Longitudinal structural and functional brain changes associated with stuttering improvement by therapy or brain lesion

Dissertation

zur Erlangung des mathematisch-naturwissenschaftlichen Doktorgrades

„Doctor rerum naturalium“

der Georg-August-Universität Göttingen

im Promotionsstudiengang Behavior and Cognition

der Georg-August University School of Science (GAUSS)

vorgelegt von

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Göttingen, 2019

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Tag der mündlichen Prüfung: 26.03.2019

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I. Summary

Stuttering is a speech fluency disorder which is affecting motor speech production and communication in the daily life of persons who stutter (PWS). The involuntarily occurring core symptoms of stuttering are sound and syllable repetitions, sound prolongations and speech blocks (Bloodstein & Ratner, 2008). In addition to these core symptoms, secondary accompanying symptoms like movements of limbs, neck and head as well as facial grimaces can appear (Guitar & McCauley, 2010). PWS repeatedly experience high communicative pressure and psychological strain. Subsequently, they often develop social withdrawal to hide their stuttering. Therefore, a reduced quality of life is measured in some PWS (Carter, Breen, Yaruss, & Beilby, 2017; Kohmäscher, 2017; Natke & Alpermann, 2010). Stuttering therapies enhance speech fluency and support patients in their handling with adverse emotions and attitudes towards their stuttering (Neumann et al., 2016). Even though the effectiveness of different types of intense stuttering therapies has been evaluated on a behavioural level, there is a research gap regarding the long-term effects of intense stuttering therapies on brain structure and function. In fact, to our knowledge, changes of white matter integrity following the participation in an intense stuttering therapy have not been investigated yet.

Therefore, the present thesis investigates the effects of therapy-induced long-term white matter plasticity changes as well as brain activation changes in adolescent and adult PWS. For this purpose, we recruited stuttering patients taking part in the “Kasseler Stottertherapie” (Euler, Gudenberg, A. W. v., Jung, & Neumann, 2009). This is an evidence-based fluency shaping therapy approach accomplished in a group setting and with a high intensity (Euler et al., 2009; Euler, Anders, Merkel, & von Gudenberg, A Wolff, 2016; Euler & Wolff v. Gudenberg, 2000; Neumann et al., 2016). In addition, a case report of a cessation of stuttering after a left cerebellar haemorrhage is presented in this thesis.

In the first study of this dissertation, we used diffusion tensor imaging (DTI) to evaluate long-term therapy-induced changes of white matter integrity in stuttering patients. For this purpose, we added two control groups and compared the longitudinal structural changes of the intervention group with the structural changes of stuttering control participants not taking part in any therapy and healthy control participants. By using Tract-Based Spatial Statistics (Smith et al., 2006; Smith et al., 2007), we investigated changes in fibre integrity within whole-brain and region of interest analyses. Our results show that the effects of therapy in the intervention group were versatile: Referring to the behavioural level, a significant decline of stuttering severity as well as of the impact of stuttering on the quality of life were detected and attributed to the stuttering treatment. Regarding white matter integrity changes, we observed a significant increase of fractional anisotropy (FA) in the left superior longitudinal fasciculus. In contrast to the intervention group, a significant decrease of white matter integrity was found in stuttering and healthy control participants. This white matter decline could have been triggered through the process of ageing. The second purpose of this study was to replicate previous findings of a reduction of white matter integrity in PWS compared to healthy controls (Cykowski, Fox, Ingham, Ingham, & Robin, 2010; Neef, Anwander, & Friederici, 2015; Sommer, Koch, Paulus, Weiller, & Buchel, 2002). We were able to confirm this reduced white matter integrity in right hemispheric brain regions including parts of the inferior longitudinal fasciculus close to the callosal body, cingulum, inferior-fronto-occipital fasciculus and the corticospinal tract. With our study, we provided first evidence that an intense stuttering therapy has the potential to change white matter plasticity in stuttering patients. Future studies are necessary to replicate this result and to relate this outcome to the aetiology of stuttering.

In the second study of this thesis, we evaluated long-term changes in brain activation induced by an intense stuttering therapy and its maintenance phase. We compared brain activation changes in the treatment group with the changes measured

in both control groups (healthy participants and stuttering participants not currently taking part in any stuttering therapy). The research aim was to investigate therapy-induced activation changes and to discuss them with regard to the therapeutic principles of action. The following results were obtained: In comparison to healthy and stuttering control participants, stuttering patients showed an increase of activity in motor (e.g. left and right rolandic operculum) and in cognition and emotion processing areas (e.g. left amygdala, right supramarginal gyrus). The effect of therapy was also traceable on the behavioural level. Only stuttering patients of the intervention group showed a significant decline of stuttering severity and a significantly decreased impact of stuttering on the quality of life. Our results underline the importance of also considering non-motor brain regions meaningful for therapeutic achievements as well as for the aetiology of stuttering.

The third part of this thesis consists of a case report about the cessaction of stuttering after a cerebellar haemorrhage. The 52 years old female patient stuttered since childhood and had taken part in a stuttering-related magnet resonance imaging (MRI) research study at the University Medical Center Göttingen. After taking part in the study, she developed a left acoustic neuroma which was subsequently surgically removed. Postoperatively, the patient presented with a cerebellar haemorrhage and, as a consequence, various neurological symptoms and impairments. After the rehabilitation period, the patient reported a cessation of her stuttering as a consequence of the cerebellar haemorrhage. We became conscious of her clinical course and invited her to a revisited measurement. The aim of this second measurement and the case report were to elucidate neurophysiological processes which are responsible for the cessation of stuttering. For the revisited measurement, we used the same behavioural measurements and functional and diffusion MRI protocols as in the previous study measurement. To compare the (functional) MRI data of the single patient with a reference group, we added a control group with healthy participants and another control group with stuttering

participants to our analyses. The study outcomes were manifold: The conducted lesion analysis indicated a large cerebellar lesion, including approximately 1/5 of the left cerebellum. The tract-based spatial statistics analysis showed a primary white matter decrease caused by the haemorrhage in the lesioned parts of the cerebellum. A secondary white matter impairment was detected in the corpus callosum, right inferior fronto-occipital fasciculus, left anterior thalamic radiation, left cingulum and right posterior corona radiata. The whole-brain functional MRI (fMRI) analysis revealed a modality-related difference in brain activity from the first to the second measurement. During covert speaking, parietal and temporal areas showed an increase of activation. This increase of activation was not traceable during covert humming. Also in our region of interest (ROI) analysis in the left and right BA 44, the single case patient showed a hyperactivation during covert speaking at the second measurement when comparing her with the control groups. This hyperactivation was again modality-related and therefore only measureable during covert speaking. Our results were discussed in respect to the cerebello-thalamo-cortical pathway. The cerebellar disinhibition caused by the lesion might have led to an overactivity in thalamus and motor cortex, represented by the hyperactivation during covert speaking. Therefore, the cerebellar disinhibition and its triggered overactivation along the cerebello-thalamo-cortical pathway might have facilitated the cessation of stuttering.

Taken together, the object of the current thesis was to evaluate the long-term longitudinal effects of an intense stuttering therapy or a brain lesion on brain structure and function in PWS. The results provide first evidence that the reduction of white matter integrity (often seen as the deficit in neural processing in PWS; see Packman, 2012) can be altered through an intense stuttering therapy. Furthermore, our research demonstrates that an intense stuttering therapy has the potential to enhance an increase of brain activation in areas that were hypoactivated before therapy. This enhancement even

takes place in non-motor regions. And last, this thesis underlines the importance of the cerebello-thalamo-cortical pathway for the aetiology of stuttering.

1 General Introduction

Stuttering is a speech fluency disorder which is known in all cultures for many thousand years. First evidence for the occurrence of stuttering was found in old Egyptian hieroglyphs (Natke & Alpermann, 2010). When a person stutters, he knows exactly what he wants to say, but cannot produce speech properly. During stuttering symptoms, persons who stutter (PWS) lose their motor control and show struggle and effort to recapture it (Guitar & McCauley, 2010). Many PWS develop avoidance behaviour, e.g. by rephrasing sentences and replacing words with synonyms or non-related words which are linguistically easier to produce. Therefore, it is not an unexpected finding that PWS often experience shame and anxiety due to the prominent stuttering core symptoms as well as the perceived time loss in communication (Iverach et al., 2011; Iverach & Rapee, 2014). In addition, PWS are often bullied and isolated. All these circumstances may result in increased (social) anxiety, low self-esteem, the fear of negative evaluation (Blood & Blood, 2016) and decreased quality of life (Carter et al., 2017; Kohmäscher, 2017; Yaruss, 2010). PWS may even experience lower socio-economic success (McAllister, Collier, & Shepstone, 2012).

To avoid these negative long-term consequences of stuttering and to ease the stuttering symptoms, various therapy approaches have been developed and evaluated (Euler et al., 2009; Jones et al., 2005; Natke & Alpermann, 2010; for an overview, see Neumann et al., 2016; Neumann et al., 2017; Nye et al., 2013). Although the success of different stuttering therapy approaches has often been investigated on the behavioural level, there is still a lack of research concerning the effect of stuttering therapy on brain structures and functions. To the best of our knowledge, no study has examined and evaluated white matter plasticity in PWS taking part in an intense stuttering intervention. While functional activation changes after a stuttering therapy have been investigated in the past, only few studies elucidated long-term activation changes after intense stuttering

therapies. Closing this gap in research may contribute to the knowledge about the aetiology of stuttering and advantageous adaptations or redevelopments in the treatment of stuttering.

Therefore, this thesis concentrates on the effects of an intense German fluency shaping therapy (Euler et al., 2009), more precisely on therapy-induced white matter plasticity and long-term brain activity changes in PWS. In addition, I introduce a case study of an adult patient who showed a cessation of stuttering elicited by a cerebellar haemorrhage. Chapter 1 contains the general introduction, where I present an overview on stuttering, the neural correlates of stuttering, stuttering therapies as well as structural and functional changes observed after stuttering therapies. The aims of my thesis are defined likewise and the presentation of the three studies I conducted is given in the chapters 2 to 4. I conclude with a general discussion of the studies' results and their implications for stuttering and its treatment (chapter 5).

1.1 Stuttering

Terminology and definition

According to the German clinical practice guideline on fluency disorders, several types of stuttering can be defined (Neumann et al., 2016; Neumann et al., 2017). Stuttering can be acquired in adolescence or adulthood (neurogenic and psychogenic stuttering), or it can be described as originary. This expression means that stuttering develops during early childhood, either as a secondary symptom of a syndrome the child presents with (e.g. Down syndrome), or idiopathically. The latter, most frequent form of stuttering is called "neurogenic non-syndromal stuttering" (Neumann et al., 2016; Neumann et al., 2017) – this is the type of stuttering I am referring to in this thesis.

There are different perspectives on how to define the complex disorder of stuttering properly. The World Health Organization (WHO) defines stuttering as being "characterised by frequent repetition or prolongation of sounds or syllables or words, or

by frequent hesitations or pauses [and it is] persistent or recurrent and of severity sufficient to markedly disrupt the fluency of speech" (World Health Organization, 2006, p. 207). An older clinically well-known and accepted definition of stuttering was suggested by Wingate. According to the author, "stuttering refers to a disruption in the fluency of verbal expression, which is characterised by involuntary, audible or silent, repetitions or prolongations in the utterance of short speech elements, namely: sounds, syllables, and words of one syllable. The disruptions usually occur frequently or are marked in character and are not readily controllable" (Wingate, 1964, p. 488).

Epidemiology

Stuttering has its onset in early childhood between two and five years of age (Yairi & Ambrose, 2013). Approximately 5% of all children develop stuttering symptoms. Interestingly, about 75% of these cases show a remission from stuttering (Guitar & McCauley, 2010; Neumann et al., 2016), especially after the first two years post onset. Girls are more likely to experience this remission. Therefore in adulthood, the sex ratio of stuttering men and women is 4:1 or 5:1 (Bloodstein & Ratner, 2008) and the general prevalence in the adult population 0.72% – 1% (Yairi & Ambrose, 2013). After puberty, a remission from stuttering is unlikely (Yairi & Ambrose, 2005). A remission can be defined as a spontaneous remission, where stuttering symptoms are reduced without intervention. It can also be determined as an assisted remission, where the decline of stuttering symptoms is facilitated through therapies (Neumann et al., 2016). Although it is still not possible to establish an individual prognosis for remission, several risk factors for persisting stuttering have been determined. These factors are: male sex of the stuttering child, a family history of (persistent) stuttering, onset of stuttering after three years of age, consistent or worsening stuttering severity, persistence of stuttering symptoms more than six months as well as aberrant phonological skills and outstanding/or delayed language skills of children who stutter (CWS) (Lattermann, 2011).

Stuttering symptoms

Stuttering symptoms can be classified as core symptoms and accompanying symptoms. The core symptoms of stuttering are (1) repetitions of sounds, syllables or one-syllable words (“T-t-t-t-taxi”), (2) sound prolongations (“wwwweather”) and (3) tense pauses (“Oh, look at this cute ----- ape”). These core symptoms appear abruptly and inadvertently – a stuttering child or adult knows exactly what he wants to say, but cannot produce this word or phrase fluently (Bloodstein & Ratner, 2008; Wingate, 1964).

Within the core symptoms, PWS lose their motor speech control and therefore often “fight” to get it back. This fighting behaviour is also called struggle behaviour in literature (e.g. Perkins, 1990; van Riper, 1973) and represents one type of the accompanying symptoms of stuttering. PWS use more phonatory pressure to overcome their symptoms, show facial grimaces or even additional head and/or limb movements to bear down the loss of motor control. Initially, struggle behaviour can be effective, but it loses its agency in the course of the stuttering history. Unfortunately, many PWS hold on to this learned behaviour – the symptom of struggle (e.g. rolling eyes when experiencing a tense pause) may then become an established accompanying behaviour the stuttering person shows regularly. This learned stabilisation of accompanying struggle behaviour leads to more prominent stuttering symptoms (Bloodstein & Ratner, 2008; Natke & Alpermann, 2010). These are often perceived as more distractive by the patient’s peer group than the solitary core symptoms.

The second type of accompanying symptoms seen in PWS is flight behaviour or avoidance behaviour. PWS often anticipate in which syllables or sounds stuttering core symptoms may occur – this anticipation allows them to use synonyms, unrelated words or rephrase sentences to avoid possible core symptoms (Natke & Alpermann, 2010). Common in PWS is also the frequent use of linguistic fillers like “huh”, “uh”, “well”, “like”, etc. These fillers can serve two different purposes. First, they can be applied to delay

the expected stuttering symptom. Second, if they are used at the beginning of a sentence, they decrease the possibility that core symptoms occur in the following phrase. Similar to fight behaviour, this flight behaviour becomes functionless over time – core symptoms still appear and are perceived more complex and distracting due to the accompanying flight (and/or fight) behaviour (Sandrieser & Schneider, 2015). In addition to core symptoms and accompanying symptoms, PWS experience 'inner' symptoms. These are specific emotions and attitudes connected with the experience of being a person who stutters. Anxiety is an emotion which is often reported by PWS. They describe anxiety towards social rejection, loss of motor control and the inability to communicate (Blood & Blood, 2016; Natke & Alpermann, 2010). In the immediate aftermath of a stuttering symptom, they often name shame, guilt, frustration and/or aggression towards themselves and their counterparts as resulting emotions (Iverach et al., 2011; Yaruss & Quesal, 2006). Social withdrawal and decline of life quality are associated with these psychosociological symptoms of stuttering (Carter et al., 2017; Kohmäscher, 2017).

Aetiology of stuttering

Even ancient Greek medical doctors and philosophers like Hippocrates hypothesised about the aetiology of stuttering. Hippocrates assumed that stuttering is caused by a dryness of the tongue (Schaffer, 1966). From this assumption up to research in the 21st century, two main insights about the constitutional factors of stuttering were obtained and reaffirmed: (1) stuttering has a genetic basis (for an overview, see Kraft & Yairi, 2012) and (2) brain structures and functions are anomalous in PWS (recent reviews: Etchell, Civier, Ballard, & Sowman, 2017; Neef et al., 2015; Neumann et al., 2016). In the field of genetics, researchers were able to estimate the heritability rate of stuttering by conducting twin-, adoption- and family-studies. For stuttering children and adults, the heritability is assumed to be between 70% and 80% (Rautakoski, Hannus, Simberg, Sandnabba, & Santtila, 2012). This refutes earlier assumptions that stuttering was

caused by the family environment or specific educational habits of the stuttering childrens' parents (Neumann et al., 2016), as supposed in former theories concerning the aetiology of stuttering (e.g. diagnosogenic theory of Johnson; Johnson, 1955). Referring to molecular genetic research, various relevant loci of the genetic predisposition of stuttering have been detected (Kang et al., 2010; Kraft & Yairi, 2012). According to those studies, genetic researchers denote stuttering as a multifactorial polygenic disorder (Kraft & Yairi, 2012), although it is not clear yet how these found loci attribute to the onset or persistence of stuttering (Neumann et al., 2016). Due to this genetic research, experts assume that the onset of stuttering is explainable via an additive risk-threshold model: the more genetic loci are involved, the higher is the risk threshold to develop stuttering (Kraft & Yairi, 2012). This risk threshold is deemed to be higher in girls, who are less likely to stutter and exhibit higher remission probabilities than boys (Dworzynski, Remington, Rijsdijk, Howell, & Plomin, 2007; Kraft & Yairi, 2012).

Structural and functional brain differences as further constitutional factors of stuttering are discussed in the following chapter.

1.2 Structural and functional brain anomalies in PWS

Before the usage of magnet resonance imaging (MRI) scanners, several single case studies provided insights in the neuropathological correlates of acquired, neurogenic stuttering. This type of stuttering develops due to cerebral and cerebellar impairments. It can emerge after stroke, traumatic brain injury and intracerebellar haemorrhage (Lundgren, Helm-Estabrooks, & Klein, 2010). Most studies report the occurrence of neurogenic stuttering after lesions in the left hemisphere, but evidence for neurogenic stuttering after right-hemispheric brain lesions has also been found (Alm, 2004). Van Borsel and colleagues (van Borsel, van Lierde, van Cauwenberge, Guldemont, & van Orshoven, 1998) describe the occurrence of neurogenic stuttering after lesions in almost all parts of the brain, sparing the occipital lobe. In addition, subcortical lesions, e.g. in

the thalamus (Abe, Yokoyama, & Yorifuji, 1993; Levine & MacDougall, 2016), basal ganglia (Nebel, Reese, Deuschl, Mehdorn, & Volkmann, 2009; van Borsel et al., 1998) and cerebellum (Tani & Sakai, 2010) were reported to elicit neurogenic stuttering.

