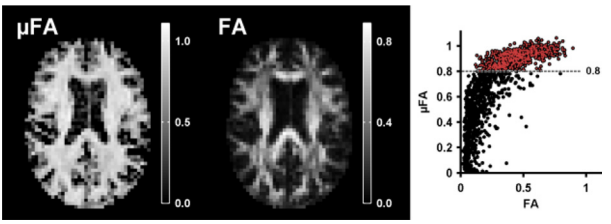

Figure 3: Left: parameter maps of μFA and FA. Right: correlation between FA and μFA. Voxels where μFA >0.8 appeared exclusively in white matter.

Simple simulations demonstrate the response of μFA and FA to three common scenarios: gradually increasing radial diffusivity corresponding to dysmyelination (Song et al., 2002), increased levels of orientation coherence, and an increasing fiber crossing angle. All three scenarios affect FA, while μFA was affected only by the simulated dysmyelination.

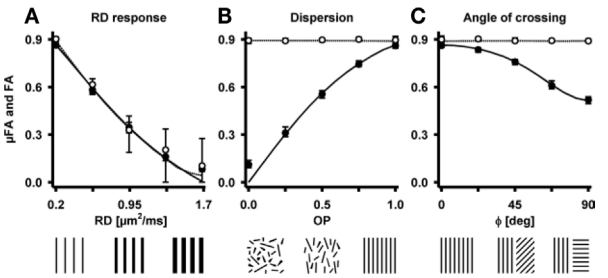

Figure 4: Simulations comparing the response of μFA and FA to varying radial diffusivity **(A)**, coherence **(B)** and the angle at which two fiber populations cross **(C)**. Error bars represent the interquartile range expected from an experiment under noise conditions typical for MRI.

CONCLUSION

We have demonstrated the feasibility of measuring microscopic anisotropy in terms of the μFA from measurements with anisotropic and isotropic encoding. Our results show that the contrast found in FA maps from DTI is strongly modulated by the amount of orientation dispersion and less so by the underlying microscopic anisotropy. Unlike the FA, μFA permits quantification of the diffusion anisotropy even in complex WM configurations. The simulations showed that μFA exhibited a more intuitive interpretation than FA and is more specific since it only responded to elevated values of RD. Since the total scan time of the protocol is acceptable for human use, we believe that μFA imaging can enhance the quality of studies of brain tissue in, e.g., studies of brain plasticity and learning.

ACKNOWLEDGMENT

This research was supported by the Swedish Foundation for Strategic Research (grant no. AM13-0090).

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Tractometry and the hunt for the missing link: a physicist perspective

Derek K. Jones[1,2*] and Markus Nilsson[3]

1. Cardiff University Brain Research Imaging Centre (CUBRIC), School of Psychology, Cardiff University, Cardiff, UK

2. Neuroscience and Mental Health Research Institute, Cardiff University, Cardiff, UK

3. Lund University Bioimaging Center, Lund University, Lund, Sweden; markus.nilsson@med.lu.se

**Cardiff University Brain Research Imaging Centre (CUBRIC), School of Psychology, Cardiff University, Park Place, Cardiff CF10 3AT, UK; jones27@cf.ac.uk*

SUMMARY

This article focuses attention on the pressing need to think carefully and deeply about the current state of the art in using measurements of tissue microstructure derived from MRI to explain individual differences in brain function, electrophysiology and or cognitive function[1]. Although initial effort in the application of microstructural imaging was on voxel-based metrics derived from diffusion tensor magnetic resonance imaging (DT-MRI), such as fractional anisotropy (FA) and the mean diffusivity (MD), there is increasing realisation of the limitations of this approach both in terms of biological specificity and in terms of interpretability of any results that emerge. This has led to the development of alternative approaches that are (i) looking at topologies of networks derived from diffusion-MRI-based fibre-tracking approaches, (ii) adopting "advanced" diffusion MRI metrics that go beyond the tensor model, or (iii) looking at data from non-diffusion-based MRI contrasts, such as those based on magnetization transfer, multi-component relaxometry, or susceptibility-weighted imaging. With the increasing availability of methods to extract such metrics, and ease of access, it should be stressed that our application of such methods is outpacing our understanding of what aspect of biology the metrics are actually capturing. As such, there is a danger of operating in an unprincipled and unstructured fashion. This article argues that the "missing link" is a non-invasive neuroimaging metric that is not only well understood, but which also can reasonably be expected to explain variance in brain function from a biological perspective, rather than a metric that is used purely as a matter of convenience.