From these previously described “neuropathological” correlates of stuttering, it became obvious that dysfunctional brain structures and networks could be causative factors for developing stuttering in adulthood. Additionally, for patients with originary neurogenic non-syndromal stuttering (childhood onset), significant structural and functional brain differences compared to fluent controls only gradually came to light in the last decades. This was enabled by new possibilities and techniques of brain imaging (for an overview, see Etchell et al., 2017).

fMRI

In the field of functional MRI (fMRI), an early meta-analysis of Brown and colleagues (Brown, Ingham, Ingham, Laird, & Fox, 2005) showed that PWS present with extended speech-related overactivations in motor regions, particularly with right-lateralised hyperactivations of motor regions like the right rolandic operculum, right Brodmann area (BA) 47 and the right anterior insula as well as a hypoactivation in auditory areas. A subsequent meta-analyses of Neef and colleagues (Neef et al., 2015) as well as the review of Budde et al. (Budde, Barron, & Fox, 2014) and Belyk and colleagues (Belyk, Kraft, & Brown, 2015) confirmed that the neural hallmarks of persistent stuttering are characterised by hyperactivity of motor regions. These were located in the right hemisphere of PWS, e.g. in the right M1, supplementary motor area (SMA) and inferior frontal gyrus (IFG), and furthermore in the right rolandic operculum and right insula (Budde et al., 2014). In addition, stuttering was associated with a reduced activation in left hemispheric motor regions and a decline of activity in the left planum temporale and middle temporal gyrus (Budde et al., 2014; Belyk et al., 2015). This frontal left-hemispheric hypoactivation and the additional hyperactivation in right motor areas were

interpreted as indicating possible dysfunctional sensorimotor processing in PWS and were therefore seen as a causative factor for stuttering (Neef et al., 2015). Supplementary to these often confirmed functional correlates of stuttering, further divergences of functional networks were found in PWS in comparison to fluent speakers. The basal ganglia of PWS exhibited functional as well as connectional aberrations (Giraud et al., 2008; Lu et al., 2010). Atypical functional connectivity was evident in the basal ganglia-thalamo-cortical pathway in PWS (Chang, Horwitz, Ostuni, Reynolds, & Ludlow, 2011). A lack of functional connectivity was also reported between insula and left laryngeal motor cortex (Howell, Jiang, Peng, & Lu, 2012) and a reduced auditory-motor coupling was measured in PWS (Watkins, 2011). In resting state functional connectivity (RSFC) studies, PWS showed a decreased connectivity between basal ganglia and bilateral superior temporal gyrus (Lu et al., 2012; Yang, Jia, Siok, & Tan, 2016) as well as a decline in connectivity between right SMA and the basal ganglia (Xuan et al., 2012; Yang et al., 2016). A decline of RSFC in the default mode network of adult PWS was also reported (Chang & Zhu, 2013; Xuan et al., 2012). The default mode network consists of parietal, prefrontal and temporal brain regions and decreases its activation in cognitive demanding and externally cued tasks (Greicius et al., 2008). It is seen as the brain's intrinsic activity (Raichle, 2015). Chang and colleagues validated atypical activations in the default mode network as well as in attention, somatomotor and frontoparietal networks (Chang et al., 2017). Taken together, fMRI findings indicate that PWS show activation aberrations in specific motor processing brain regions and networks - right hemispheric frontal overactivations (e.g. Neef et al., 2015) seem to be the most frequently reported neural hallmarks of stuttering. Nevertheless, it becomes obvious that also non-motor networks might be a constituent of the pathogenesis of stuttering.

DTI

In the field of diffusion MRI, Sommer and colleagues (2002) were the first group of researchers who demonstrated an anomalous white matter integrity in PWS. This was characterised by a reduction of fractional anisotropy (FA). FA expresses the directionality of the mobility of water molecules – it is elevated along but not perpendicular to white matter axons and, as a consequence, represents a quantification method for the thickness of the tract structure (Smith et al., 2006). In the study of Sommer et al. (2002), a decline of FA was observed in the left rolandic operculum in the vicinity of areas processing motor speech movements – Sommer et al. concluded that this finding might represent a constitutional factor for the aetiology of stuttering. In the following years, many researchers were able to reproduce the result of a reduced white matter integrity in mostly left frontal brain areas in PWS (e.g. Chang et al., 2011; Cykowski et al., 2010; Watkins, Smith, Davis, & Howell, 2008). A meta-analysis (Neef et al., 2015) evaluated all diffusion tensor imaging studies (DTI) studies concerning white matter integrity deficits in PWS – three major clusters of reduced white matter integrity became apparent in the left superior longitudinal fasciculus as well as in the posterior midbody of the corpus callosum. Recently, lower FA values were not only detected in left-hemispheric regions of stuttering participants, but also in the right hemisphere. Kronfeld-Duenias and colleagues (Kronfeld-Duenias, Amir, Ezrati-Vinacour, Civier, & Ben-Shachar, 2016) could show diminished white matter integrity in the right inferior longitudinal fasciculus and right cingulum, while Chang et al. (Chang, Zhu, Choo, & Angstadt, 2015) and Cai et al. (Cai et al., 2014) demonstrated reductions of white matter integrity in different portions of the right superior longitudinal fasciculus. Additionally, a recent study of Neef and colleagues (Neef et al., 2018) demonstrated FA reductions in the right superior longitudinal fasciculus as well as in the junction of the right frontal aslant tract. Similar results were evident in CWS: compared to fluent children, they exhibited diminished

white matter integrity in the right frontal aslant tract (Misaghi, Zhang, Gracco, Nil, & Beal, 2018).

Conclusions

Structural and functional differences found in PWS during the last 30 years of imaging could contribute crucially to the understanding of the pathogenesis in stuttering. Yet, it is not clear if these deviations are causative for stuttering or if they are compensational mechanisms in reaction to already existing stuttering core behaviour, concomitants and inner symptoms. The fact that a reduction of white matter integrity was even found in children as young as three years of age (Chow & Chang, 2017; Misaghi et al., 2018; Watkins et al., 2008) might hint to a causational component of structural brain differences towards stuttering. Indeed, Chow and Chang (2017) were able to affirm a different FA maturation rate in children with persistent stuttering compared to fluent and from stuttering recovered children in the left arcuate fasciculus and corpus callosum. The authors infer that FA increases are likely to mirror compensatory neuroplasticity processes where alternative anatomical connections are built. CWS with a higher growth rate of FA might be able to build more alternative connections, thus developing successful compensational motor speech processing and enhancing the chance to recover from stuttering. By contrast, children with a decreased FA growth might not be able to compensate stuttering symptoms properly and therefore develop persistent stuttering (Chow and Chang, 2017).

This finding could have the potential to explain the association between the constitutional factors of stuttering “genetic abnormalities” and “neurophysiological brain abnormalities”. A delayed and incomplete myelination of white matter in CWS could elevate the breakdown susceptibility of the motor speech production in children and could cause stuttering – this delayed and/or incomplete myelination is probably primarily caused by changed or lesioned genes (May & Gaser, 2006). An increase of FA as reported by

Chow and Chang (2017) might, in this case, characterise the compensational reaction of the child's (and later on adult's) brain to the already established weak myelination caused by genetic aberrations (Guitar & McCauley, 2010; Kell et al., 2009; Neumann et al., 2016). Future longitudinal studies with advancing imaging technologies can probably provide secure and stable results to fortify this assumption.

1.3 Stuttering therapies for stuttering adolescents and adults and therapy-induced functional and structural brain changes

Concerning CWS, stuttering therapies are aiming for the recovery of stuttering. Since a recovery is only rarely observed after puberty (Yairi & Ambrose, 2013), a relief towards coping with the speech fluency disorder is the therapeutic objective in treatments for stuttering adolescents and adults (Natke & Alpermann, 2010). In detail, therapies should facilitate the quantitative and qualitative reduction of stuttering core symptoms, of accompanying symptoms and of negative inner symptoms and emotions related to stuttering. Stuttering therapies can have a positive impact on the quality of life as well as participation and activities in society (Neumann et al., 2016). We know that there are effective therapeutic components which should be, at least partly, included into a therapy programme for adolescents and adults. These components are (1) the intense practice of initial slowed-down speech, (2) soft voice onsets, (3) rhythmic speech, (4) control of breathing and (5) self-management to change attitudes towards stuttering (Andrews, Guitar, & Howie, 1980). The review of Bothe and colleagues adds the factors (6) group therapy, (7) transfer exercises into everyday life situations (called "in-vivo tasks"), (8) maintenance programmes, and (9) the attempt to gain a naturally appearing speech to the effective therapy components (Bothe, Davidow, Bramlett, & Ingham, 2006).

In Germany, two major stuttering therapy approaches which are conducted in a group setting are offered for adolescents and adults. The first approach is called *"speech restructuring"*. It is based on behavioural therapeutic methods where patients learn a

globally applied new motor pattern of speech. This new motor speech pattern prevents the occurrence of stuttering symptoms (Natke & Alpermann, 2010; Natke, Alpermann, Heil, Kuckenberg, & Zückner, 2010). A popular therapy form of the speech restructuring approach is “fluency shaping”, where patients speak initially with less velocity and soft voice onsets. When the patient is able to implement this new speech pattern properly, the velocity of speech is increased step by step to regain a more natural speech prosody. The transfer of these techniques to everyday life is supported by hierarchically structured in-vivo tasks and an accompanied maintenance-phase including refresher-courses (Natke & Alpermann, 2010; Neumann et al., 2016). A prevalent German evidence-based stuttering intervention that can be allocated to this fluency shaping/respectively speech structuring approach is the Kasseler Stottertherapie (Euler et al., 2009). Various studies reported a long-term speech fluency improvement and a decrease of negative emotions towards stuttering after therapy (Euler et al., 2009; Euler et al., 2016; Euler & Wolff v. Gudenberg, 2000). Participants of the Kasseler Stottertherapie (from now on called Kasseler stuttering therapy) train an interconnected and soft speech pattern with a slower velocity of speech, assisted by a biofeedback computer programme. The therapy begins with an intensive course of 2 weeks duration. One and ten months after the onset of therapy, two refresher courses are offered. After leaving the therapy centre, patients continue the biofeedback-training at home.

The second major therapy approach for adolescents and adults is called *stuttering modification* (also named as *“non-avoidance”* approach). In contrast to the speech structuring approach, the speech pattern is not changed generally. Only the stuttered parts of speaking are ‘modified’ via speech techniques (Starke, 1997; van Riper, 1973). The aim of non-avoidance therapies is to achieve a kind of stuttering that is characterised by relaxed, short core symptoms without any struggle, accompanying symptoms and negative emotions (Natke et al., 2010; Natke & Alpermann, 2010). Therefore, the training of the perception of own core and accompanying symptoms as well as incriminating

emotions towards stuttering are one major component of this therapy approach. In a second step, a desensitisation against stuttering symptoms, reactions of the audience and the trained speech-techniques follows (Decher, 2011). Subsequently, patients are training the modification of stuttered parts of speech with specific speech techniques. In-vivo tasks allow the patients to transfer their successful modified stuttering to stressful everyday life communication situations. A maintenance phase as well as refresher sessions are components of most stuttering modification therapies (e.g. (Breitenfeldt & Rustad Lorenz, 2002; Zückner, 2014). The “Intensiv Modifikation Stottern” (IMS) (Zückner, 2014) is an evidence-based stuttering modification therapy frequently applied in Germany (Euler, Lange, Schroeder, & Neumann, 2014). A study of Natke and colleagues (Natke et al., 2010) showed stable long-term effects of an enhanced speech fluency and an improved coping with stuttering.

From evidence-based studies it became obvious that stuttering patients taking part in the previously described therapy programmes improved in behavioural scales like stuttering severity or life quality. Due to the fact that neurophysiological abnormalities seem to be constitutional factors of stuttering, researchers were not only interested in behavioural changes caused by stuttering therapy, but also in neurophysiological changes.

In terms of *fMRI research*, direct intervention effects of the Kasseler stuttering therapy have been investigated. Participants where measured before and after an intense therapy course of three weeks duration taking place at the therapy centre. At post-measurement, Neumann and her colleagues reported more extended brain activity than before, especially in frontal motor speech and temporal regions (Neumann et al., 2004; Neumann et al., 2005). Furthermore, Neumann and colleagues found evidence for a shift of activity from right to left-hemispheric frontal regions. In these regions, a decrease of white matter plasticity had been reported before. At the end of the intense therapy course, patients also showed a modified activation of basal ganglia (Giraud et al., 2008),

a normalised auditory-motor-coupling and integration of somatosensory feedback (Kell, Neumann, Behrens, Gudenberg, & Giraud, 2018) as well as a normalisation of cerebellar activity (Lu et al., 2012). Studies with a follow up longer than six months after an intense stuttering therapy are rare. Such an extended time frame has the benefit of evaluating the therapy success during the maintenance phase of stuttering therapy. De Nil et al. (De Nil, Luc F., Kroll, Lafaille, & Houle, 2003) affirmed an activation shift from right- to left-hemispheric brain areas as well as a reduced overactivation of bilateral and right motor regions after a one year follow-up measurement. Neumann and colleagues (Neumann et al., 2004) stated that after a follow-up of two years, five stuttering patients still exhibited left-frontal hypoactivations, but showed reduced overactivations in the right hemisphere motor regions. Recently, Neumann et al. (2018) reported a pre-treatment hypoactivation of the left inferior frontal gyrus and anterior insula in stuttering patients which was normalised after therapy. To sum up, the body of evidence for long-term effects of intense stuttering therapies on brain activation is sparse and inconsistent.

Referring to *DTI research*, to the best of our knowledge there is no study published up to date that evaluated white matter plasticity changes after an evidence based intense stuttering therapy. From studies investigating other populations and disorders, we know that white matter integrity changes after intense trainings and therapies occurred. For example, an increase of white matter integrity in aphasic patients was present after an intense training of melodic intonation therapy (Schlaug, Marchina, & Norton, 2009; Zipse, Norton, Marchina, & Schlaug, 2012) and in children with cerebral palsy and dysarthria, a facilitation of white matter tract integrity was shown after an intense Lee Silverman Voice treatment (Reed, Cummine, Bakhtiari, Fox, & Boliek, 2017). Due to these study results from patients with other disorders, I expect that an intense stuttering therapy might have comparable effects on white matter plasticity.

1.4 Objective of the dissertation

The scope of this dissertation is to evaluate the long-term effects of an intense evidence-based German stuttering therapy on white matter plasticity and brain activation.

Due to the small body of literature, it remains unclear how functional changes related to stuttering therapy develop if one includes the maintenance phase into the follow-up period. Furthermore, it is an open question if and how white matter plasticity changes due to an intense stuttering treatment. Especially because PWS show a reduction of white matter plasticity in the left and right hemisphere and this reduction might be a constitutional factor of stuttering, one could consider that an intense treatment might facilitate FA growth. This growth could be a compensational boost for PWS which might support a gain of speech fluency.

In the first study (chapter 2), the research aims are manifold. To replicate the previous research findings of a decreased FA in PWS compared to healthy controls is a primal intent. With our unique population of PWS, we can contribute supplementary evidence to the divergent findings of a declined white matter integrity in PWS and furthermore establish a relation to the neuropathological mechanisms of stuttering. In addition, we evaluate changes of white matter plasticity in PWS participating in an intense stuttering treatment. For this purpose, we add two control groups. One group includes stuttering participants who do not take part in any therapy, the other group consists of healthy control participants. We apply tract based spatial statistics (TBSS, Smith et al., 2007) within whole-brain and ROI analyses to detect FA changes in the patient group as well as differences in FA change between groups. Another important aim is to discuss observed white matter plasticity in relation to neurophysiological and neuropathological processes in the stuttering brain.

With the second study (chapter 3), we aim to detect long-term changes in brain activation evoked by an intense stuttering therapy and its maintenance phase. Again, we compare

brain activity changes in the intervention group to the changes present in both control groups (stuttering participants not taking part in any stuttering therapy, healthy participants). We wanted to detect therapy-induced activation changes which are only evident in the group of stuttering patients taking part in the therapy, and to discuss them with regard to the therapeutic mechanisms of action.

In the third study presented in this thesis (chapter 4), the stuttering of a single case patient did not vanish due to therapy, but was reduced as a consequence of a cerebellar haemorrhage. With this case study, we want to discuss neurophysiological mechanisms responsible for the cessation of stuttering and draw possible consequences to the neural hallmarks of stuttering.

2 Long-term white matter plasticity changes in persons who stutter induced by stuttering therapy[1]

2.1 Introduction

Stuttering is a speech fluency disorder that is characterised by its involuntary core symptoms sound prolongations, speech blocks and sound and syllable repetitions (Andrews & Harris, 1964; Bloodstein & Ratner, 2008; Guitar & McCauley, 2010). These described speech dysfluencies often occur together with secondary symptoms like facial grimaces, head, neck and limb movements. Stuttering also has an enormous impact on communication - persons who stutter (PWS) often develop avoiding-strategies including paraphrasing and restructuring of sentences, substitution of words or social withdrawal to reduce or hide the appearance of their symptoms. They often experience a high communicative pressure and psychological strain including anxiety, shame, embarrassment and low self-esteem (Boyle & Fearon, 2018; Zückner, 2017). It is not surprising that a reduced life quality can be measured in some PWS (Carter et al., 2017; Kohmäscher, 2017; Yaruss, 2010).

There are several forms of stuttering known from clinical experience and research. The fundamental difference between these types of stuttering is if stuttering is originary and develops during childhood or if stuttering is acquired after puberty. Psychogenic as well as neurogenic stuttering are forms that are normally acquired during adulthood. In contrast, the “idiopathic” stuttering which is also defined as “originary neurogenic non-syndromal stuttering” in the German guidelines for speech fluency disorders (Neumann et al., 2017) already has its onset in early childhood at the ages of 2 to 6 years (Yairi & Ambrose, 2013). The life-span incidence of this originary form of stuttering is approximately 5% in children, while it affects approximately 1% of the adult population (Guitar & McCauley, 2010; Yairi & Ambrose, 2013). Originary stuttering (from now on

[1] Manuscript in preparation together with Peter Dechent and Martin Sommer

simply called “stuttering”) has a high spontaneous recovery rate of approximately 70% - 80% during childhood, but persists in a small number of children. Different risk factors for the persistence of stuttering are known nowadays (e.g. male sex, history of stuttering in the family; see Guitar & McCauley, 2010), but still no individual prognosis for the remission of stuttering is possible.

The aetiology of stuttering is not fully comprehended yet, but a lot of diverse research studies illuminated different possible pathomechanisms and abnormalities that are likely associated with stuttering.

First, a genetic aetiology of stuttering became evident through twin studies that confirmed a heritability of 70% - 80% (Rautakoski et al., 2012). Research concerning molecular genetics found diverse loci probably involved into stuttering – therefore stuttering is regarded as a multifactorial polygenic disorder (Kraft & Yairi, 2012).

Second, stuttering can be characterised as an impairment of brain structure and/or function. The inconsistent appearance of symptoms is an additional challenge for research to find the underlying neurological mechanisms. Growing evidence for an altered neurophysiology was found in different brain imaging studies with PWS in the last years (for an overview, see Etchell et al., 2017), though study outcomes are sometimes conflicting and therefore discussed controversially.

In the field of functional MRI, the meta-analyses of Belyk et al. (2015) and Budde and co-authors (2014) evaluated the neural correlates of persistent stuttering. A prominent finding of several studies is the hyperactivation of right-hemispheric motor areas which PWS show. This is often combined with a left-hemispheric decrease of activation in motor regions (Neef et al., 2016; Neumann et al., 2005). These phenomena are considered as the neurophysiological hallmarks of stuttering. Some study authors interpret these hallmarks as an impairment of the sensorimotor integration and a potential cause for stuttering. Studies that evaluated changes in brain activation induced

by stuttering therapies could show a change of the before-seen patterns of hyperactivation in PWS. For example, a therapy-related reduction of the over-activation in the right IFG (Neumann et al., 2004; Neumann et al., 2005) or a reduced cerebellar hyperactivation after intervention (Lu et al., 2012; Toyomura, Fujii, & Kuriki, 2015) was reported.

Concerning structural MRI, Sommer an colleagues (2002) were the first researchers who found evidence for a reduction of fractional anisotropy (FA) in PWS. FA represents the directionality of water molecule mobility which is high along white matter axons and therefore a quantification for how strong the direction of the tract structure is (Smith et al., 2006). Thus, it can be seen as an entity of white matter integrity. Sommer and colleagues (2002) found a reduction of FA in the left rolandic operculum, which is close to the motor speech representation of the articulators and the arcuate fasciculus. They concluded that this finding had the potential to explain the dysfluencies PWS experience.

Sommer's study outcome was replicated by various other studies, although the exact areas where a reduction of FA was found varied (Cykowski et al., 2010). Neef and colleagues (2015) published a meta-analysis of all published DTI studies on FA reductions in PWS. They identified three primary clusters of lower FA values in PWS. The first cluster was located in the left superior longitudinal fasciculus of the inferior parietal lobe. The second cluster was situated in the left superior longitudinal fasciculus and included fibres of the arcuate fasciculus and the third cluster was determined in the posterior midbody of the corpus callosum (Neef et al., 2015).

Due to the previously described study outcomes, there is reliable evidence for differences in white matter integrity between PWS and fluent controls. As far as we know, there have been no studies conducted yet which investigate the longitudinal effects of stuttering therapy on brain structure. It is therefore unclear how the reduced white matter integrity found in PWS might be influenced by a therapeutic intervention.

Stuttering therapy can help to alleviate the symptoms of stuttering and to optimise coping strategies with inner, psychological symptoms. Well-known therapy approaches that are evidence-based and conducted world-wide are stuttering modification (e.g. Intensiv Modifikation Stottern, Natke et al., 2010), fluency shaping (e.g. Kasseler Stottertherapie, Euler et al., 2009) and behavioural therapy (e.g. Lidcombe Program, Packman & Onslow, 2012). All of these therapies facilitate speech fluency and reduce stuttering symptoms, though relapses may occur in some patients and the amount of stuttering symptoms might increase at some point after therapy. A structured aftercare including a relapse management plan is therefore a necessary component of a stuttering therapy (Craig, 1998; Cream, O'Brian, Onslow, Packman, & Menzies, 2009; Neumann et al., 2017).

If one considers that stuttering therapy helps to improve speech fluency on a behavioural level, one could also assume that it might change the abnormalities in white matter integrity which has been found in PWS.

First cues that add support to this hypothesis were found in studies with healthy participants and patients with other disorders than stuttering: Scholz and colleagues (Scholz, Klein, Behrens, & Johansen-Berg, 2009) showed an increase of FA due to an intense juggle training in healthy participants, while Keller and colleagues detected an increase of white matter integrity in poor readers after an intense phonological training (Keller & Just, 2009). Schlaug et al. (2009) as well as Zipse et al. (2012) reported an increase of fibre volume in stroke patients that followed from an intense intervention consisting of Melodic Intonation Therapy. The same group of researchers also found a fibre increase in descending motor tracts after a specific motor training in stroke patients (Zheng & Schlaug, 2015). Also Reed et al. (2017) recently detected an enhancement of white matter tract integrity in children with cerebral palsy and dysarthria – it was observed after an intensive application of Lee Silverman Voice Treatment and was prominent especially in the posterior corpus callosum and bilateral cingulum.

Not only a white matter integrity increase but also a decrease was an outcome of a study looking for fibre changes after intensive interventions. In the study of Wan and colleagues, improvements in speech production after an intense period of Melodic Intonation Therapy were associated with reductions of FA in parts of the right (contralesional) arcuate fasciculus (Wan, Zheng, Marchina, Norton, & Schlaug, 2014). The authors concluded that an increase of FA due to intervention could be associated with a higher alignment of fibres and improved myelination, while a decrease of FA could be a sign of less fibre alignment as well as axonal sprouting or branching (Wan et al., 2014).

Overall, changes of white matter integrity can be observed as a result of a training or therapy in healthy participants as well as in patients with different disorders.

Therefore, we assume that changes in white matter plasticity can also be observed in PWS taking part in an intense stuttering therapy. As far as we know, no study has been conducted yet which investigates the effect of stuttering therapy on white matter integrity. Especially because PWS show a reduction of FA in structures important for motor speech production (e.g. arcuate fasciculus, corpus callosum, superior longitudinal fasciculus, see Neef et al., 2015), we would expect an increase of white matter integrity in these regions after therapy. This fibre growth could be the trigger for a boost in speech fluency and would probably express the overcoming of the poorly wired brain regions existent in PWS.

To investigate the influence of stuttering therapy on brain structure, we studied a group of adolescents and adults who stutter before and approximately 11 months after a well-established intensive stuttering therapy called “The Kasseler Stottertherapie” (Euler et al., 2009; Neef et al., 2015). This therapy is an evidence-based stuttering therapy (Euler et al., 2009, Euler et al., 2009; Euler et al., 2014; Euler et al., 2016; Ingham, Ingham, Euler, & Neumann, 2017; Neef et al., 2017; Neumann et al., 2017) which can be

classified as a fluency shaping therapy and is often conducted in Germany and in some other countries in the world.

For our study, we carefully controlled unspecific and not therapy related effects by adding two control groups to the group of stuttering patients taking part in the intervention: one group of adults who stutter but were not enrolled in any form of stuttering therapy during the study period and one fluent speaking control group with healthy participants. We decided not just to evaluate changes in white matter integrity in the whole brain, but also conducted region of interest (ROI) analyses in the regions showing a significant decrease of FA in the meta-analysis of Neef and co-authors (2015) and in the first study of Sommer and co-authors (2002). Therefore, we used tract based spatial statistics (TBSS; Smith et al., 2006) within the FMRIB Software Library (FSL) (Smith et al., 2004).

In addition to these longitudinal research objectives, we wanted to replicate the previous findings of FA decrease in PWS compared to healthy controls in our own independent sample of patients. Because reduced FA values in PWS have been found in diverse brain regions so far, outcome replications can be helpful to further examine the neurophysiological background of stuttering properly.

We applied the following research hypotheses for our purposes:

1. At baseline, PWS show a FA decrease in left and right hemispheric brain regions compared to healthy controls; the regions are in line with the literature mentioned before. To check this hypothesis profoundly, we include age and total SSI score as a covariate of no interest to our model to exclude age and stuttering severity effects on fibre plasticity.
2. Intense stuttering therapy leads to an increase in white matter integrity in:
    a. the left rolandic operculum

   b. the three clusters showing decreased FA in PWS calculated in a meta-analysis of Neef and colleagues (Neef et al., 2015)
   c. other brain areas.
3. The white matter integrity of both healthy and stuttering control groups will, in contrast to the stuttering patients, not change in the time of the longitudinal data acquisition.

2.2 Material and methods

2.2.1 Participants

To explore the aims of the study, three different groups of participants were included: persons who stutter about to begin an intense stuttering therapy after the first study measurement (stuttering patients, SP; n = 17); persons who stutter but do not take part in any stuttering therapy at the time of the study (stuttering controls; SC; n = 15) and fluent speakers (healthy controls, HC; n = 25) (Sommer & Primaßin, 2017, 2018).

Groups of SP and HC were matched for sex, age, handedness (Oldfield, 1971) and years of formal education (1=school; 2 = high school; 3 = <2 years college; 4 = 2 years college; 5 = 4 years college; 6 = postgraduate; see also Neef et al., 2016). The group of SC was older and though better educated compared to HC and SP; participants in the SC group were still matched for sex and handedness in comparison to both SP and HC groups (see Table 1).

Table 1. Participants and demographic information.

	SP	SC	HC	p-value SP - HC	SP - SC	SC - HC
n	17	15	25			
Age in years (mean)	27.3 (SD 12.3)	35.1 (SD 7.5)	24.0 (SD 4.9)	0.320 [a]	0.041 * [a]	0.000 * [a]
Sex (male)	15 (88.2%)	13 (86.7%)	22 (88%)	1 [b]	1 [b]	0.371 [b]
Handedness (mean LQ)	77.9 (SD 47.4)	84.2 (SD 16.9)	69.7 (SD 52.4)	0.609 [a]	0.630 [a]	0.212 [a]
Education (median)	1	5	2	0.076 [c]	0.000 * [c]	0.002 * [c]
Mean time difference between pre- and post-measurement (months)	11.5 (SD 1.1)	11.7 (SD 1.5)	11.5 (SD 0.8)	0.761 [a]	0.770 [a]	0.530 [a]
Age of stuttering onset in years (mean)	4.2 (SD 2.4)	5.3 (SD 3.8)	n/a	n/i	n/i	n/i
Number of participants with PWS in their families	7 (41.2%)	7 (46.7%)	n/a	n/i	n/i	n/i

Note. SP (stuttering patients); SC (stuttering controls); HC (healthy controls); p-value derived from group-pairwise statistical testing with the following methods: [a] (T-test); [b] (Fisher's exact test); [c] (Mann-Whitney-U-test); n/a (not applicable); n/i (not investigated) SD (standard deviation); * (significant result, p<.05).

Apart from the stuttering in SP and SC, the participants met the following criteria for inclusion into the study: 1) general MRI compatibility, 2) native German speakers, 3) normal or corrected-to-normal vision, 4) no pregnancy, 5) no history of dementia or other central nervous system (CNS) or psychiatric diseases and 6) no history of speech and language disorders. In the HC group, no family-history of stuttering was present.

All participants of the SP group were recruited at the therapy centre via information events directly hosted by the centre. Regarding the SC group, recruitment was

completed via stuttering support groups. Healthy, fluent speaking controls (HC) were recruited via advertisements at the University of Göttingen. Informed written consent for participating in the study was obtained from each subject. The study was approved by the local ethics committee of the University Medical Center Göttingen and conformed to the Declaration of Helsinki. Participants received 21 Euros for each measurement; travel costs were refunded.

2.2.2 Stuttering Therapy

Stuttering participants which took part in an intense stuttering therapy were recruited from the therapy centre of the Kasseler stuttering therapy (located in Bad Emstal, Germany). At this therapy centre, therapists are providing intense group therapies for PWS (Iven & Hansen, 2017; Sommer & Primaßin, 2017).

The Kasseler stuttering therapy (Euler et al., 2009) is a computer-assisted intensive biofeedback therapy and based on the fluency shaping stuttering therapy approach. Participants are training a specific soft and bound speech pattern which they have to use constantly during speaking. The therapy starts with a 2-week-intensive course in Kassel. It is followed by two refresher-weekends in Kassel, conducted one month and approximately ten months after the two-week-intensive-course. In between these stationary therapy courses at the therapy centre, the patients have to accomplish a computer-assisted bio-feedback-training of 20 minutes daily. In addition; online-therapy sessions are conducted. Several studies and guidelines could confirm the effectiveness of the Kasseler stuttering therapy (Euler et al., 2009; Iven & Hansen, 2017; Kell et al., 2009; Neumann et al., 2017), showing there is a long-lasting improvement of speech fluency as well as an reduction of stuttering-related negative emotions after therapy. The Kasseler stuttering therapy is finished after ten to twelve months of intense practice (depending on the patient’s personal time schedule).

2.2.3 Research Design

A pre-post-test design was used to evaluate therapy-induced changes in SP as well as changes of brain function in SC and HC. In SP, the pre-test took place just before intense therapy started. All measurements were conducted at the University Medical Center, Göttingen; they contained an (f)MRI-Measurement as well as clinical speech analysis and behavioural examinations to collect information about the stuttering severity and different attitudes and emotions towards stuttering and fluent speech. The post-test in SP took place after the therapy-closure-weekend. The pre-post-test interval of SC and HC was comparable to the pre- and post-test interval of the SP group (see Table 1). Pre- and post-test measurements consisted of identical clinical and (f)MRI examinations.

2.2.4 Clinical and behavioural examinations

The following tests were applied in the study:

Clinical speech analysis with the SSI-4

Stuttering severity was assessed by using the *Stuttering Severity Instrument 4* (SSI-4, Riley, 2009). For obtaining the stuttering severity score for each participant and measurement; the frequency and duration of stuttered syllables as well as physical concomitants of stuttering have to be counted and rated. Therefore, we videotaped samples from reading aloud as well as spontaneous speech (participants were asked to describe their daily routine, hobbies and favourite TV series or books). 500 syllables of reading as well as 500 syllables of spontaneous speech were included into the analysis.

Subjective stuttering severity

To also explore the *subjective stuttering severity degree*, we asked the participants to rate the severity of their stuttering on a scale from 1 to 9, where 9 was representing a very high and 1 a very low degree of stuttering severity.

Behavioural questionnaires

The German version of *the Overall Assessment of the Speaker's Experience with Stuttering* (OASES, Yaruss & Quesal, 2006) was employed to evaluate the participants' experience of stuttering and the entirety of the disorder. It is also evaluating the impact of stuttering on communication and life quality and therefore able to measure stuttering treatment outcomes (Kohmäscher, 2017).

The WHO-5 Well-Being Index (WHO-5) is a short self-report questionnaire consisting of five questions that reflect one's well-being. It has been applied as an outcome measure in diverse clinical trials (Topp, Ostergaard, Sondergaard, & Bech, 2015; Wit, Pouwer, Gemke, Reinoud, J. B. J., Delemarre-van de Waal, Henriette A., & Snoek, 2007) and should reflect possible changes due to stuttering therapy in the current study.

The *Beck Depression Inventory* (BDI, Beck, Ward, Mendelson, Mock, & Erbaugh, 1961) is a self-report questionnaire which is measuring the severity of depression. Is has been used by clinicians and researchers in different settings (Richter, Werner, Heerlein, Kraus, & Sauer, 1998). We applied it to control therapy-induced changes in terms of depressive behaviour.

To ensure that the communication behaviour of the healthy control participants was not associated with anxiety or social phobia and did not change during the longitudinal course of the study, we conducted the German *State-Trait Anxiety Inventory* (STAI, Laux, Glanzmann, Schaffner, & Spielberger, 1981) in this specific group.

2.2.5 Imaging Acquisition

We used a 3 Tesla MR system (Siemens Magnetom Tim Trio, Siemens Healthcare, Erlangen, Germany) as well as a standard 8-channel phased-array head coil. Participants were placed into the scanner in supine position. They wore headphones for

noise protection and MR-compatible LCD goggles (VisuaStim XGA, Resonance Technology Inc., Northridge, CA, USA).

First, a T1-weighted anatomical 3D turbo fast low angle shot FLASH sequence was accomplished (repetition time (TR) 2250 ms, inversion time 900 ms, echo time (TE) 3.26 ms, flig angle = 9°, voxel size 1x1x1 mm^3).

Second, diffusion-weighted images were carried out using spin-echo EPI with 64 diffusion sensitized gradient directions (TR interval 10100 ms, TE 93 ms, b-values 0,1000 s/mm^2, 74 axial slices, voxel size 1.9x1.9x1.9 mm^3, phase-encoding in anterior-to-posterior direction). We additionally acquired one volume without diffusion-weighting and opposite phase encoding direction (posterior-to-anterior).

2.2.6 Data analyses

2.2.6.1 Analysis of behavioural data

Interrater reliability calculation of the SSI-4 data

Two experienced speech and language pathologists (one of them was A.P.) analysed each 50% of the full sample of SSI-4s according to the SSI-4's manual instructions (Riley, 2009). The SSI-4s were distributed pseudorandomly to each rater; every pathologist rated pre- and post-recordings of one participant and also rated an analogous amount of SPs', SCs' and HCs' SSI-4s.

Before the interrater-reliability calculation and the main analysis started, we conducted an analysis-training with both raters (duration: 30 hours). During this training, all SSI-4-manual guidelines were checked and inconsistencies were clarified. In addition, 3 different SSI-4 (subset of full sample) which were not part of the interrater-reliability calculation were evaluated by both raters independently. Results were then compared to extinguish still existing differences in the analysis.

After this training was completed, we calculated the interrater-reliability. Both speech and language pathologists analysed 9 SSI-4 (subset of the full sample; 3 of the SP group, 3 of the SC group and 3 of the HC group) independently and the results were statistically assessed with Krippendorf's Alpha Reliability Estimate (KALPHA) in SPSS, using 10000 bootstrapping samples and the ordinal data level (Hayes & Krippendorff, 2007).

Statistical analysis of behavioural questionnaires

We conducted the SSI-4, WHO-5 and BDI in all three groups of participants. To compare for behavioural differences between the three groups at one point of time, we used the Kruskal-Wallis-Test for ordinal scaled data. Pairwise-comparisons tests were included to correct p-values with the Dunn-Bonferroni method for multiple comparisons. To check for longitudinal changes in the behavioural questionnaires from pre- to post-measurement, the Wilcoxon signed-rank test was executed and corrected with the Holm-Bonferroni method for multiple comparisons.

The OASES and subjective stuttering severity score were executed in the group of stuttering patients and stuttering controls. To compare for behavioural differences between both groups at one measurement, we applied the Mann-Whitney test for ordinal scaled data and corrected for multiple comparisons with Holm-Bonferroni. For evaluating changes over time from pre- to post-test, we calculated Wilcoxon signed-rank tests for the paired ordinal data in each group, respectively. The obtained p-values from the Wilcoxon signed-rank test for each calculation were corrected with the Holm-Bonferroni-method for multiple comparisons.

The STAI was only applied in the group of healthy participants. Here, we used the Wilcoxon signed-rank test for finding significant differences in the score from pre- to post-test and also executed the Holm-Bonferroni-method.

For all statistical tests of behavioural data, the effect size estimate *r* was calculated as $r = \frac{z}{\sqrt{N}}$ (z = z-score that SPSS calculates; N = number of total observations on which z is based (Field, 2011, p. 295)).

2.2.6.2 Analysis of DTI data

After checking for artefacts in the DTI data, we used the FMRIB's Diffusion Toolbox vers. 5.0.9 (Smith et al., 2004; Smith et al., 2006; Woolrich et al., 2009; www.fmrib.ox.ac.uk/fsl) for all steps of analysis. For the preprocessing, the distortion due to magnetic field inhomogeneities in the DTI data was corrected with TOPUP (Andersson, Skare, & Ashburner, 2003). Here, an additional dataset without diffusion-weighting and opposite phase encoding direction is used, resulting in pairs of images with distortions going in reverse directions. From these pairs the susceptibility-induced off-resonance field was estimated and applied to correct the magnetic field inhomogeneities of the DTI dataset. In TOPUP, we also created a brain extracted, undistorted mask for the performance of EDDY, where we corrected for head motion and eddy current artefacts. Next, we ran DTIFIT to fit diffusion tensors to the data, resulting in the FA images for each participant.

Subsequently, we conducted Tract-Based Spatial Statistics (TBSS; Smith et al., 2006; Smith et al., 2007) of the patient as well as the control group as recommended in the FSL guidelines. In brief, FA images were preprocessed and registered to the FMRIB58_FA template provided by the FMRIB's Diffusion Toolbox to produce a mean FA skeleton in the MNI152 standard space. Pre and post-specific FA values were projected onto this mean FA skeleton to explore variations in FA at both timepoints. Here, a FA threshold of 0.2 was used to exclude non-white matter from analysis. For statistical analysis, we applied permutation based statistics within the white-matter skeleton using FSL's RANDOMISE command. In RANDOMISE, we performed diverse general linear models (GLMs) along the WM skeleton, where we compared the desired

contrasts for our research hypotheses (see Table 2). Threshold-free cluster enhancement (TFCE) was used. If voxels in white-matter survived the family-wise error (FWE) correction for multiple comparisons, we report them with $p<0.05$. For the TBSS analyses inside of a ROI mask, we used the described coordinates of interest and created a sphere of 2 mm around them by using FSL's command line tools. After this, we integrated the spheres as a mask to our TBSS analyses.

Table 2. Contrasts for statistical anylsis of DTI data, derived from the research hypotheses.

Hypotheses	**GLM type in randomise**	**Contrasts**
1. At baseline, PWS show compared to healthy controls a FA decrease in left and right hemispheric brain regions; the regions are in line with the before mentioned literature. Covariates of no interest: - Age in months - Total SSI score	Two-group difference	**Whole-brain analysis:** PWS > HC PWS < HC
	Two-group difference adjusted for covariate	**Whole-brain analysis with covariate 'age in months':** PWS > HC PWS < HC
	Two-group difference adjusted for covariate	**Whole-brain analysis with covariate 'total SSI score':** PWS > HC PWS < HC
2. Intense stuttering therapy leads to an increase in white matter integrity in: a. the left rolandic operculum	One-Sample T-Test using an timepoint-difference-image; calculation within ROI-mask	**ROI analysis in C_RO {-48, -15, 18}:** SP post > pre SP pre > post
b. the three clusters showing decreased FA in PWS calculated in a meta-analysis of Neef and colleagues (Neef et al., 2015)	One-Sample T-Test using an timepoint-difference-image; calculation within ROI-mask	**ROI analysis in** **C1 {-41, -53, 42}** **C2 {-38, -22, 30}** **C3 {3, -22, 25}:** SP post > pre SP pre > post
c. other brain areas	One-Sample T-Test using an timepoint-difference-image	**Whole-brain analysis:** SP post > pre SP pre > post
	Two-group difference using timepoint-difference-images (post-pre) for each group	**Whole-brain analysis:** SP_post-pre > SC_post-pre SP_post-pre < SC_post-pre SP_post-pre > HC_post-pre SP_post-pre < HC_post-pre
3. The white matter integrity of both healthy and stuttering control groups will, in contrast to the group of stuttering patients, not change over the time of the longitudinal data acquisition.	One-Sample T-Test using an timepoint-difference-image	**Whole-brain analysis:** HC post > pre HC pre > post SC post > pre SC pre > post

Note. SP (stuttering patients), SC (stuttering controls), HC (healthy controls), GLM (general linear model).