Keywords: axons, connectivity, DTI, graph theory, microstructure, myelin, networks, tractography, tractometry, white matter

INTRODUCTION

The UK national rail network

The United Kingdom is served by a "national rail network" that comprises mainline and local train services that link small villages to large cities. An internet search for images of the "UK rail network" yields multiple schematic depictions of the available routes and connections between different stations distributed throughout the UK. Any of these schematic diagrams would lead an alien visitor to believe that all lines in the network are largely equivalent. In other words, the ability to travel from one station to another station is the same no matter where you are in the network. In other words, all train lines are created equally. However, anyone who has attempted to use this network on a regular basis knows that the ability to use the trainline is variable, with "leaves on the line" being a perennial problem, now part of humour in UK train-users (see http://www.networkrail.co.uk/timetables-and-travel/delays-explained/leaves/).

What this tells us is that while it is *necessary* to have a schematic map of the network connections in order to understand possible routes that one may take to visit different parts of the network, the information is *insufficient* to determine *a priori* how effectively one may use any particular parts of the network, and therefore the efficiency of using the network as a whole. Thus, while the designer of a rail network may be gratified to have minimized the travel distance between any two train stations, in terms of time or number of train changes required, and perhaps at the amount of steel needed to make that network, suggesting that the *layout* of the network is efficient, the passenger's perspective is very different. If the condition of the track is such that the passenger is unable to travel efficiently, incurs unacceptable delays, misses a vital interchange, or does not arrive at the same station at the same time as a friend travelling on another route, frustration is ensured. As far as the passenger is concerned, there is a dysfunction of the network.

1 Henceforth, we will use the term "function" as a generic term to include measurements of activity as observed by neuroimaging, measurements of electrophysiology and/or performance on a task, unless we specify a specific aspect of "function."

Brain networks

Networks are studied increasingly in neuroscience (Sporns, 2010), whether these are derived from mapping statistical relationships between time-varying "functional" signals (Biswal et al., 1995; Schoffelen and Gross, 2009; Friston, 2011), correlation of structural attributes such as cortical thickness (He et al., 2007; Chen et al., 2008), or by inferring continuous white matter pathways between cortical nodes using white matter *tractography*. Here we focus on the latter. In most cases, the researcher employs an algorithm that will use diffusion MRI data to derive discrete estimates of a continuous white matter pathway, either in a deterministic or probabilistic fashion, to infer a continuous trajectory, which is then asserted as a "tract" or a "connection" (Jones, 2008; Behrens and Sporns, 2012).

If a deterministic algorithm is used, then one obtains something akin to a national rail network map, with each connection being represented "democratically." With a sufficient number of pathways thus reconstructed, the data can be subjected to a graph theory analysis (Iturria-Medina et al., 2007; Hagmann et al., 2008; Bullmore and Sporns, 2009; Kaiser, 2011), allowing one to compute measures of network integration and segregation, including measures such as clustering coefficient, minimum path length, "small-worldness" and efficiency (Hagmann et al., 2008; Bullmore and Sporns, 2009; Kaiser, 2011). Using this approach, researchers have found differences in graph theory metrics in disease (Petrella, 2011; Griffa et al., 2013) and associations between graph theory metrics and cognition, for example (e.g., van den Heuvel et al., 2009; Ajilore et al., 2014).

When such analyses are performed purely on the connections themselves by considering "binary" edges (present or not), one might be surprised by these results since they implicitly assume that the only difference between individuals is in the *layout* of the wiring, and that any individual differences in the *make up* of the white matter fibres is less interesting/irrelevant/non-existent (or, implicitly, correlates strongly with the presence of an edge in the graph). They implicitly disregard as uninteresting any individual differences in the make up of the fibres. Of course, such a supposition makes no sense whatsoever when considering single pathways between two nodes, or a single fascicle, such as the arcuate fasciculus when studied in a language paradigm. Discarding the microstructural information (and even that on shape/length) and reducing the information to "there is a connection" leaves no variance in information. In addition, the criteria by which the binary edges are determined are often arbitrary and can have a huge effect on graph theory analysis results (Langer et al., 2013; Drakesmith et al., 2014).

In practice, of course, researchers have adopted several approaches to "weight" the edges of a graph. The first class of approach is a "frequency" approach. Some apply information obtained from a probabilistic tracking algorithm, and use the variance in the number of successfully reconstructed pathways between two nodes to explore covariance with "function" (e.g., Parker et al., 2005; Broser et al., 2012). The "probabilistic tractography score" is often interpreted as a quantitative marker of "connectivity" in this context. This approach to obtaining inter-individual or inter-tract variances has also been employed in graph theory analyses to "weight" the edges of the graph (Vorburger et al., 2013; Weiler et al., 2014). A closely related approach to probabilistic tracking is simply to use cortical nodes with an extended area, i.e., containing multiple voxels, and count how many "streamlines" can be reconstructed successfully between the nodes, often referred to as "streamline count" (Wang et al., 2012; Hecht et al., 2013). This is, however, a challenging parameter to work with due to sources of bias such as length and curvature (e.g., Jones, 2010).

The second class of approach is to sample localised quantitative metrics of tissue microstructure (and thus obtain a distribution of the metric) along a particular pathway or fascicle, i.e., graph edge (e.g., Jones et al., 2005a,b). To this end, the most commonly used metrics are those derived from the tensor model, i.e., fractional anisotropy (Basser and Pierpaoli, 1996; Pierpaoli and Basser, 1997), mean diffusivity, "longitudinal" and "radial" diffusivities. For critical perspectives on these metrics see Wheeler-Kingshott and Cercignani (2009) and Szczepankiewicz et al. (2015). A small number studies have used other indices such myelination as estimated by magnetization transfer imaging (e.g. van den Heuvel et al., 2010). Looking at individual fasciculi, this approach is termed "tract-specific" analysis (Kanaan et al., 2006), while in graph-based analyses it is referred to as a "weighted graph" approach.

Network usage

At this stage, we should return to our analogy of the passenger on the train network and the importance of being able to predict our ability to travel along the train track as intended, so that we might arrive at the right place at the right time, and synchronise with others travelling on the same network. It is this aspect that will explain a large amount of variance in differences in efficiency between rail networks or in brain function between individuals. The question is this: to what extent does the probabilistic score, streamline count or tensor-based metric, either on a tract-specific basis, or in a graph analysis, give us *relevant* information?

The probabilistic score is simply how many times one can reconstruct a pathway between two points (Jones, 2010). While microstructural information may be used in the derivation of those pathways (e.g., low anisotropy used to terminate the propagation of a particular instance of a streamline), the probabilistic score does not provide a measure of the microstructural make up *per se*. DT-MRI-based metrics are heavily influenced by the microstructural make up of the tissue, and so perhaps take us one step closer to ascertaining the *quality* of the connection. However, as has been discussed many times in the past, the shape of the tensor is influenced by many factors including "interesting"