2.3 Results

2.3.1 Behavioural data

2.3.1.1 SSI-4

SSI-4 -interrater reliability

For the interrater reliability analysis, we obtained results >0.80 in each tested category of the SSI-4, pointing towards a good interrater reliability (Krippendorff, 2013). Especially the KALPHA result for the 'Total SSI score' interrater agreement was >0.95, so a high consensus and compliance between both raters became evident (see Table 3).

Table 3. Outcome of interrater-reliability analysis of SSI-4.

(Sub-)scores of SSI-4	KALPHA
Reading Score	.8436
Spontaneous Speech Score	.9809
Duration Score	.8528
Concomitants Score	.8868
Total SSI Score	**.9578**

Note. KALPHA was calculated with the SPSS macro of Hayes (Hayes, 2017; Hayes & Krippendorff, 2007) using 10000 bootstrapping samples and the ordinal data level.

SSI-4 – differences between groups at pre- and post-test

A significant difference in the SSI-4 score between all participants groups was found at the *pre-test* (H(2) = 42.6, p = 0.000). Pairwise comparisons with Dunn-Bonferroni adjusted p-values for multiple comparisons showed that the scores differed significantly between stuttering patients and healthy controls (p = 0.000, r = 0.96, large effect) as well as stuttering controls and healthy controls (p = 0.000, r = 0.66, large effect). No significant difference was present between stuttering controls and stuttering patients (p = 0.26, r = 0.3, medium effect).

Testing for differences in the SSI-4 score between all participants groups at the *post-test*, we again obtained results that show significant differences between all groups (H(2) = 208, p = 0.000): stuttering patients and healthy controls (p = 0.000, r = -0.7, large effect) as well as stuttering controls and healthy controls (p = 0.000, r = -0.82, large effect) differed significantly, while there was no significant difference between stuttering controls and stuttering patients (p = 0.397, r = 0.12, small effect).

SSI-4 – longitudinal changes in each group

Comparing the *changes of SSI-Scores from pre- to post-test in each group, respectively,* we saw a significant decrease of the SSI-Score in the group of stuttering patients (median_pre = 26, median_post = 9, z = -3.625, p = 0.000, r = -0.88, large effect; see Figure 1). No significant changes in the SSI-4 from pre- to post-test were detectable in the group of stuttering controls (median_pre = 14, median_post = 13, z = -0.566, p = 0.571, r = -0.15, small effect) as well as healthy controls (median_pre = 0, median_post = 0, z = -1.26, p = 0.208, r = -0.252, small effect; see Figure 1).

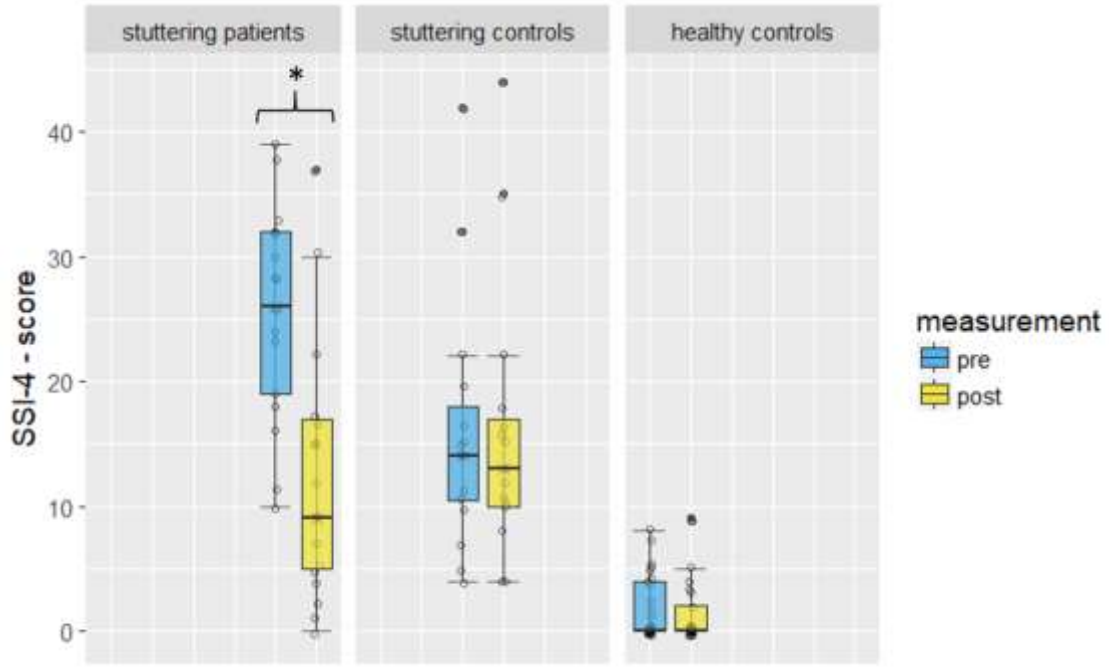


Figure 1. SSI-4 scores of all study groups and measurements. *(Significant difference between post- and pre-measurement; p = 0.000, r = -0.88).

2.3.1.2 Subjective stuttering severity

Subjective stuttering severity – differences between groups at pre- and post-test

At pre-test, the subjective stuttering severity score in the SP group (median_pre = 5) was significantly higher compared to the group of stuttering controls (median_pre = 3), U = 61, z = -2.553, p = 0.011, r = -0.45 medium effect. At the post-measurement, no significant difference in the subjective stuttering severity score could be shown between stuttering patients (median_post = 2) and stuttering controls (median_post = 3), U = 99.5, z = -1.085, p = 0.278, r = -0.19, small effect.

Subjective stuttering severity – longitudinals changes in each group

For the subjective stuttering severity, we found a significant decrease of severity in the group of stuttering patients from pre- to post-test (median_pre = 5, median_post = 2, z = -3.241, p = 0.000, r = -0.79, large effect, see Figure 2). For the stuttering controls, no significant change was detected (median_pre = 3, median_post = 3, z = -0.905, p = 0.366, r = -0.23, small effect).

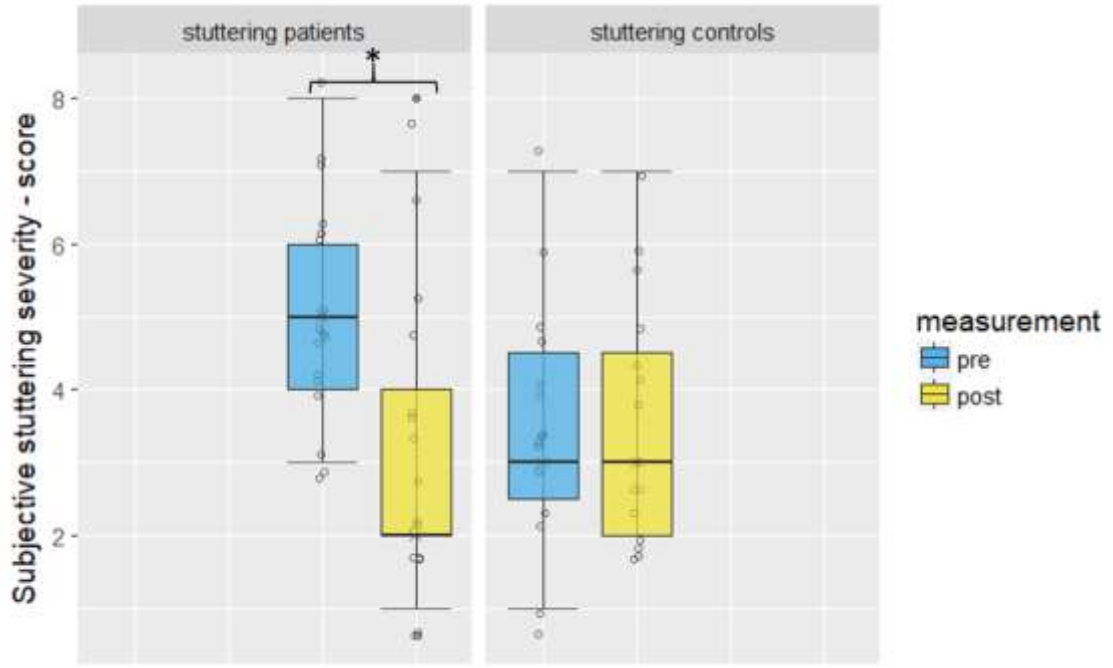


Figure 2. Subjective stuttering severity scores of stuttering patients and stuttering controls. *(Significant difference between post- and pre-measurement; p = 0.000, r = -0.79).

2.3.1.3 OASES

OASES – differences between groups at pre- and post-test

At pre-test, the OASES score in the SP group (median_pre = 3.1) was significantly higher compared to the group of stuttering controls (median_pre = 2.08), U = 14, z = -4.286, p = 0.000 corrected with Holm-Bonferroni for multiple comparisons, r = -0.76, large effect. At the post-measurement, no significant difference in the OASES score could be shown between stuttering patients (median_post = 1.9) and stuttering controls (median_post = 1.98), U = 112, z = -0.585, p = 0.558, r = -0.10 , small effect).

OASES – longitudinals changes in each group

In the OASES, a significant decrease was found in the group of stuttering patients from pre- to post-test (median_pre = 3.1, median_post = 1.9, z = -3.621, p = 0.000, r = -0.87, large effect). No significant difference between both measurements could be shown in the stuttering controls (median_pre = 2.08, median_post = 1.98, z = -0.341, p = 0.733, r = -0.08, small effect, displayed in Figure 3).

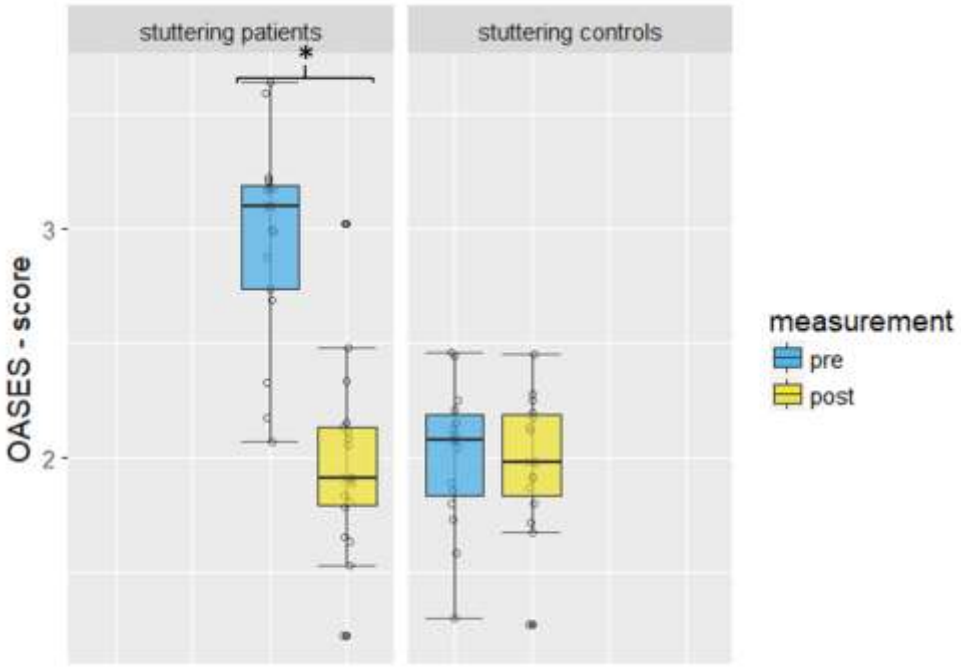


Figure 3. OASES score of stuttering patients and stuttering controls. *(Significant difference between post- and pre-measurement; p = 0.000, r = -0.87).

2.3.1.4 WHO-5

WHO-5– differences between groups at pre- and post-test

Testing for differences between the three groups at the *pre-test*, we found no significant difference (H(2) = 1.008, p = 0.604; see Figure 3) in the WHO-5-score.

Post, we found a significant difference between the groups (H(2) = 7.298, p = 0.026; see Figure 3).

Pairwise comparisons showed the latter could be found between stuttering controls and healthy controls (p = 0.032, r = -0.40, medium effect). No significant difference was present between stuttering controls and stuttering patients (p = 0.87, r = 0.38, medium effect) as well as stuttering patients and healthy controls (p = 1, r = -0.30, medium effect).

WHO-5 – longitudinal changes in each group

Elucidating the *changes of WHO-5-Scores from pre- to post-test in each group, respectively,* there was no significant change detectable (see Table 4, Figure 4).

Table 4. Statistical testing of longitudinal changes in the WHO-5 score in each group.

Group	median pre	median post	z	p	r
SP	17	18	-1.407	0.159	-0.34
SC	18	16	-1.815	0.069	-0.46
HC	17	18	-1.163	0.245	-0.23

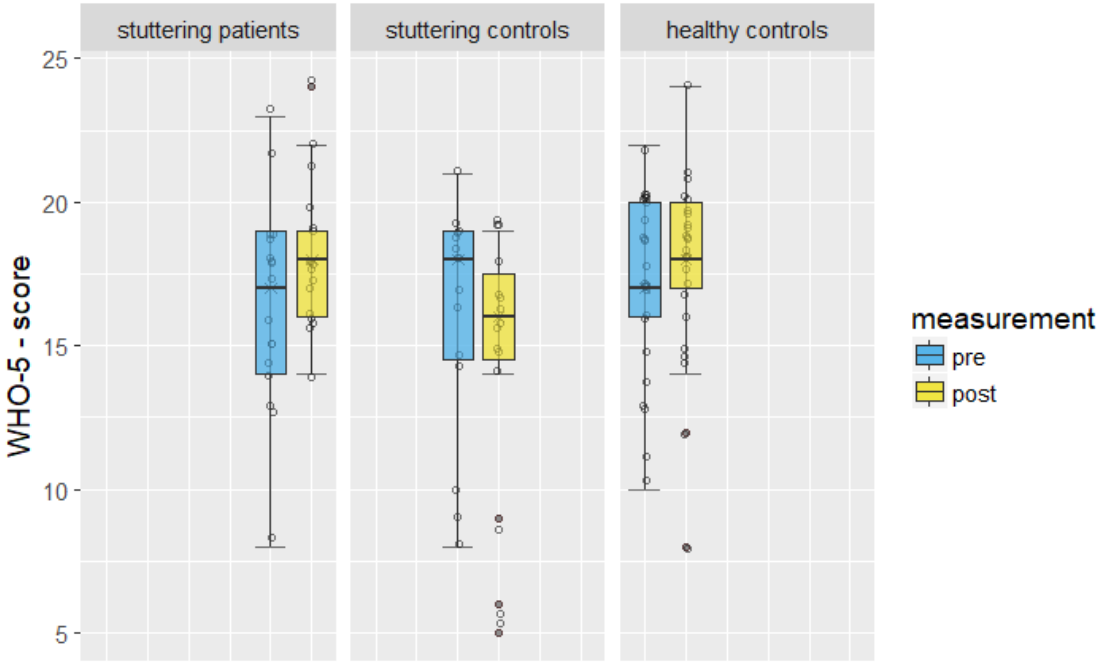


Figure 4. WHO-5 scores of all study groups and measurements.

2.3.1.5 BDI

BDI – differences between groups at pre- and post-test

Testing for differences between the three groups at *pre-test* (H(2) = 0.696, p = 0.706) as well as *post-test* (H(2) = 0.789, p = 0.674), we found no significant difference.

BDI – longitudinals changes in each group

Comparing the *changes of BDI scores from pre- to post-test in each group, respectively,* there was solely a significant difference in the group of stuttering patients (p = 0.004, r = -0.69, large effect, set out in Table 5, Figure 5).

Table 5. Statistical testing of longitudinal changes in the BDI-score in each group.

Group	median pre	median post	z	p	r
SP	3	1	-2.852	0.004	-0.69
SC	3	1	-1.384	0.166	-0.36
HC	2	1	-0.365	0.715	-0.07

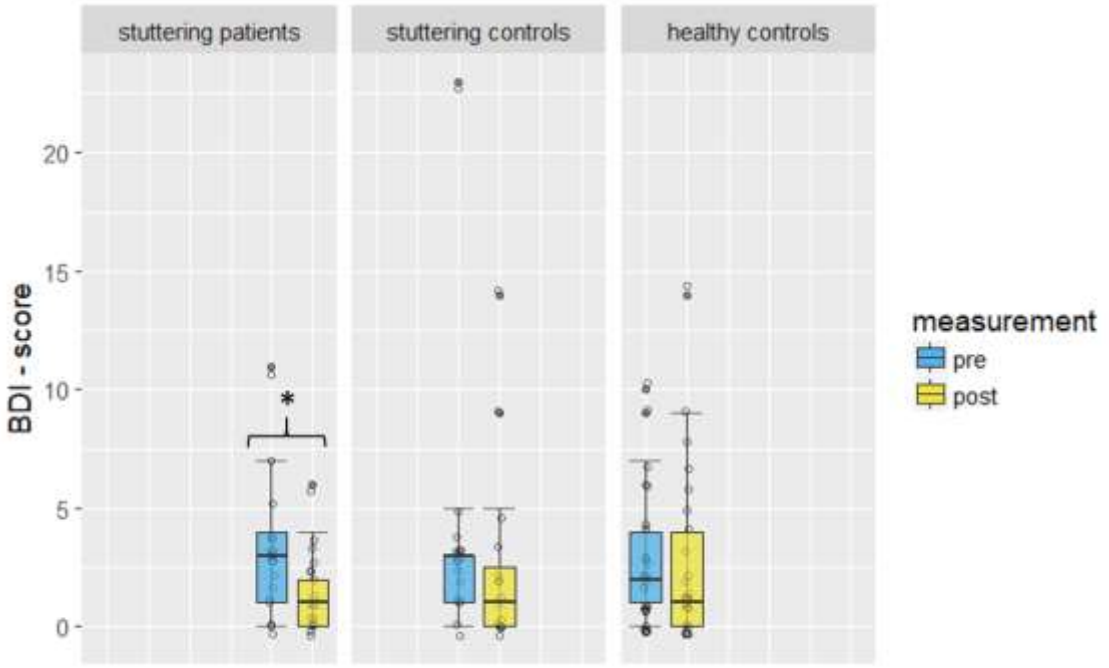


Figure 5. BDI scores of all study groups and measurements. *(Significant difference between post- and pre-measurement; p = 0.004, r = -0.69).

2.3.1.6 State-Trait Anxiety Inventory

For both STAI subtests X1 and X2, there was no significant change between both measurements in the group of healthy participants (see Table 6, Figure 6).

Table 6. Statistical testing of longitudinal changes in the STAI-score in the healthy controls. X1 (state), X2 (trait).

Group	subtest	median pre	median post	z	p	r
HC	X1	29	29	-0.503	0.651	-0.10
	X2	29	28	-0.387	0.699	-0.08

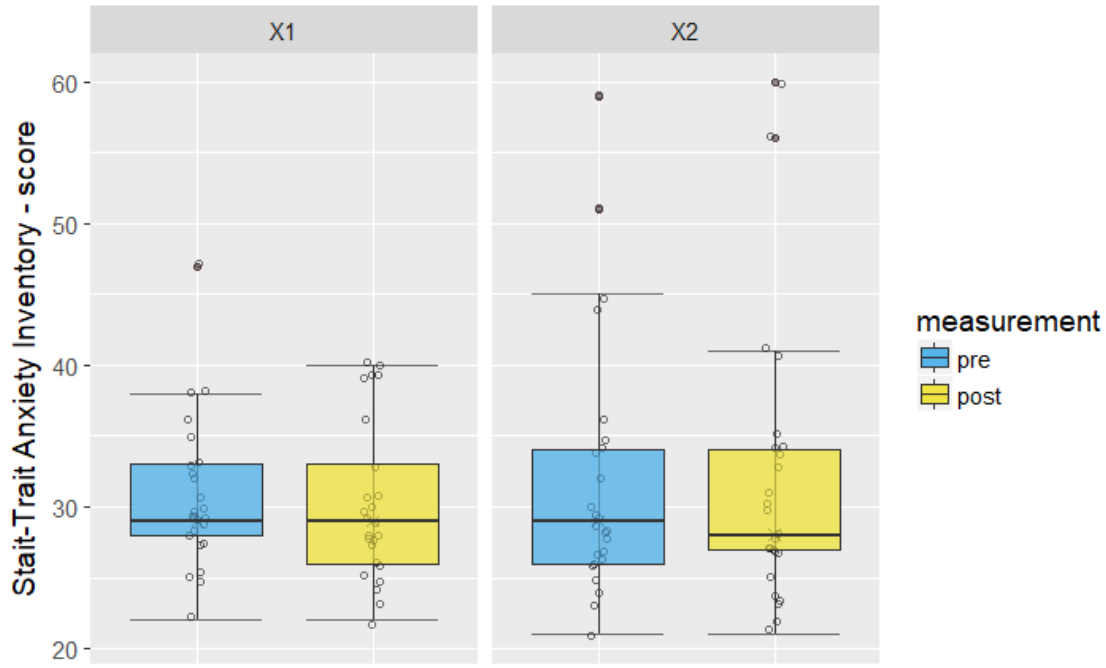


Figure 6. STAI scores of healthy controls. X1 (state), X2 (trait).

2.3.2 Results of the TBSS analysis

We conducted a TBSS analysis to evaluate structural properties of white matter in the participants. For calculating statistics within the analysis, we used the determined contrasts associated with our research hypotheses (see Table 2).

2.3.2.1 TBSS-results - difference between stuttering and healthy participants at pre-measurement

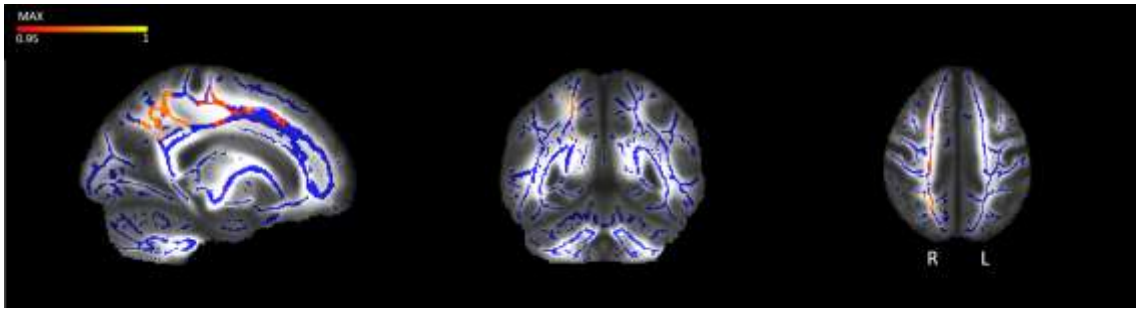


Figure 7. Significantly decreased FA of PWS compared to HC at pre-test visually presented on the participants' white-matter skeleton.

We ran TBSS on the diffusion data of 32 PWS and 25 matched healthy controls at baseline using the contrasts PWS > HC and PWS < HC. In PWS < HC, six clusters in the group of PWS showed significantly decreased FA compared to the healthy controls ($p<0.05$, FWE, cluster size > 10 voxels, see Table 7, Figure 7). All of them were found in the right hemisphere. The largest cluster was located in the cingulum, the others were found in the inferior longitudinal fasciculus as well as in the white matter acoustic radiation, in the inferior-fronto-occipital fasciculus and in the corticospinal tract (see Table 7). For the contrast PWS > HC, no voxels survived the TFCE after an FWE correction.

Table 7. Decreased FA of PWS compared to HC at pre-test – significant clusters of TBSS analysis.

Cluster	Voxel	MAX	MAX X	MAX Y	MAX Z	JHU	ICBM	Jülich
6	3263	0.975	15	-57	32	6% Cingulum (Cingulate gyrus) R	n.s.	n.s.
5	66	0.954	41	-28	7	3% Inferior longitudinal Fasciculus R	n.s.	22% WM Acoustic radiation R 37% GM Primary auditory Cortex TE1.1 R
4	59	0.957	31	28	14	3% Inferior fronto-Occipital fasciculus R	n.s.	n.s.
3	53	0.956	29	39	-2	18% Inferior fronto-Occipital fasciculus R 3% Uncinate fasciculus R	n.s.	6% WM Callosal body
2	24	0.955	38	-37	13	3% Inferior longitudinal Fasciculus R	n.s.	9% WM Callosal body
1	10	0.952	21	-13	4	21% Corticospinal Tract R	Posterior limb Of internal capsule R	30% Wm Potic radiation R 79% WM Corticospinal tract Right

Note. Outcome of the TBSS analysis, conducted with randomise (TFCE, FWE, $p<0.05$). MAX (significance level; p = 1-MAX); MAX X – MAX Z (Coordinates in mm); JHU (JHU white-matter tractography atlas (Mori, Wakana, van Zijl, & Nagae-Poetscher, 2005)); ICBM (ICBM-DTI-81 white-matter labels atlas (Mori et al., 2005)); Jülich (Jülich histological (cyto- and myelo-architectonic) atlas (Eickhoff et al., 2005)). Including clusters ≥ 10 voxel.

Due to the fact that the group of stuttering controls was older than both other groups, we included age in months as a covariate of no interest to the analysis. In this adjusted analysis, we still found three clusters showing significant decreased FA in the group of PWS compared to healthy controls (PWS<HC, p<0.05, FWE).

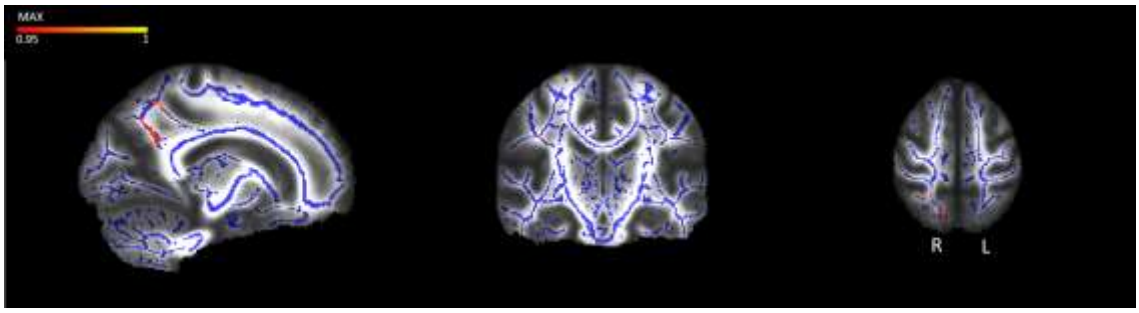


Figure 8. Significantly decreased FA of PWS compared to HC at pre-test, including age as a covariate of no interest - visually presented on the participants' white-matter skeleton.

Again, all clusters were located in the right hemisphere, and anatomically represented the anterior thalamic radiation underneath the superior parietal lobule 5M, the superior longitudinal fasciculus as well as white matter regions lying underneath the inferior parietal lobule (see Table 8, Figure 8 and Figure 9). For the contrast PWS > HC, no voxels survived the TFCE including an FWE correction.

Table 8. Decreased FA of PWS compared to HC at pre-test – significant clusters of TBSS analysis including age in months as a covariate of no interest.

Cluster	Voxels	MAX	MAX X	MAX Y	MAX Z	JHU	ICBM	Jülich
3	1205	0.969	18	-53	49	3% anterior thalamic radiation R	n.s.	10% GM Superior parietal lobule 5M R 8% GM Superior parietal lobule 5L R
2	98	0.953	35	-24	27	42% Superior longitudinal fasciculs R 19% superior longitudinal fasciculus (temporal part) R	Superior longitudinal Fasciculus R	19% WM Superior longitudinal Fasciculus R 2% GM Insula Ig1 R
1	22	0.951	49	-53	18	n.s.	n.s.	13% GM Inferior parietal lobule Pga R

Note. Outcome of the TBSS analysis, conducted with randomise (TFCE, FWE, $p<0.05$). MAX (significance level; p = 1-MAX); MAX X – MAX Z (Coordinates in mm); JHU (JHU white-matter tractography atlas (Mori et al., 2005)); ICBM (ICBM-DTI-81 white-matter labels atlas (Mori et al., 2005)); Jülich (Jülich histological (cyto- and myelo-architectonic) atlas (Eickhoff et al., 2005)). Including clusters ≥ 10 voxel.

The extracted mean FA from the TBSS analysis adjusted for age as a covariate of no interest are set out in Figure 9. The significant differences in mean FA between HC and PWS are apparent in the data distribution illustrated by violin plots.

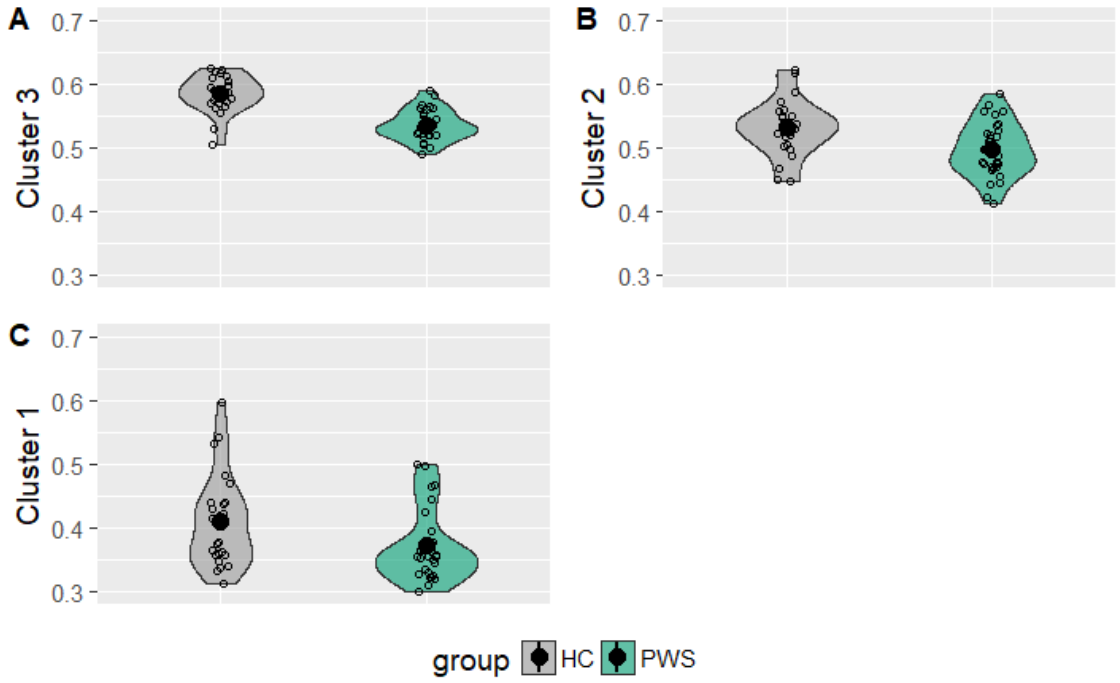


Figure 9. Mean FA of the three significant clusters with a reduced FA in PWS, determined by TBSS analysis with age as a covariate of no interest. Violin plots show individual data points of the participants for the mean FA in the specific cluster, the black dot represents the group mean.On the y-axis, the mean FA in the specific cluster is presented.

After including age as a covariate of no interest, we used the total SSI score as a covariate of no interest to see if the stuttering severity degree had an influence on the decreased FA in the PWS. No significant results were found (PWS < HC, p = 0.233, FWE).

2.3.2.2 TBSS-results – increase of white matter integrity in the group of stuttering patients as an intervention effect

2.3.2.2.1 TBSS-results – increase of white matter integrity in the left rolandic operculum

Because of the a priori hypothesis that stuttering patients taking part in an intense therapy will show an increase of white matter integrity in the left rolandic operculum, we created a 2-mm sphere around the significant coordinate of Sommer and colleagues (2002) and ran the TBSS analysis inside of this sphere to detect an increase of mean

FA in this ROI. We were not able to show any increase of white matter in the left rolandic operculum (SP post > pre, p = 0.29, FWE).

2.3.2.2.2 TBSS-results – increase of white matter integrity in the three clusters showing decreased FA in PWS calculated in a meta-analysis of Neef and colleagues

We ran three TBSS analyses, including each cluster-coordinate of Neef and colleagues (2015) as a ROI with a 2 mm sphere around it, respectively. With this approach, we could evaluate if there was a significant increase of FA in the stuttering patients after therapy. For the first cluster {-41, -53, 42} which was located in the left superior longitudinal fasciculus, we found a significant increase of mean FA after therapy (p = 0.035, FWE, see Figure 10, Figure 11). For the other two clusters, no evidence was found for an increase of white matter integrity (cluster-coordinate 2: {-38, -22, 30}; p = 0.76, FWE; cluster-coordinate 3: {3, -22, 25}, p = 0.76, FWE).

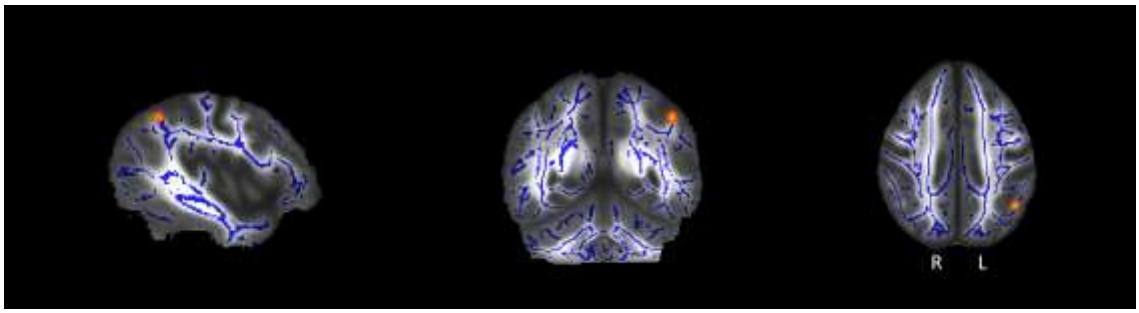


Figure 10. Significant cluster-coordinate 1 of Neef and colleagues.

Figure 11 illustrates the extracted mean FA of the stuttering patients in cluster 1. The significant increase of mean FA from pre- to post-measurement is detectable.

To check if the FA increase in this ROI would correlate with changes in the total SSI score or the OASES score from pre- to post-measurement, we calculated Spearman's rank order correlations. No correlations were detectable between changes of FA values and changes of SSI scores (p = 0.648, r = 0.119) as well as between changes of FA values and changes of OASES scores (p = 0.333, r = 0.25).

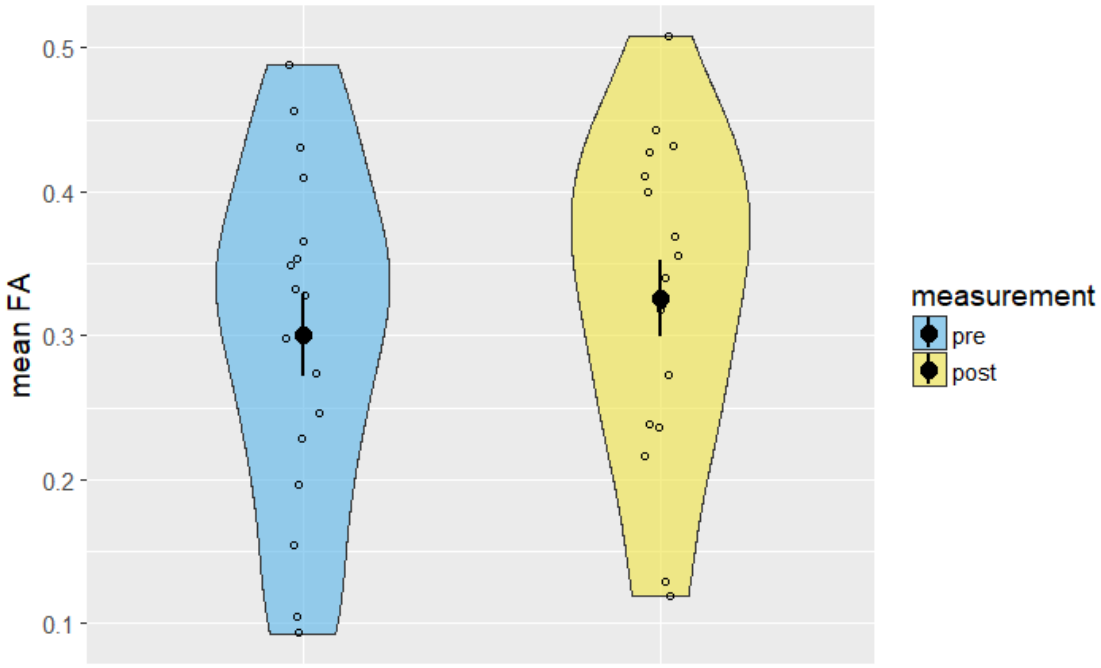


Figure 11. Significant increase of mean FA pre and post therapy in the group of stuttering patients. Analysed in ROI - cluster 1 of Neef and colleagues, 2015. Violin plots show individual data points of the participants for the mean FA in the specific cluster, the black dot represents the group mean and the black vertical lines indicate the standard error.

2.3.2.2.3 TBSS-results – increase of white matter integrity in other brain areas

To check if there are therapy-related structural changes in the whole brain without considering a specific ROI, we ran the TBSS analysis without any ROI mask.

First, we focused on the change of white matter plasticity in the group of stuttering patients itself without comparing the fibre change in this groups to the other control groups: The contrast SP post>pre ($p = 0.126$, FWE) showed no significant increase of FA.

Second, we compared the structural changes over time in the SP group with structural changes over time in the SC and HC group, respectively. The comparison with the healthy controls showed that the stuttering patients' change of white matter integrity from pre to post measurement was significantly larger than in the group of healthy controls (SP_post-pre > HC_post-pre, $p = 0.023$, FWE, see Figure 12, Table 9). In comparison

to healthy controls, stuttering patients showed more white matter increase in the right corticospinal tract as well as in the right superior longitudinal fasciculus and the body of the corpus callosum (see Table 9, Figure 12).

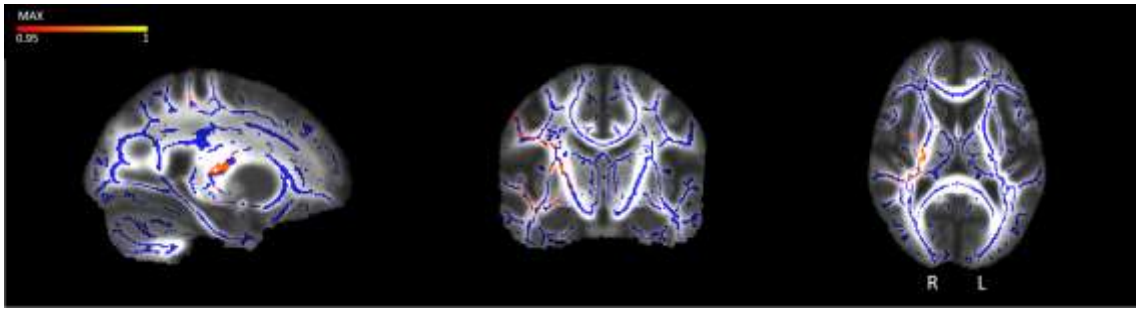


Figure 12. Significantly increased FA from pre- to post-measurement in the SP group compared to the HC group - visually presented on the participants' white-matter skeleton.

Table 9. Increased FA from pre- to post-measurement in the SP group compared to the HC group (SP_post-pre > HC_post-pre) – significant clusters of TBSS analysis.