sources of variances, such as axonal density, diameter, myelination (Beaulieu, 2002), but one "uninteresting" source of variance dominates, i.e., the intra-voxel orientational dispersion or "*architectural paradigm*" (Beaulieu, 2002; Szczepankiewicz et al., 2015). To the best of our knowledge, the relative orientation of one axon to its neighbours in a voxel has no impact on its ability to carry an action potential. Thus, as previously noted, we should not be surprised when a DT-MRI-based metric does not explain variance in brain function. Rather, we might be more surprised when it does! It is, perhaps, in these instances that individual differences in "uninteresting" sources of variance are small, e.g., the function under assessment is reliant on information along a fibre pathway that is relatively *invariant* in intra-voxel orientational dispersion. It is in these situations that the DT-MRI metrics may be biased more towards the interesting sources of variance, such as myelin and axon morphometrics, explaining our previous proposal that "diffusion tensor MRI does well only some of the time" (De Santis et al., 2014).

A consequence of this argument would be that DT-MRI is probably more informative when comparing groups that have similar overall brain structure than when comparing widely different brains. For example, interpreting DT-MRI when comparing brains of Alzheimer's disease patients to healthy controls is less informative than comparing the brains of a strictly defined control group (e.g., males, 25 years old, minimal variation in intracranial volume) before and after intervention such as cognitive training.

Tractometry and going beyond the tensor

Several DT-MRI studies have demonstrated a significant group difference or within-group microstructure-function correlation when taking the average parameter along a specific pathway (reconstructed with tractography) when a voxel-based search of the same data reveals nothing significant (Keedwell et al., 2012; Postans et al., 2014; Bracht et al., 2015). While only conjecture, this is likely to be attributable to the increased statistical power derived from averaging along the tract, effectively grouping the estimates from multiple noisy voxel-wise estimates, rather than considering each voxel in isolation. This then motivates the need for "tractometry" (Bells et al., 2011), which is the derivation of microstructural metrics along specific white matter pathways, whether it is averaging the parameter across the whole tract, or just a segment thereof.

The missing link: current status

So, what of the "Missing Link"? The provocative title refers to the fact that we have yet to come up with a principled white matter metric to explain variance in brain function. More specifically, to the best of our knowledge, there is a marked absence of any formal *theoretical* link between individual differences in any MRI-derived measure of tissue microstructure and individual differences in brain function. Thus, for example, while DT-MRI does indeed do well "some of the time" (De Santis et al., 2014) in that, for example, there are numerous reports of correlations between DT-MRI metrics and cognition (Johansen-Berg, 2010; Kanai and Rees, 2011; Roberts et al., 2013a), but doubtless there are at least an equal number of studies that have been conducted that have NOT found any correlation or group difference which have not been reported in the literature. Further, only a small number of studies with "counter-intuitive" results have appeared in the literature (e.g., choice reaction time correlating positively with FA in the visual pathway (Tuch et al., 2005), or negative correlation between years of training in karate and FA (Roberts et al., 2013b). While not specific to diffusion MRI, or even neuroimaging more broadly this positive reporting bias (Fanelli, 2010; Ioannidis, 2011; Francis, 2014; http://www.badscience.net/2011/08/brain-imaging-studies-report-more-positive-findings-than-their-numbers-can-support-this-is-fishy/) and subsequent plethora of positive results in the literature clearly has an impact on researchers coming into the field looking for a structural substrate. When coupled with the modest data acquisition requirements for a DT-MRI experiment, increasing availability of easy-to-use and push-button analysis packages, the increasing ubiquity of DT-MRI based studies is understandable. The exquisite sensitivity of diffusion-based metrics, and thus their tendency to yield some form of difference or correlation in many instances, makes them particularly attractive. Granted, in cases where a correlation or group difference is found, they yield *some* insight in that they show that *something* in the white matter explains differences, but can go no further. Given the degenerate nature of the metrics, one is simply unable to say whether this "*something*" relates to axonal morphometrics, myelin morphometrics, some combination of the two or even, perhaps, something entirely unrelated such as subject motion (Yendiki et al., 2013).

Thus, while DT-MRI yields sensitivity, it comes at a price of lack of biological *specificity.* It is this degeneracy that has partly motivated the gradual adoption of neuroimaging approaches to offer increased biological specificity, attempting to hone in and capture variance in just one particular attribute of neural microstructure (Alexander et al., 2011; Assaf et al., 2013), e.g., "axonal" markers (Assaf et al., 2004, 2008; Alexander et al., 2010; Nilsson et al., 2013a), or "myelin" markers (MacKay et al., 1994; Henkelman et al., 2001; Laule et al., 2007; Deoni et al., 2008; Wharton and Bowtell, 2012, 2014; Liu et al., 2014; Haacke et al., 2015), summarised below. Ultimately, the hope is that by increasing the biological specificity, one might also increase the sensitivity by being able to invest scan-time resources in the most informative metrics.

Summary of microstructural imaging approaches and what they offer

Diffusion MRI utilizes diffusing water molecules as a probe of tissue microstructure. Diffusion tensor MRI, diffusional kurtosis imaging (DKI) (Jensen and Helpern, 2010; Wu and Cheung, 2010), and q-space diffusion MRI

(King et al., 1994; Assaf et al., 2002; Cohen and Assaf, 2002) are techniques that use statistical tools to model and extract features of the molecular displacement probability distribution, or diffusion propagator. DT-MRI yields the average diffusion tensor, while DKI and related methods yield the intra-voxel variance of diffusion coefficients or tensors, in addition to the average. In q-space MRI, features such as the width of the diffusion propagator are extracted, which can be associated with axon diameters (Assaf et al., 2008; *Alexander et al., 2010*). While these techniques are highly sensitive, they cannot tell which specific feature is responsible for a certain level of change, because they provide single metrics that cannot disentangle the contributions of features of the tissue such as axonal orientation, density, myelination *et cetera* (Cohen and Assaf, 2002; Szczepankiewicz et al., 2015).

Biophysical models hold the potential to at least partly provide the missing link. The CHARMED model (Yendiki et al., 2013) predicts the diffusion-weighted MRI signal in terms of axon density and average diameter, while extensions such as AxCaliber (Assaf et al., 2004) extend the average diameter with a distribution described by its mean and variance. The NODDI model (Zhang et al., 2012) assumes an effective axonal diameter of zero, but includes axonal (or "neurite") orientation dispersion. Estimation of the axon diameter is demanding in terms of MRI hardware performance (Dyrby et al., 2012; Setsompop et al., 2013; McNab et al., 2014; Huang et al., 2015). Just as in light microscopy, diffusion MRI has a resolution limit below which axon diameters cannot be reliably quantified (Nilsson et al., 2013a). Although the resolution limit does depend on the analysis model, protocol and the design of the gradient waveforms, it is ultimately determined by the maximal amplitude of the magnetic field gradient that the scanner can produce. Current clinical scanners and present analysis models do not permit accurate quantification of axon diameters below 2–4 μm; below this limit axon diameters are inseparable from zero. This is why the effective diameter is assumed as zero in the NODDI model, given that most axons are smaller than the limit. All of these three models (CHARMED) (Assaf et al., 2004), AxCaliber (Assaf et al., 2008) assume slow exchange between the intra-axonal and the extracellular spaces, but inter-compartmental exchange can under limited circumstances be modelled and estimated using the modified Kärger model (Nilsson et al., 2013a) and extensions such as filter-exchange imaging (FEXI) (Lasič et al., 2011; Nilsson et al., 2013b). From a more general perspective, the diffusion MRI signal can be modelled using multiple components, each described by a scalar or a distribution on the anisotropy, orientation and the size or diffusivity of the component. When testing a multitude of different models with different compositions on in vivo or ex vivo data, three tissue components are typically required (extracellular, intracellular, and water confined in spherical cells or freely diffusing cerebrospinal fluid), but it is not entirely clear that the data supports reliable estimation of the axon diameter (Ferizi et al., 2014). Parameters such as axon density, the amount of extracellular "free" water, and orientation dispersion, have higher explanatory power and can be reliably quantified using optimized protocols (Zhang et al., 2012). These parameters can be beneficial for localizing brain regions impacted by diseases, but improved models and hardware (e.g. Nilsson et al., 2013a,b; McNab et al., 2014; Huang et al., 2015) will be needed to provide accurate estimates of axon diameters below the current resolution limit.