Cluster	Voxels	MAX	MAX X	MAX Y	MAX Z	JHU WM Tract	ICBM	Jülich
5	4385	0.977	25	-16	11	50% Corticospinal tract R	Posterior limb of internal Capsule R	89% WM Corticospinal tract R
4	9	0.951	20	-21	38	3% Corticospinal tract R	Superior corona radiata R	47% WM Callosal body 23% WM Corticospinal tract R 8% WM Superior occipito-frontal fascicle R
3	8	0.951	20	-42	28	x	Splenium of corpus callosum	82% WM Callosal Body
2	5	0.951	30	-23	43	3% Superior longitudinal Fasciculus R	x	62% Gm Primary somatosensory cortex BA3a R 61% WM Corticospinal tract R 32% WM Superior longitudinal fascicle R 29% GM Primary motor cortex BA4p R
1	4	0.951	15	-26	31		Body of corpus callosum	89% WM Callosal Body

Note. Outcome of the TBSS analysis, conducted with randomise (TFCE, FWE, p<0.05). MAX (significance level; p = 1-MAX); MAX X – MAX Z (Coordinates in mm); JHU (JHU white-matter tractography atlas (Mori et al., 2005)); ICBM (ICBM-DTI-81 white-matter labels atlas (Mori et al., 2005)); Jülich (Jülich histological (cyto- and myelo-architectonic) atlas (Eickhoff et al., 2005)).

On the contrary, the fibre increase of the stuttering patients compared to the FA increase in stuttering controls over time was not significant (SP_post-pre > SC_post-pre, p = 0.067, FWE).

2.3.2.3 TBSS-results – white matter changes over time in both control groups

We hypothesised that the white matter integrity in both healthy and stuttering controls would not change over time because there was no intervention or training for these participants taking place. To evaluate this hypothesis, we checked for fibre increase as well as decrease in both control groups.

The tested contrasts for fibre increase in both groups were not significant (HC post > pre, p = 0.945, FWE; SC post > pre, p = 0.966). On the opposite, the contrasts for evaluating a fibre decrease were both signifcant (HC pre > post, p = 0.021, FWE; SC pre > post, p = 0.024, FWE). In the group of healthy controls, the fibre decrease took place in the left and right superior longitudinal fasciculus, left and right corticospinal tract as well as in right inferior fronto-occipital fasciculus, right inferior longitudinal fasciculus and in the right anterior-thalamic radiation (see Table 10, Figure 13).

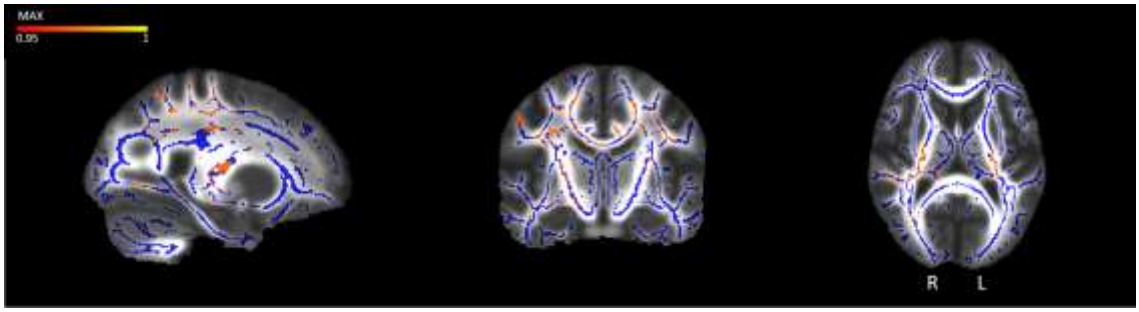


Figure 13. Significantly decreased FA from pre- to post-measurement in the group of HC - visually presented on the participants' white-matter skeleton.

Table 10. Decreased FA from pre- to post-measurement in the HC group – significant clusters of TBSS analysis.

Cluster	Voxels	MAX	MAX X	MAX Y	MAX Z	JHU	ICBM	Jülich
10	6660	0.979	38	-57	33	5% superior longitudinal fasciculus R	n.s.	10% GM Anterior intra-parietal sulcus hIP1 R
9	3500	0.979	-34	-33	25	29% Superior longitudinal fasciculus L 16% Superior longitudinal fasciculus (temporal part) L	n.s.	10% Secondary somatosensory cortex / Parietal operculum OP1 L 7% GM Inferior parietal lobule Pfcm L 6% GM Insula Ig1 L
8	393	0.979	-25	-17	11	34% Corticospinal Tract L	Posterior limb of internal capsule L	57% Corticospinal tract
7	352	0.971	40	-41	-4	47% Inferior fronto-occipital fasciculus R 26% Inferior longitudinal fasciculus R	Sagittal stratum (include inferior longitidinal fasciculus and inferior Fronto-occipital fasciculus) R	73% Callosal body 21% WM Optic radiation R
6	120	0.961	13	-25	-12	50% Corticospinal Tract R	Cerebral peduncle R	27% GM medial geniculate body R 7% WM Acoustic Radiation R
5	97	0.953	28	-9	-12	3% Inferior longitudinal Fasciculus R	n.s.	54% GM Amygdala_laterobasal Group R 34% GM Amygdala_centromedial group R
4	24	0.951	25	-18	-9	n.s.	n.s.	10% Hippocampous cornu ammonis R 29% GM Lateral geniculate body R 14% WM Corticospoinal tract R 14% GM Hippocampus cornu Ammonis R
3	23	0.951	30	-55	-12	n.s.	n.s.	n.s.
2	22	0.953	32	-65	-9	21% Inferior longitudinal fasciculus R 8% Inferior fronto-	n.s.	29% GM Visual cortex V4 R 1% WM Optic radiation R

						occipital fas-Ciculus R		
1	14	0.951	31	-26	-5	3% Anterior thalamic radiation R	Fornix (cres) / Stria terminalis	50% WM Optic radiation R 15% Gm Hippocampus cornu ammonis R 10% WM Fornix

Note. Outcome of the TBSS analysis, conducted with randomise (TFCE, FWE, p<0.05). MAX (significance level; p = 1-MAX); MAX X – MAX Z (Coordinates in mm); JHU (JHU white-matter tractography atlas (Mori et al., 2005)); ICBM (ICBM-DTI-81 white-matter labels atlas (Mori et al., 2005)); Jülich (Jülich histological (cyto- and myelo-architectonic) atlas (Eickhoff et al., 2005)). Including clusters ≥ 10 voxel.

In the group of stuttering controls, the fibre decrease was detectable mainly in the left hemisphere. Specifically, it was traceable in the left anterior thalamic radiation, corticospinal tract, inferior fronto-occipital fasciculus, in the forceps minor, cingulum and superior longitudinal fasciculus (see Table 11, Figure 14).

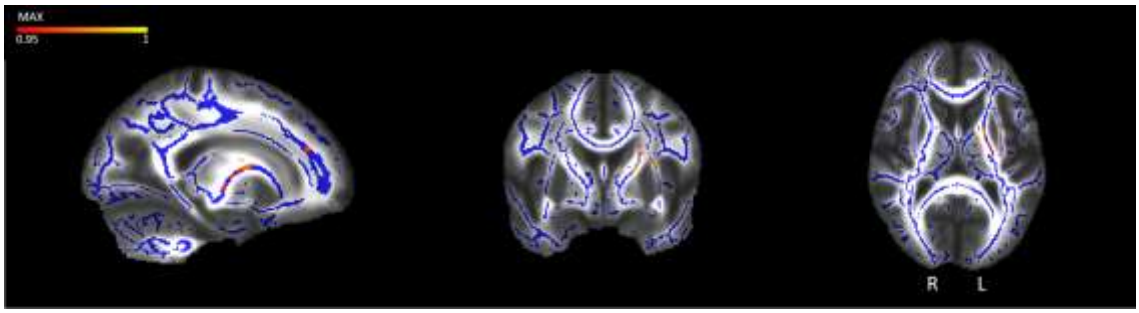


Figure 14. Significantly decreased FA from pre- to post-measurement in the group of SC - visually presented on the participants' white-matter skeleton.

Table 11. Decreased FA from pre- to post-measurement in the SC group – significant clusters of TBSS analysis.

Cluster	Voxels	MAX	MAX X	MAX Y	MAX Z	JHU	ICBM	Jülich
11	478	0.976	-18	1	10	16% Anterior thalamic radiation L	Anterior limb of internal capsule L	8% WM Corticospinal tract L
10	276	0.966	-22	-10	10	3% Corticospinal tract L	Posterior limb of internal capsule L	60% WM Corticospinal tract
9	31	0.956	-27	-25	15	16% Corticospinal Tract L	Retrolenticular part of internal capsule L	27% WM Corticospinal tract L
8	30	0.961	-32	-14	11	3% Inferior fronto-occipital fasciculus L	External capsule L	34% GM Secondary somatosensory cortex / parietal operculum OP3 L
7	30	0.965	-19	38	23	29% Forceps minor 8% Anterior thalamic radiation L	n.s.	n.s.
6	22	0.965	-12	-6	-3	34% Anterior thalamic radiation L	Posterior limb of internal capsule L	n.s.
5	17	0.960	-29	-16	18	5% superior longitudinal fasciculus L	External capsule L	18% WM Corticospinal tract 12% WM Superior longitudinal fascicle L 10% GM Secondary somatosensory cortex/ Parietal operculum OP2
4	16	0.956	11	18	21	n.s.	Body of corpus callosum	93% WM Callosal body 6% WM Cingulum R
3	14	0.959	-13	33	10	74% Forceps minor 14% Cingulum (cingulate gyrus) L	genu of corpus callosum	100% WM callosal body
2	13	0.955	-25	-15	14	21% Corticospinal tract L	Posterior limb of internal capsule L	88% WM Corticospinal tract L
1	12	0.955	-12	31	12	68% Forceps minor	Genu of corpus callosum	100% WM callosal body 1% WM Cingulum L

Note. Outcome of the TBSS analysis, conducted with randomise (TFCE, FWE, $p<0.05$). MAX (significance level; p = 1-MAX); MAX X – MAX Z (Coordinates in mm); JHU (JHU white-matter tractography atlas (Mori et al., 2005)); ICBM (ICBM-DTI-81 white-matter labels atlas (Mori et al., 2005)); Jülich (Jülich histological (cyto- and myelo-architectonic) atlas (Eickhoff et al., 2005)). Including clusters ≥ 10 voxel.

After obtaining these results, we controlled if a fibre decrease over time was also evident in the group of stuttering patients, but no significant fibre decrease in the whole brain was present in the stuttering patients' group (SP pre > post, p = 0.763, FWE).

2.4 Discussion

Our prime study objective was to analyse the influence and effects of an intense stuttering therapy on white matter integrity in PWS. Subsidiary, we wanted to replicate former results of reduced FA in an independent sample of PWS – these reductions are seen as a neural hallmark of stuttering. Because brain regions with less amount of FA differed slightly in previous studies, we illuminated this circumstance again in our study.

There are multiple outcomes of this study:

Regarding the *behavioural measurements*, a reduced occurrence of stuttering symptoms in the patient group taking part in the intervention was characterised by the significant decrease of the total SSI-score in this group. Additionally, a significant shrinkage of the subjective stuttering severity as well as the OASES score was shown, pointing towards a subjectively perceived grown speech fluency and a reduced impact of stuttering on the life of PWS. These positive effects seem to be associated with the intervention, since the stuttering control group did not show any of the described changes.

While the WHO-5 revealed no significant longitudinal changes in each group, the BDI score displayed a significant decrease from pre- to post-measurement in the patient group. This can probably be interpreted as a result following stuttering therapy – the more secure the patients could implement their new-learned speech pattern, the less negative emotions and attitudes they had to deal with (Euler et al., 2016). The healthy controls did not show any significant changes in the STAI between pre- and post-measurement. This is underlining the stable psychological state of this group concerning anxiety in general and towards different communication situations.

According to the *FA differences between PWS and healthy controls*, we were able to show that PWS demonstrate significant lower FA values in specific brain regions compared to healthy controls at pre-test. This result is replicating the outcome of previous research studies (for a review, see Etchell et al., 2017; Neef et al., 2015). Regions with significant reduced FA values in PWS of our current study sample were all in the right hemisphere and included the cingulum, parts of the inferior longitudinal fasciculus (close to the white matter acoustic radiation and the callosal body), the inferior-fronto-occipital fasciculus as well as the corticospinal tract.

The reduction of white matter integrity in PWS was still consistent when adding age as a covariate of no interest to the whole brain TBSS-analysis, but yielded only three significant clusters of FA reduction. These were situated again in the right hemisphere, comprising the anterior thalamic radiation, the superior longitudinal fasciculus as well as white matter regions underneath the inferior parietal lobule. The TBSS analysis which was conducted using the SSI total score as a covariate of no interest did not reveal any significant FA reduction. Therefore, the reduction of FA in PWS seems to be influenced by age, but not by different stuttering severity degrees.

Concerning the *influence of an intense stuttering therapy on FA values*, the whole-brain TBSS analysis revealed no FA increase in the group of stuttering patients. Also in the ROI analysis in the left rolandic operculum, no significant FA increase became evident. Nevertheless, we were able to verify a significant growth of FA measured from pre- to post-test in the first cluster identified by the meta-analysis of Neef and colleagues (2015). It was situated in the left superior longitudinal fasciculus, but did not correlate with the change of scores in the SSI or OASES scales in stuttering patients. Therefore, we could not confirm an association between the fibre increase facilitated by therapy and improvements in the most meaningful, standardised and international well established stuttering-related behavioural scales. We conclude that SSI and OASES scores do not

have a prognostic potential for predicting an enhanced structural plasticity as a consequence of a therapeutic success.

As opposed to our expectations, we found a change in FA from pre- to post-test in both control groups. The participants of these groups demonstrated a significant fibre integrity decrease in the whole brain TBSS analysis from pre- to post-test, which was absent in SP.

White matter integrity differences in PWS compared to healthy controls

Specifying the differences in FA values between PWS and healthy controls, former studies mostly reported reduced fibres in left hemispheric brain regions, e.g. the left rolandic operculum (Sommer et al., 2002), left perisylvian regions (Cykowski et al., 2010) and the left mid motor cortex (Cai et al., 2014). Also the meta-analysis of Neef and colleagues revealed clusters with reduced FA values in PWS in the left superior longitudinal fasciculus of the parietal lobe, the left arcuate fasciculus and the midbody of the corpus callosum (Neef et al., 2015). They did not find a significant cluster in the right hemisphere.

Nevertheless, studies published within the last five years reported reductions of white matter integrity in PWS in the right hemisphere: Cai and colleagues (2014) as well as Chang and co-authors (2015) discovered reduced FA values along different parts of the right superior longitudinal fasciculus, while Kronfeld-Duenias et al. (2016) found FA reductions in the anterior callosum as well as the right inferior longitudinal fasciculus and the right cingulum. Cieslak et al. (Cieslak, Ingham, Ingham, & Grafton, 2015) also discovered missing portions of the bilateral arcuate fasciculus in PWS. A recent study evaluating the FA values of CWS in comparison to fluent controls reported a reduction of FA in the right frontal aslant tract (Misaghi et al., 2018). Moreover, Neef and colleagues (2018) detected a FA reduction in the left and right superior longitudinal fasciculus as well as in the junction of the right frontal aslant tract in their newest study.

These studies illuminate that also right hemispheric reductions of FA exist in PWS and are partly in line with the results of our study. We were able to show that our stuttering study population exhibited reduced FA in the right cingulum and in parts of the inferior longitudinal fasciculus (as previously shown by Kronfeld-Duenias et al., 2016) and in parts of the right corticospinal tract (as demonstrated by Cai et al., 2014; Watkins et al., 2008). Additionally, we exhibited a decline of FA in the right inferior-fronto-occipital fasciculus. This region has not been reported by any study examining a reduction of white matter integrity so far.

The decline of FA in PWS was still consistent when adding age as a covariate of no interest to the whole brain TBSS analysis, but yielded only three significant clusters of FA reduction. These were situated again in the right hemisphere, comprising the anterior thalamic radiation, the superior longitudinal fasciculus as well as white matter regions underneath the inferior parietal lobule.

The right superior longitudinal fasciculus was frequently obtaining less FA in PWS in former studies (Cai et al., 2014; Chang et al., 2015; Etchell et al., 2017; Neef et al., 2018) as well as in our study. This fibre bundle connects the perisylvian speech areas in the parietal lobe and posterior and inferior frontal lobe interhemispherically. Together with the arcuate fasciculus, this region is known to support e.g. vocalization control in humans (García, Zamorano, & Aboitiz, 2014). Therefore it seems plausible that a reduced white matter integrity in these bundles found in PWS might play a major role in the pathophysiology of stuttering. Especially the fact that an impaired vocalization control is present within the core symptoms of stuttering underlines this plausible finding. Neef and colleagues illuminate that the superior longitudinal fasciculus belongs to the dorsal fibre tracts which have different, hemispheric-related functions according to diverse speech production models (Guenther, Hampson, & Johnson, 1998). Dorsal tracts in the left hemisphere are important for language acquisition and articulation, while dorsal tracts in the right hemisphere process the control of deranged auditory and somatosensory

feedback during speaking (Golfinopoulos et al., 2011; Neef et al., 2018). This is why the observed FA reductions in PWS in the right superior longitudinal fasciculus might point to a disturbance of feedback processes in stuttering which could be part of the stuttering aetiology.

When not cancelling out age effects in the group of PWS, we discovered reduced FA values also in the right cingulum, inferior longitudinal fasciculus, the inferior fronto-occipital fasciculus and in the right corticospinal tract.

The cingulum contains association fibres and connects the cingulate cortex with the parahippocampal gyrus as well as the medial prefrontal cortex and the medial cortex areas of parietal and occipital lobes (Schmahmann et al., 2007). The cingulate gyrus which is innervated by the cingulum is seen as the neural basis for attention control (Greicius et al., 2007). The cingulum itself was found to regulate attention and to support the control of emotional conflicts in patient with major depressive disorder (Keedwell et al., 2016). Furthermore, a reduced FA in the cingulum was demonstrated in patients with post-traumatic stress disorder (Fani et al., 2012). This is why the reduction of white matter integrity in PWS in this region could be related to the dealing with learned fear, emotional processing and the coping of psychological strain – all of these conditions occur often in the disorder of stuttering.

The inferior longitudinal fasciculus links the inferior temporal lobe with the occipital lobe (Duffau, 2012). In the right hemisphere, this fibre bundle is primarily associated with visual object recognition (Tavor et al., 2014). In the field of stuttering, Chang and colleagues demonstrated that the FA in the right inferior longitudinal fasciculus was increased in CWS (Chang, Erickson, Ambrose, Hasegawa-Johnson, & Ludlow, 2008). Kronfeld-Duenias and Colleagues, on the opposite, exhibited a bilateral reduction of FA in this tract in adult PWS (Kronfeld-Duenias, Civier, Amir, Ezrati-Vinacour, & Ben-Shachar, 2018). Our study outcome of an decreased FA in this tract might additionally

stress the importance of this bundle as a neural correlate of stuttering. The fact that CWS demonstrated an increased white matter integrity in this fibre bundle, while adults showed the opposite pattern, might also lead to the conclusion that a reduced FA in adults could be a compensatory response to the stuttering and its symptoms. Therefore, an increase of FA in childhood might be a causative factor for the onset of stuttering.

Interestingly, the inferior longitudinal fasciculus is also known to show reduced FA in patients with autism spectrum disorder (Boets et al., 2018). These patients experience an impact of their disorder on social and communication behaviours -this holds also true for stuttering patients. Moreover, studies have shown an increase of FA in toddlers with autism spectrum disorder. This increase turned into a decrease of FA in grown-up patients with autism (Boets et al., 2018; Solso et al., 2016) – the same pattern of FA increase in childhood vs. FA decrease in adulthood is observable in stuttering patients in the inferior longitudinal fasciculus. More studies with a larger number of patients should therefore gather further insight on the inferior longitudinal fasciculus as a possible neural correlate for emotional and social processing in patients with stuttering.