Methods other than diffusion MRI can also contribute to the "missing link". Such methods take advantage of relaxometry (MacKay et al., 1994; Laule et al., 2007; Deoni et al., 2008), magnetization transfer (Wolff and Balaban, 1989; Henkelman et al., 1993, 2001; Sled and Pike, 2001; Xu et al., 2014) and magnetic susceptibility (Wharton and Bowtell, 2012, 2014; Haacke et al., 2015). In their basic implementation, these techniques yield voxel-averaged metrics that are sensitive to changes in the tissue structure, but that lack specificity in the same manner as FA from DT-MRI does. Relaxometry is sensitive to the proton density (PD) and the longitudinal and transverse relaxation rates (T_1 and T_2, respectively), which reflect on the chemical environment of water molecules. Multi-echo experiments demonstrate a distribution of these values within the voxels (MacKay et al., 1994), where different T_1 and T_2 values can be associated to myelin water, intracellular water, and extracellular water. Methods such as multi-component driven equilibrium single pulse observation of T_1 and T_2 [mcDESPOT (Deoni et al., 2008)] provide a means to estimate these compartment-specific values in clinically realistic times. The water fraction with short T_2 has been designated as the myelin water fraction (MWF) (MacKay et al., 1994). Since brain function may be modulated by the myelin content but not the white matter T_2-value, the MWF is one step closer to contributing to the "missing link" than T_2. However, the MWF is blind to whether a change in myelin content occurs in small or large axons, and may thus not be sufficient to predict individual differences in brain function. Another approach to quantify white matter content is to utilize magnetization transfer (Wolff and Balaban, 1989; Henkelman et al., 1993, 2001; Sled and Pike, 2001; Ramani et al., 2002), where the MR signal is sensitized to macromolecular content, i.e., myelin density, by a process where macromolecular protons (such as those found in myelin) are saturated by an off-resonance ["magnetisation-transfer" (MT) pulse] while in constant exchange with free water. By comparing the MR signal with and without the application of the MT pulse, the magnetization transfer ratio (MTR) can be obtained (Wolff and Balaban, 1989). High MTR in WM is believed to be associated with the proteins and lipids in myelin. However, the MTR value depends on many aspects of the protocol in use, including the RF pulses, B_0 and B_1 homogeneity (particularly at higher field strengths) as well as intrinsic MR properties such as the T_1. This led to the development of several approaches to model out these additional sources of variance, to move toward a quantitative parameter (the macromolecular proton fraction) that gets closer to a physiological property of tissue, and therefore one step closer to the assembling the missing link. Finally, quantitative susceptibility mapping (e.g., Wharton and Bowtell, 2012, 2014; Liu et al., 2014; Haacke et al., 2015) shows promise to add another dimension to imaging of brain structure and function, especially at ultra-high field strengths. Due to the geometrical arrangement of myelinated axons, and the differential in magnetic

susceptibility between white matter components and surrounding tissue, they distort the surrounding magnetic field in a characteristic way, which may be utilized to estimate properties such as axonal orientations (Wharton and Bowtell, 2012) and potentially also axonal morphometrics such as myelin thickness (Wharton and Bowtell, 2014). Remarkably, few attempts have been made to provide a joint model of white matter that predicts the outcome of diffusion, relaxation, magnetization transfer and susceptibility mapping experiments simultaneously.

In contrast to DT-MRI, these approaches have not yet found widespread adoption in neuroscience, which is likely attributable to several reasons. Firstly, the neuroscience community has wholesale adopted diffusion tensor MRI. Consequently, in any study reporting on white matter microstructure, reporting the fractional anisotropy, for example, is expected. Moreover, as previously noted, given the likely "success" in getting a positive result with DT-MRI, any reluctance to relinquish a grip on DT-MRI would be completely understandable. Additional metrics require additional acquisition time, invoking additional scan costs, resource contention, and participant tolerance. Secondly, many of these approaches are still under active development. Thus, while for DT-MRI there are commonly found strategies for data acquisition (e.g., $b = 1000$ s/mm^2, 30–60 uniformly distributed directions) (Jones et al., 1999) and standardized protocols (Jones and Leemans, 2011), metrics of anisotropy (FA being the most prevalent), and data analysis (e.g., Smith et al., 2006; Leemans et al., 2009), the same cannot be said of the other metrics. Third, even if there was consensus, there is relatively limited availability of user-friendly interfaces. Fourth, while DT-MRI is widely used *despite* us not really having an understanding of what the metrics are telling us from a biologically specific perspective, it seems that the community developing these alternative approaches is more hesitant in releasing them for general use, while efforts to understand and interpret what they are telling us are on-going.

Quantitative, interpretable metrics – now what?

Consider a Utopian world, in which we have developed fully robust non-invasive methods to quantify axonal morphometrics (including axon diameter, axon density, membrane permeability), myelin metrics (including myelin thickness), g-ratio (Stikov et al., 2011; Melbourne et al., 2014; Stikov et al., 2014; Campbell et al., 2014) and so forth. Moreover, each metric has been validated using more invasive methods, and finally there is a consensus on the optimal way to acquire, pre-process and analyse the data. What then? We would then be equipped with a set of tools to quantify disparate attributes of white matter microstructure and would be in the position of being able to repeat the exercise of looking for correlations between these new metrics and aspects of brain function. We may, for example, find a positive association between a myelin metric and task performance, or between coherence in electrophysiological recordings between two cortical regions and the mean axon diameter in the pathway connecting them. This gives us yet further insights into associations between microstructure and function – but remains unprincipled. In other words, to the best of our knowledge, there is no theory that links task performance to axon diameter or myelin thickness. Granted, we understand that action potentials are conducted more quickly in axons with larger diameters (Hursh, 1939), which has recently been explored with diffusion MRI and EEG by Horowitz et al. (2014), and that myelin further increases the conduction velocity (Waxman and Bennett, 1972; Waxman, 1980). Moreover, we understand that there is a theoretical optimal ratio of the outer diameter of the axon (axon + myelin) to the inner axon diameter, characterized by the "g-ratio" (Rushton, 1951), in that the conduction velocity is optimised when the g-ratio is 0.6. However, consider a thought experiment in which all axons happened to be of the same diameter across our cohort. In this special case, one may reasonably anticipate that deviations from the optimal g-ratio, and therefore (in our *Gedanken* that all axon diameters are the same), variance in our myelin metric might be expected to explain variance in conduction velocity, albeit non-linearly since both more and less myelin results in a departure from the optimal g-ratio. However, the likelihood of there being no intra- or inter-individual variance in axon diameter seems particularly low, especially in light of histological evidence (e.g. Aboitiz et al., 1992). If, as is more likely, there is variance in axon diameter, then the impact of any additional variance in myelin metrics may be less significant. It is, of course, important to consider the distance between nodes of Ranvier, which also impacts on conduction through the axon. To the best of our knowledge, there has been no MR-based approach that allows quantification of the inter-nodal distance, although it has been shown that inter-nodal distance correlates with axon diameter, at least in healthy rabbits (Hess and Young, 1952). Moreover, it is not just the mean but also the *distribution* of axon diameters that is likely important to consider. In myelinated axons, a diameter of 0.7 µm appears to optimize the energy per transmitted bit of information (Perge et al., 2009). The presence of large axons, whose diameter correlates with brain size between species in contrast to mean diameters of myelinated and unmyelinated axons (Wang, 2008; Wang et al., 2008) indicates they must support another type of function than small axons that motivate the extra energy required to use them.