The reduced FA in the inferior frontal occipital fasciculus observed in our study is a rare result in the field of stuttering. The functional role of this fibre bundle is still not clear –it is known for its role in non-verbal semantic processing (Herbet, Moritz-Gasser, & Duffau, 2017). Or it is characterised as a “multi-function” bundle that might play a role in semantic, emotional and behavioural processing as well as in sensory-motor integration (Sarubbo, Benedictis, Maldonado, Basso, & Duffau, 2013). Our result should be replicated by other studies to gather further knowledge about the involvement of the inferior frontal occipital fasciculus in stuttering.

The reduction of FA in the right corticospinal tract we report here was previously found in CWS (Chang et al., 2008), but also in stuttering adults (Cai et al., 2014; Watkins et al., 2008). The decrease of white matter integrity near the posterior limb of the right

internal capsule might have an impact on the motor production of spoken words and syllables and therefore be related to the stuttering core symptoms. Surely, it is not alone responsible for the emergence of stuttering symptoms – as our results and previous studies show, reductions of FA are reported in diverse brain regions in PWS. Complex structural as well as functional network collaborations including some of these mentioned regions might be responsible for the onset of stuttering symptoms in the end.

Therapy-induced effects in white matter integrity

When solely regarding the group of stuttering patients that took part in the therapy, we could not detect any effects on white matter integrity in the whole-brain TBSS analysis, but found an increase of FA in a ROI analysis. This ROI was the first cluster showing decreased FA in PWS compared to healthy controls in the meta-analysis of Neef et al (2015) and was located in the third part of the left superior longitudinal fasciculus in the inferior parietal lobe, close-by the angular gyrus and the posterior supramarginal gyrus.

Neef and colleagues showed with deterministic DTI tractography that this cluster is connected to the postcentral gyrus, the ventral premotor cortex and the posterior-ventral area of the pars opercularis of the inferior frontal gyrus (BA 44, Broca's area, see Neef et al., 2015).

These reconstructed connections are highlighting the important role of this area for the motor speech output of our stuttering patients: while the postcentral gyrus is responsible for processing sensorimotor information and contains the sensory homunculus (Trepel, 2004), the ventral premotor cortex overlaps partly with Broca’s area in the dominant cerebral hemisphere and is built of BA 44 and 45. According to diverse studies, it constitutes of an execution-observation matching system (mirror neurons) as well as the motor cortex homunculus (Binkofski & Buccino, 2006). The inferior frontal gyrus’ pars opercularis is congruent with BA 44/Broca’s area and represents the key centre in the brain for producing articulated language. Broca’s area is known to play a role in the

processing of speech production and semantic, syntactic and phonologic processing (Friedrich et al., 2018; Heim, Eickhoff, & Amunts, 2008; Price, 2010), furthermore in rhythm and music processing as well as the working memory for pitch (Koelsch & Siebel, 2005; Platel et al., 1997).

Altogether, these speech-related functions are processed in the brain regions which are connected via the superior longitudinal fasciculus to the cluster 1 identified by Neef et al. (2015). In this cluster, our stuttering patients showed an increase of FA after therapy. The FA increase we observed in this region seems to be caused by the intense learning of a new articulation pattern in the Kasseler stuttering therapy, as it is a unique feature of the intervention group. This white matter integrity growth could have facilitated the improvement of speech fluency seen in the SSI-4 as well as for the improvement of the life quality of patients measured by the OASES.

It is up for discussion what this ROI-specific FA increase might evoke on a neuronal level. Chow and Chang (2017) conducted a longitudinal DTI study with CWS and showed that a slower FA growth with age can be found in children with persistent stuttering compared to children with recovered stuttering and fluent controls. The regions where this reduced FA growth rate was shown were the left arcuate fasciculus and the corpus callosum. This result suggests that the growth of FA in certain brain areas in CWS might be a predictor for the unassisted remission of stuttering. In our study, we examined the assisted recovery of stuttering evoked by therapy. Our observed white matter integrity growth in the ROI analysis could be interpreted as a hallmark for the assisted alleviation of stuttering: the successful therapy might have facilitated fibre growth in the described region, which was supporting the gain of speech fluency in the patients.

Concerning the biochemical level this ROI-specific FA increase might express a higher alignment of fibres or an improved myelination, like Schlaug and colleagues suggest

(Wan et al., 2014; Zheng & Schlaug, 2015). This improved myelination could be responsible for a facilitated intrahemispheric interaction. A more detailed analysis using additional tractography to reconstruct the tracts of interest and then directly measuring their FA values or more advanced methods that can analyse axon diameter distributions (Assaf et al., 2008) would have helped to get more insight on neural cellular processes.

Interestingly, on a whole brain analysis, we could not demonstrate that stuttering patients showed a significant increase of white matter integrity. Solely when we compared the longitudinal changes of FA from post- to pre-test between healthy controls and stuttering patients on a whole-brain level, we found a significant white matter increase in stuttering patients compared to healthy controls in the right corticospinal tract, the right superior longitudinal fasciculus and in the corpus callosum (Figure 12, Table 9). This white matter increase was not demonstrated in the longitudinal comparison to the stuttering control group. We interpret this in relation to the outcome discussed in the next section: because of the measured FA decline in both control groups (which was more widespread in healthy controls than in stuttering controls), the stuttering patients showed an increase of FA in comparison to healthy controls. The latter is evoked by the contrast of significant decline of FA in healthy controls on the one hand and the increase of FA in stuttering patients on the other hand.

Indeed, no correlations were evident between increases of FA values and improvements in the SSI-4/OASES, so the association between the behavioural enhancement and the white matter integrity growth in the evaluated ROI is debatable. We were not able to show evidence for a prognostic potential of SSI-4 and OASES. In the current study, both scores are not able to predict an enhanced structural plasticity as a consequence of a therapeutic success.

On the contrary, in a few studies where correlations between behavioural measurements and white matter integrity were investigated, a positive result was found: Connally and

colleagues (Connally, Ward, Howell, & Watkins, 2014) were able to show an association between the white matter integrity in the left angular gyrus (close to Neef and colleagues' (2015) cluster 1 where we found an increase of FA in the intervention group) and the SSI score. They observed that the higher the stuttering severity index is, the lower the white matter integrity in the left angular gyrus is, and the greater the white matter connectivity in the left corticobulbar tract is. This result underlines that behavioural measurements like the SSI relate in some studies to white matter characteristics, but in other studies, no associations are found (e.g. Cai et al., 2014 as well as our current study). The reasons for this might be manifold – they could be found in different study population characteristics as well as in different analysis and statistical approaches. In addition, the current study is the first one observing longitudinal changes in the white matter integrity of PWS after therapy – therefore, more longitudinal studies in this field are needed to clarify if any prognostic factors for a fibre increase induced by therapy can be determined.

Age-induced FA decrease in controls groups

Before starting the study, we assumed that white matter integrity of both stuttering and healthy control groups would not change because of the relatively small follow-up-time of the measurements and the fact that no intervention was applied to these groups. Contrary to our expectations, we observed a significant fibre decrease in the healthy control group as well as in the stuttering control group from pre- to post-test.

In the group of healthy controls, the FA decrease was relatively widespread and verifiable in both hemispheres, e.g. in parts of the left and right superior longitudinal fasciculus, corticospinal tract and in the right hemisphere in the inferior fronto-occipital fasciculus, the right inferior longitudinal fasciculus and in the right anterior-thalamic radiation.

In comparison to this outcome, the group of stuttering controls also showed a significant fibre decrease which was less widespread and solely detectable in the left hemisphere. In specific, decreases of white matter integrity were found in the left anterior thalamic radiation, corticospinal tract, inferior fronto-occipital fasciculus, the forceps minor, cingulum and the superior longitudinal fasciculus.

Because the participants in these groups experienced no form of treatment, intervention or a specific training, we assume that the effect of a significant reduction of white matter integrity could be caused by ageing effects that become obvious during our longitudinal study design. The second measurement took place approximately 11.5 months after the first measurement. This is a rather long timespan where neural plasticity could have changed.

Several studies shed a light on the changes of white matter integrity during the life span. A review of Seidler and colleagues (2010) shows that both quality as well as quantity of white matter decrease over the life span. The “last-in-first-out-hypothesis” of brain ageing (Raz, 2001) contains the theory that white matter regions which develop lately in life are more vulnerable to progressive age and exhibit an earlier atrophy than regions which develop earlier. According to this hypothesis, the greatest longitudinal reduction of FA should be expected in association fibres and after that in commissural fibre bundles. The least changes should be assumed for projection fibres (Bender, Völkle, & Raz, 2016).

Indeed, several studies reported a decline of white matter integrity with age; in addition, they found region-specific effects on white matter integrity on an anterior-to-posterior gradient. The more anterior the evaluated white matter fibre bundles were, the lower the FA values were. Davis and co-authors (2009) demonstrated this behaviour for long white matter tracts like the uncinate fasciculus, the cingulum, the inferior longitudinal fasciculus and the corpus callosum. Also Zahr et al. demonstrated that lower FA represented an age-related effect in their study; the decline of white matter integrity was prominent in

anterior tracts, specifically in the genu, fornix and uncinate fibres (Zahr, Rohlfing, Pfefferbaum, & Sullivan, 2009). Kochunov and colleagues (Kochunov et al., 2012) found evidence for an age-related reduction of FA especially in the genu, the anterior part of the corpus callosum. They summarized that white matter tracts which mature later in life demonstrate higher age-associated reductions of FA than earlier developing motor and sensory tracts (Kochunov et al., 2012) Their findings were therefore in line with the “last-in-first-out-hypothesis”.

Unfortunately, the study design of these previously described studies did not include multiple longitudinal measurements. In our study, we measured the same stuttering and healthy controls with a follow-up-period of 11 to 12 months, so the question is if a reduction of white matter integrity we have seen in our healthy and stuttering control group became evident in other studies with a similar interval between two longitudinal measurements.

Bender and colleagues (2016) created a longitudinal study in which they investigated age-related white matter changes at 4 different measurements. The time interval between measurement 1 and 2 of their study was 14.93 months (SD 1.38) and the interval between measurement 2 and 3 was 15.58 months (SD 2.65). The DTI data of all four measurements were included into their linear mixed effects models. As an outcome, Bender et al. (2016) showed that, from middle age onward, age is associated with an FA decline in commissural, projection as well as association fibres, with the steepest decline in association regions.

But also for participants with a baseline-age from 20 years on, Sullivan and colleagues reported that normal ageing is characterised by a decline of FA (Sullivan, Rohlfing, & Pfefferbaum, 2010). Also Sexton et al. (2014) included participants between 20 and 84 years of age and were able to demonstrate decreases in FA, though these decreases grew with age.

All in all, it seems plausible that the FA decline observed in our healthy and stuttering control group is attributable to ageing effects evoked by our longitudinal study design.

However, it is a notable outcome that healthy controls show an FA decline in different areas compared to stuttering controls – the decrease of white matter integrity was visible in bilateral and right hemispheric association fibre tracts (superior longitudinal fasciculus, inferior fronto-occipital fasciculus, inferior longitudinal fasciculus and anterior thalamic radiation) as well as projection fibres (bilateral corticospinal tract). It is observable that mainly association fibres are affected by the FA decline. This is in line with the results of Bender and colleagues (2016) and shows that the “last-in-first-out-hypothesis” might also pertain for ageing processes in our healthy control group.

Contrary, an FA decrease in the group of stuttering controls was solely evident in the left hemisphere, mostly in association fibres (anterior thalamic radiation, inferior fronto-occipital fasciculus, cingulum, superior longitudinal fasciculus), but also in commissural fibres (forceps minor) and projection fibres (corticospinal tract). For this group of participants, the “last-in-first-out-hypothesis” also seems valid. Nevertheless, it is debatable if an FA decline which is detectable within an opposite hemispherical pattern in comparison to healthy controls might point to a different ageing physiology or probably pathophysiology in PWS. Future studies involving longitudinal DTI measurements and investigating FA changes over time in PWS should be conducted to elucidate this interesting observation.

No decline of white matter integrity in participants of the intervention group

A clinical relevant finding in this context is that in the whole brain analysis, stuttering patients who took part in the therapy intervention did not show any significant decline of white matter integrity in the whole brain analysis. On the contrary, in the ROI analysis, they demonstrated an increase of FA in the first cluster of Neef and Colleagues (2015) that was situated in the left superior longitudinal fasciculus.

Can an intense stuttering therapy be beneficial for suspending an age-related reduction of white matter integrity in PWS, and simultaneously facilitate the white matter plasticity in motor speech-related brain regions? In reference to the studies that investigated training effects of linguistic (Keller & Just, 2009), motor (Scholz et al., 2009; Zheng & Schlaug, 2015) and motor speech interventions (Reed et al., 2017) on white matter integrity, a facilitating effect of these trainings was characterised by the change of FA values. In most studies, FA increased while the evaluated and trained function also improved due to the training. This holds also true for our study: the increase of white matter in the described ROI can definitely be regarded as a direct therapeutic effect. It is not clear if this therapy-induced effect might have been able to stop or inhibit the pattern of an age-related whole-brain FA decline that was traceable in both control groups.

Indeed, some studies evaluating training effects on age-induced white matter changes might further elucidate this question: Colcombe and co-authors (2006) could demonstrate that older adults who were enrolled in an aerobic fitness intervention for six months showed an increased brain volume in gray matter (especially in motor regions) as well as in white matter (corpus callosum). Furthermore, they reported that elderly participants with higher cardiovascular fitness levels required less error monitoring (characterised by a decreased activity in the anterior cingulate cortex) and improved in their motor performance. These results portray a convincing argument for the possibility that the stuttering therapy in our study might have been able to stop an age-related FA-decline: the therapy consisted of an intense training of a new motor pattern for speech production – this might have produced similar beneficial effects on the white matter integrity as the aerobic fitness intervention did.

Another interesting outcome was reported by Luk et al. (Luk, Bialystok, Craik, & Grady, 2011). They investigated the effects of bilingualism on age-related changes in white matter integrity and found higher FA values in lifelong bilinguals compared to

monolinguals. These white matter integrity increases were existent in the corpus callosum, with extensions to the superior and inferior longitudinal fasciculi. In addition, bilingual participants demonstrated stronger anterior to posterior functional connectivity than monolinguals. Luk and colleagues concluded that a maintained white matter integrity is in their study a “brain reserve” (Luk et al., 2011, p. 16808) that was enhanced by the lifelong intense and extreme phonological and linguistic training of speaking two different languages on a high quality level.

The similarities to our study are obvious: PWS are learning a new motor speech pattern in the Kasseler stuttering therapy which is demanding as complex brain networks and resources as speaking and comprehending two languages at the same time. This is why the group of stuttering patients in our study might not have developed a decline of FA and, on the contrary, revealed a growth of white matter integrity in the investigated ROI. Learning new motor speech programmes that inhibit stuttering symptoms might also be a “brain reserve” (Luk et al., 2011, p. 16808) that positively influences the white matter integrity during ageing.

2.4.1 Limitations and future directions

Several limitations should be discussed in terms of this study. First, concerning the characteristics of participants, the quality of the study would have been improved by adding perfectly matched control groups to the intervention group. The stuttering patient group was well matched to the healthy control group, but the group of stuttering controls was older and therefore better educated than both other groups. Although we used age as a covariate in some of our analyses, the age difference might have influenced the presented results.

Also the circumstance that we were not able to solely include right-handed participants was not optimal, but at least, we were able to match the laterality quotient of all participants. Stuttering is a speech disorder which is, in contrast to a language disorder,

not dependent on a hemispheric dominance – this is another reason why different handedness lateralities in this study can be accepted.

Concerning the DTI analysis, we focused on the evaluation of FA, which is one of the most frequently used unit and method in the field of investigating white matter integrity. Although this method and its analysis via TBSS are widely spread in research, it contains several disadvantages. The explanatory power of FA is limited when it comes to regions were two fibre populations cross, the underlying white matter microstructure might yield false positives in this case. Therefore, using a crossing-fibre measure as well as tractography to reconstruct specific fibre tracts and then directly analyse their FA values would have been beneficial to this study.

Furthermore, Zatorre and colleagues (Zatorre, Fields, & Johansen-Berg, 2012) resume in their review of gray and white matter neuroplasticity that recent neuroimaging approaches do not give insights about the directly underlying cellular and biochemical mechanisms which are causative for diverse study outcomes. They also stress that specific structural findings of changes or anomalies in white matter integrity are probably not only the result of a single brain process, but a result of complex network changes, including alterations in diverse cell types. Despite of all the knowledge derived from neuroimaging studies so far, the interpretation of results and the impact of these results on interventions like an intense stuttering therapy are still insecure and hard to develop.

In our study, we were able to find the first evidence that an intense stuttering therapy is able to increase white matter integrity in a part of the left superior longitudinal fasciculus which is connected to important motor speech processing brain areas. However, the neuronal processes that evoked this white matter integrity increase (e.g. stronger myelination or higher alignment of fibres), as well as its influence on improvements in behavioural measures like speech fluency or psychological strain are still not clear.

Future research including a larger number of participants who stutter and take part in an intense, evidence based stuttering therapy should be conducted. Replications of our result will help to understand how stuttering therapy works on a neuronal level. The finding of other, additional brain locations where changes of white matter integrity due to the intervention can be found will provide further information how we can improve therapies.

The outcome of future studies might also enhance the usage of non-invasive brain stimulation techniques in PWS – the knowledge of suitable target brain regions for stimulation is crucial for a successful intervention. Furthermore, future studies that investigate white matter changes after stuttering therapies could be able to broaden our knowledge of causative coherences in the aetiology of stuttering – it might be easier to identify the neural correlates of stuttering and their prognostic function if we know how, why and in what extent white matter integrity changes due to different therapy approaches.

In addition to the discussed limitations, PWS show large individual differences concerning personal traits, the quality and quantity of stuttering symptoms, the application of compensatory strategies and the talent to realise new learned therapy techniques. All these individual factors are hardly to control in research studies and might have influenced the outcomes of the current study. They might be partly responsible for different outcomes of previous studies which have investigated or future studies that will investigate white matter integrity and plasticity.

2.4.2 Conclusion

To our knowledge, this is the first study demonstrating that an intense stuttering therapy is able to cause an increase of white matter integrity. After therapy, stuttering patients demonstrated increased FA values in a portion of the left superior longitudinal fasciculus which is connected to brain regions that process important motor speech functions.

Therefore, we assume that the increase of white matter integrity in this area could have facilitated the improvements in speech fluency and in life quality the stuttering patients experienced.

Furthermore, our study yields first clues for a beneficial effect of the stuttering intervention on age-related white matter integrity declines that were solely observable in the control groups.

Finally, we were also able to replicate the findings of newer research studies: we demonstrated that PWS show, in comparison to healthy controls, a decline of FA in right hemispheric brain regions. Our results indicate that the right superior longitudinal fasciculus as well as the right cingulum, inferior longitudinal fasciculus, inferior fronto-occipital fasciculus and corticospinal tract might be considered to play a role in the multi-factorial aetiology of stuttering.

3 Long-term brain activity changes in persons who stutter induced by stuttering therapy[2]

3.1 Introduction

Stuttering has a significant impact on speech fluency as well as on communication in everyday life. In terms of speech fluency, affected patients present with the involuntary occurring core symptoms (1) sound and/or syllable repetitions, (2) sound prolongations and (3) speech blocks (Andrews & Harris, 1964; Bloodstein & Ratner, 2008; Guitar & McCauley, 2010). These core symptoms are often accompanied by secondary symptoms like limb and body movements, head movements and facial grimaces. In terms of communication, PWS undergo communicative pressure and psychological strain, caused by the loss of motor control during core symptoms and by the often negatively-marked reactions of their audience towards their stuttering symptoms. Anxiety, shame, a low self-esteem and embarrassment are emotions and feelings PWS frequently experience (Boyle & Fearon, 2018; Zückner, 2017). As a consequence, the quality of life is affected by this speech fluency disorder (Carter et al., 2017; Kohmäscher, 2017; Yaruss, 2010).