This one example presupposes that conduction velocity is the primary target of interest. However, the conduction *time*, in other words, the time to propagate a signal from one region to another will also be a function of the length of the connection between them. This, alone, argues against a voxel-wise search for correlations between these microstructural metrics and function. Now, suppose that we have a forward model [e.g., based on cable theory (Tasaki and Matsumoto, 2002)] that allows us to predict conduction velocities from tractometry of relevant quantities and, through robust tractography, the length of a pathway, to enable prediction of conduction delays. What then? Is there a theory that indicates that the conduction delay should always be minimised? And if so, are

conduction delays "absolute" (so that one might expect correlation between conduction delay and task performance across a cohort), or "relative" (i.e., different individuals operate at overall different "rates" – so that we should not anticipate correlations across a cohort). Fields argues that what is important for optimal brain function is not that the conduction velocity is as high as possible, or that conduction delays are minimized, but that signals arriving from different parts of the brain arrive *in synchrony* (Stanford, 1987; Sugihara et al., 1993; Pajevic et al., 2014). Extrapolating, one might expect that deviations from synchrony may lead to deviations from optimality and therefore reduction in performance/function. However, deviation from synchrony does not necessarily mean deviation from optimality. Many EEG/MEG task elicit reductions in synchrony as well as increases, with the best known example being an increase in alpha synchrony when eyes are closed compared to open. More synchronous signals have lower entropy and suggest that increase in synchrony reflect *less* information processing (e.g. Anderson and Jakobsson, 2004; Qi et al., 2004). One possibility is that the conduction velocities of different fibre populations coming into an area of cortex should be tailored to maximise *the amount of information* received by the cortex, rather than synchrony *per se.*

The complexity of the problem of deriving the missing link in the "Tractometry" framework is further exacerbated by evidence for conduction velocity varying *along* white matter pathways (Baker and Stryker, 1990; Traub and Mendell, 1988); thus the tissue should be characterised at each point of every axon and used in the forward model. Recently, Tomassy et al. (1988) have also reported evidence for wide variation in myelin content along single axons, further exacerbating the problem of establishing the missing link between measurements of microstructure at the voxel scale to function. Thus, the ability to use microstructural data in a forward model to predict accurately individual differences in function in terms of differences in white matter structure seems a long way off.

CONCLUSION

In conclusion, while diffusion tensor imaging is a sensitive technique, and therefore yields useful information that *something* in the white matter might be different, it lacks the biological specificity needed to gain any further insight. This is acknowledged and many groups are developing complementary approaches to provide higher biological specificity. While promising, these have not yet enjoyed widespread dissemination nor, therefore, widespread adoption. There would be considerable change of behaviour needed for these metrics to REPLACE diffusion tensor imaging.

However, even if adopted into widespread practice, we would be only be able to relate individual differences in function to differences in specific white matter attributes in a phenomenological manner at best. A more principled approach that generates forward models, predicting function from structure, and states *a priori* exactly how and why a particular imaging metric (or combination thereof) will explain variance in a particular aspect of brain function, would fashion the deployment of advanced microstructural imaging in neuroscience into a more rigorous science. However, before this can happen, much more work is needed on the "hunt for the missing link."

ACKNOWLEDGMENTS

The authors wish to thank Dr Mark Drakesmith, Cardiff University for helpful comments on the manuscript. DKJ is also grateful to the Wellcome Trust, United Kingdom, for supporting this work through an Investigator Award.

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