There are several types of stuttering. The “idiopathic” stuttering which is also defined as “originary neurogenic non-syndromal stuttering” in the German guidelines for speech fluency disorders (Neumann et al., 2017) is the type of stuttering we are referring to in this paper (from now on simply called “stuttering”). It has its onset in early childhood, approximately between the age of 2 and 6 years (Yairi & Ambrose, 2013). 5% of children experience an onset of stuttering (life-span incidence), while in adults, stuttering is traceable only in 1% of the population (Guitar & McCauley, 2010; Yairi & Ambrose, 2013). The difference between incidence and prevalence rate is explainable by the high

[2] Manuscript in preparation together with Sarah Wolter, Roberto Goya-Maldonado and Martin Sommer

rate of spontaneous remission in stuttering: approximately 70-80% of the children who began to stutter in their childhood recover. Still, in a small portion of children, the stuttering persists onto adulthood. Previous research made it possible to identify various risk factors for persisting stuttering (e.g. history of stuttering in the family, male sex, Guitar & McCauley, 2010) though an individual prognosis for the likelihood of recovery is not possible yet.

So far, the aetiology of stuttering is not entirely understood, even though various research projects found different abnormities and pathomechanisms that are potentially related to stuttering.

Initially, a genetic influence on the origin of stuttering became evident. Twin studies affirmed a heritability of 70% - 80% (Rautakoski et al., 2012). Research that included molecular genetics identified several loci that are likely incorporated into stuttering – this is why stuttering is seen as a multifactorial polygenic disorder (Kraft & Yairi, 2012).

Research studies in the field of brain imaging found growing evidence for the theory that an impairment of brain structure and/or function play a meaningful role in the aetiology of stuttering. However, the outcomes of these studies are partly contradictory and therefore discussed controversially.

In studies using diffusion MRI measurements, evidence for a reduction of white matter integrity in PWS was found. Various authors reported a decline of white matter integrity in predominantly left hemispheric areas like the left rolandic operculum (Sommer et al., 2002), the left superior longitudinal fasciculus and the midbody of the corpus callosum (Neef et al., 2015). Newer studies also demonstrated a reduction of white matter integrity in right hemispheric regions in PWS, e.g. in the right aslant tract and in the right superior longitudinal fasciculus (Neef et al., 2018).

In the field of functional MRI, the meta-analyses of Belyk et al. (Belyk et al., 2015) and Budde and co-authors (Budde et al., 2014) evaluated the neural hallmarks of persistent

stuttering. A prominent finding of several studies is the hyperactivation of right-hemispheric motor areas that PWS show. This hyperactivation is consistent in several studies and represented in the right primary motor cortex, the right pre-SMA and SMA and the IFG, as well as in the right insula and rolandic operculum. Contrary to this right-hemispheric over-activation, a reduced activation of motor regions like the larynx area within the primary motor cortex as well as a decreased activity in the planum temporale and middle temporal gyrus were found in the left hemisphere of PWS (Budde et al., 2014; Belyk et al., 2015). Some authors interpret the right-hemispheric hyperactivation and the left-hemispheric hypoactivation as characteristic traits of an impaired sensorimotor integration in PWS and therefore as a potential cause for stuttering (for a review, see Neef et al., 2015; as well as Neef et al., 2016; Neumann et al., 2005).

In studies evaluating functional connectivity in PWS, connectivity deficits between the pre-motor cortex and BA 44, a hyper-connectivity in right-hemispheric motor areas (Chang et al., 2011) as well as a reduced auditory-motor coupling (Watkins, 2011) became evident. RSFC studies demonstrated a reduced connectivity between right SMA and the basal ganglia for PWS (Xuan et al., 2012; Yang et al., 2016). This decreased connectivity was also found between basal ganglia and bilateral superior temporal gyrus (Lu et al., 2012; Yang et al., 2016).

Interestingly, also brain regions and networks that are not associated with motor- or motor-speech-processing showed abnormalities in PWS: Chang et al. (2017) demonstrated anomalous resting state networks in CWS in the default mode network as well as in attention, frontoparietal and somatomotor networks. Their findings are partly in line with the study of Xuan and colleagues (2012).These researchers demonstrated a reduced connectivity within the default mode network of PWS. For CWS, the same authors reported an increased connectivity in the sensorimotor networks. Also O'Neill and colleagues (2017) were able to find evidence for altered neurometabolisms in brain areas associated with attention (e.g. inferior frontal and superior temporal gyri, caudate)

- for adults and children who stutter, this evidence was found in a study using proton chemical shift imaging.

Stuttering therapy facilitates speech fluency and helps patients to develop coping strategies concerning psychological strain and ‘inner’ symptoms evoked by their dysfluencies. There are certain evidence-based therapy approaches that are conducted world-wide: (1) fluency shaping (e.g. Kasseler Stottertherapie, Euler et al., 2009) (2) stuttering modification (e.g. Intensiv Modifikation Stottern, Natke et al., 2010) and (3) behavioural therapy approaches (e.g. Lidcombe Program, Packman & Onslow, 2012). It was shown that these therapies promote speech fluency and reduce stuttering symptoms as well as inner, psychological symptoms significantly (Euler et al., 2014; for an overview, see Neumann et al., 2016). However, in some patients relapses happen after the end of therapy and the amount of stuttering symptoms rises. This is why a carefully structured aftercare programme including a plan for the relapse management is an essential part of a successful stuttering therapy (Craig, 1998; Cream et al., 2009; Neumann et al., 2017).

The changes that stuttering therapies evoke, e.g. an improvement of speech fluency, are not only detectable on a behavioural level. In recent years, neuroimaging studies found evidence for an influence of stuttering therapy also on brain activations.

However, study outcomes were manifold in terms of in which areas brain activation changed after a completed therapy and how these activity changes were interpreted as possible hallmarks of the stuttering brain or possible prognostic determinants for a successful therapy. The different outcomes probably exist due to different imaging techniques that were used (e.g. a positron emission tomography (PET), fMRI, RSFC) and due to different tasks and paradigms conducted in the scanner. Different factors like group sizes of participants, diverse therapy approaches that were investigated and the

varying durations of the follow-up-measurements might be causative for heterogenous results.

Particularly, in most of the studies the follow-up-measurement was taking place promptly, namely directly after the intense therapy course where therapy techniques are trained. The therapy phase where patients implemented these techniques into their daily routine was not included in the investigation of brain activity changes in these studies. The following therapy-induced effects on brain activation were found after these rather short intervention periods:

In 2005, Neumann and colleagues investigated the effects of a 3 weeks intense course of the Kasseler stuttering therapy (Euler et al., 2009) in 9 male stuttering patients. They measured the patients within 12 weeks after the 3 weeks course had ended. Neumann et al. reported a more widespread activation in frontal speech and language areas as well as in temporal areas bilaterally, but more pronounced in the left hemisphere after therapy. They also showed that the increase of activation in the left hemisphere after therapy was traceable in the region of the left rolandic operculum – the region in which Sommer and colleagues (Sommer et al., 2002) were able to show a decline of white matter fibres. Neumann et al. (2005) concluded that the fluency shaping therapy caused a neuronal reorganisation between both hemispheres, activating bigger portions of the left hemisphere which was found to be hypoactivated before therapy.

Giraud and co-workers (2008) were able to find evidence for a correlation between stuttering severity and basal ganglia activity in 9 stuttering patients – furthermore, they could show that the activity in the basal ganglia was modified by a 3 weeks intensive course of fluency shaping therapy (Kasseler Stottertherapie, Euler et al., 2009). Before the intensive therapy course, a negative correlation was reported between stuttering severity and bilateral inferior temporal areas. This correlation vanished after the intervention, while another negative correlation between stuttering severity and the

precuneus as well as the anterior nucleus of the thalamus was found. The authors concluded that the basal ganglia play a prominent role for the aetiology of stuttering. A diminished basal ganglia functionality could be caused by the structural anomalies PWS show, this structural anomaly might impair neuronal connections between Broca's area and the motor cortex.

Kell and colleagues (2009) also investigated the effects of the Kasseler stuttering therapy directly after the 3 weeks intense therapy course and compared the functional brain changes during the assisted recovery of 13 stuttering patients with brain activity in healthy controls as well as with brain activities in individuals who recovered from stuttering spontaneously (without intervention). The authors reported that untreated PWS with persistent stuttering showed an over-activation of a large right hemispheric motor network, also including the Broca's homologue, the right frontal operculum, right premotor areas as well as right auditory cortices. This activity pattern was interpreted to have a compensational function. In the intervention group, the hyperactivation of this right hemispheric network was no longer traceable after therapy, i.e., a partial lateralisation to the left hemisphere took place. Nevertheless, the over-activation of the right BA 47/12 (orbitofrontal cortex) as well as in mesial cortices and the right planum temporale was still detectable. Striking was that participants who experienced a spontaneous recovery from stuttering in their past exhibited an over-activation in the left homologue of BA 47/12. This is why Kell and colleagues (2009) characterised the activation in left BA 47/12 as a repair activation – the functional recruitment of this region might lead to the spontaneous, unassisted remission of stuttering. The left BA 47/12 is also close to brain regions where Kell et al. (2009) as well as previous studies found a decline of white matter integrity in PWS – therefore, the authors interpreted it as a perianomalous functional reorganisation.

Kell and colleagues reanalysed their in 2009 presented data and investigated changes in cortical functional connectivity in PWS, induced by the intense Kasseler stuttering

therapy (Kell et al., 2018). In their new analysis, they demonstrated that persistent stuttering was associated with a reduction of auditory-motor coupling and an intensified integration of somatosensory feedback between supramarginal gyrus and pre-frontal cortex. After the 3 weeks intervention course of the Kasseler stuttering therapy, the hyper-connectivity between supramarginal gyrus and pre-frontal cortex was no longer detectable, while the auditory-motor coupling was normalised. In the participants with spontaneous, unassisted recovery, both functional connectivity measures were normalised and activity in the superior cerebellum as well as in the left orbitofrontal cortex seemed to be detached from the speech production network. Kell et al. (2018) concluded that unassisted as well as assisted recovery induced by stuttering therapy facilitated the auditory-motor mapping and normalised left hemispheric speech networks.

Lu and colleagues (2012) investigated the effect of a short-term intervention on RSFC of stuttering patients. The intervention group learned a new speech pattern over 7 consecutive days, therefore the second measurement took place one week after the baseline measurement. Compared to healthy controls, stuttering patients showed reductions of RSFC in the left pars opercularis and increases of RSFC in the cerebellum before intervention. The short-term intervention was successful in reducing stuttering symptoms and also changed the RSFC in the intervention group: it evoked a decrease of RSFC in the cerebellar vermis to the level of healthy controls. Because the RSFC was still reduced in the left pars opercularis after therapy, Lu et al. (2012) concluded that the left pars opercularis might be involved in the aetiology of stuttering. Furthermore, they assumed that the therapy-induced neural reorganisation in the cerebellum might be a compensatory response to stuttering in PWS.

Ingham and colleagues (Ingham, Wang, Ingham, Bothe, & Grafton, 2013) investigated the prognostic factors of brain activity changes induced by two different stuttering therapy approaches, Modifying Phonation Intervals (MPI) as well as the prolonged speech program (PS). In their PET study, they measured healthy controls as well as the

participants of the intervention group up to 6 times (at the end of each phase of the treatment programme) with a follow-up interval of 17-43 weeks. In the intervention group, brain activity changes of patients with successful and unsuccessful therapy was compared. A region which was able to predict therapy success was the left putamen, regardless of which therapy approach they participated in.

Toyomura and researchers (2015) reported the effects of an 8 week externally triggered speech training on basal ganglia functional activity in PWS and healthy controls. A low activity in the basal ganglia in PWS was no longer traceable after the speech training. Also, the cerebellar vermis exhibited a decreased activity compared to the pre-test, while on a behavioural level, speech fluency improved after therapy. Toyomura and colleagues (2015) therefore interpreted the pathology mechanism of stuttering as a deficient motor control during self-paced speech, where basal ganglia as well as cerebellum are impaired in their function. Externally triggered speech patterns trained in the 8-week-programme were able to improve the pathomechanisms and to enhance speech fluency.

In these previously described studies, the second measurement investigating therapy-induced changes on brain activity took place directly or early after therapy. The disadvantage of this procedure is that the successful implementation of therapy techniques into the daily routine and its influence on brain activation of PWS are not taken into account. Furthermore, if a relapse occurs and the stuttering symptoms increase again after finishing the intense therapy course, the coping with this relapse in the after-care therapy programme and the later-on development is not considered.

Studies with a follow up >6 month after an intense stuttering therapy might therefore offer a different perspective and new insights into the neural effects of stuttering therapy on functional brain plasticity. Unfortunately, this study design has rarely been realised during the last years. As far as we know, three studies not only examinated a change of

brain function in PWS taking part in an intervention directly after the intense course, but also longer than six months after the therapy onset.

De Nil et al. (2003) were the first researchers taking longer-term therapy effects on brain activity into consideration. They conducted a positron emission tomography (PET) study and measured the stuttering participants before and after a 3 weeks therapy programme, as well as one year after accomplishing the therapy. The fluency shaping therapy consisted of an intensive 3 weeks module (individual fluency treatment for 6 hours each day), as well as a one-year-maintenance programme. In addition to this, cognitive intervention strategies were trained. Before the stuttering therapy, PWS showed a hyperactivation in cerebral and cerebellar (speech-) motor brain regions compared to healthy controls. These were found bilaterally in the superior temporal gyrus, in the pre- and post-central gyrus, in the insula and cerebellum as well as right-hemispherically in the medial frontal gyrus, the anterior cingulate and the putamen. Immediately after the 3 weeks treatment programme, the activation of PWS shifted more to the left hemisphere. Also in the one-year-follow-up measurement, the activity-shift to the left hemisphere was still traceable, the over-activation of bilateral and right motor regions PWS showed in the pre-treatment measurement was reduced (De Nil, Luc F. et al., 2003).

Neumann and colleagues were the first researchers exploring brain changes after participation in the Kasseler stuttering therapy. In one of their studies (Neumann et al., 2004), they measured therapy-effects directly after the three-weeks-intensive-therapy course, but also provided long-term data of five participants who had a follow-up-measurement two years after therapy start. In the pre-treatment measurement, PWS exhibited larger and more widespread neuronal activation as well as a left frontal hypoactivation compared to healthy controls. After the three-weeks-therapy-course, the activation became more distributed and more shifted to the left hemisphere, including frontal and temporal brain regions as well as the putamen. Two years after the first measurement, the over-activations PWS showed before were reduced but more right

sided. The left-frontal hypo-activations were still traceable. Therefore, Neumann et al. (2004) interpreted this left-hemispheric hypoactivation as a dysfunctional hallmark of stuttering.

In 2018, Neumann and colleagues again explored the long-term effects of the Kasseler stuttering therapy – this time, they focused on brain activation changes related to dysprosody. fMRI measurements took place before treatment, directly after the intense therapy course and 1 year after the therapy onset. During the generation of emotional and linguistic prosody, PWS showed a reduced activation of the left inferior frontal gyrus and the anterior insula before therapy. This hypoactivation was normalised at the 1-year-follow-up measurement. Neumann and colleagues (2018) discuss that dysprosody does not seem to be a pathomechanism leading to the dysfluencies, because dysprosody was not correlated with stuttering severity. In their opinion, a training of prosody might indirectly facilitate the improvement of stuttering.

Due to this small amount of studies exploring the stuttering therapy effects on functional brain activity with a follow-up period bigger than six months, the long-term activity changes induced by stuttering interventions are not well-known until now.

We decided to gather knowledge of how the training of newly learned speech techniques, their regular application during day-to-day life, as well as possible relapses and their management after an intense stuttering therapy are changing brain activation of stuttering patients over a long-term period. We therefore investigated the effects of an evidence-based intense German stuttering therapy (Kasseler Stottertherapie, Euler et al., 2009) on brain activity changes, measured with a follow-up period of approx. 11 months. This follow-up period reassured that not only brain changes directly after the initial intense therapy course at the beginning of the therapy were taken into account, but also changes caused by the long-term therapy training – including the self-training

of the patients, the refresher-courses after one and ten months of therapy and the everyday application of the newly-learned speech patterns.

For our study, we carefully controlled unspecific and not therapy related effects by adding two control groups to the group of stuttering patients taking part in the intervention: one group of adults who stutter but were not enrolled in any form of stuttering therapy during the course of the study and one fluent speaking control group with healthy participants.

In addition to investigating therapy-induced changes in behavioural measurements like stuttering severity, psychological strain and inner symptoms related to stuttering, we analysed longitudinal brain activity changes evoked by stuttering therapy during a covert-speaking fMRI paradigm. The paradigm mirrors motor (speech) processes. Therefore, it is expected to be sensitive to therapy-induced activation changes in motor speech processing brain regions (Neef et al., 2016; Tian, Zarate, & Poeppel, 2016). Another reason to choose this established covert-speaking paradigm for the current study was the activation bias that might have been evoked by applying an overt-speaking paradigm. During overt speaking, large functional activity in speech motor processing regions becomes evident and might have exacerbate the imaging analysis (Callan et al., 2006). By using an established covert speaking paradigm, we were able to circumvent this difficulty.

For the imaging analysis, we used the Statistical Parametric Mapping software (SPM8; Wellcome Trust Centre for Neuroimaging, UK). Within the second level analysis, we calculated a two-factorial ANOVA (group, time).

According to the neurological hallmarks of stuttering described in the reviews and meta-analyses of Belyk et al. (2015), Budde et al. (2014) and Neef et al. (2015), we addressed the following hypotheses:

1. After therapy, we expect a normalisation of brain regions hyperactivated before therapy in the group of stuttering patients. This normalised activation will be traceable especially in motor (speech) regions (e.g. left rolandic operculum, IFG, SMA).
2. We expect a greater change of functional activity in participants of the intervention group compared to participants of both control groups in the previously mentioned motor (speech) regions.
3. We assume there is a positive correlation between the intensity of the stuttering severity and the strength of the functional activity in (speech) motor processing brain regions. This would be in line with the above discussed literature, pointing to the difference between stuttering severity in healthy controls (low stuttering severity score, no hyperactivity in motor regions) and stuttering severity in PWS (higher stuttering severity score, hyperactivation of motor regions).

3.2 Material and methods[3]

3.2.1 Participants

To explore the aims of the study, three different groups of participants were included: Persons who stutter about to begin an intense stuttering therapy after the first study measurement (stuttering patients, SP; n = 17); persons who stutter but did not take part in any stuttering therapy at the time of the study (stuttering controls, SC; n = 16) and persons who do not stutter but speak fluently (healthy controls, HC; n = 25); see also (Sommer & Primaßin, 2017, 2018).

[3] Parts of the chapters 3.2.1 and 3.2.5, as well as the whole chapters 3.2.2, 3.2.3, 3.2.4, 3.2.7.1 are copied from chapter 2 of the current thesis [Primaßin (2019)].

Groups of SP and HC were matched for sex, age, handedness (Oldfield, 1971) and years of formal education (1 = school; 2 = high school; 3 = <2 years college; 4 = 2 years college; 5 = 4 years college; 6 = postgraduate; see also Neef et al., 2016).

The group of SC was older and better educated compared to HC and SP (see Table 12).

Table 12. Participants and demographic information.

	SP	**SC**	**HC**	**p-value SP - HC**	**SP - SC**	**SC - HC**
N	17	16	25			
Age in years (mean)	26.4 (SD 12.1)	34.7 (SD 7.5)	24.8 (SD 7.6)	0.620 [a]	0.025 * [a]	0.000 * [a]
Sex (male)	16 (94,1%)	14 (87.5%)	21 (84%)	1 [b]	0.125 [b]	1 [b]
Handedness (mean LQ)	68.3 (SD 64.4)	85.2 (SD 16.9)	76.4 (SD 44.3)	0.621 [a]	0.317 [a]	0.483 [a]
Education (median)	1	5	2	0.120 [c]	0.000 * [c]	0.001 * [c]
Mean time difference between pre- and post-measurement (months)	11.7 (SD 0.9)	11.6 (SD 1.5)	11.3 (SD 0.6)	0.078 [a]	0.647 [a]	0.548 [a]
Age of stuttering onset in years (mean)	4.1 (SD 2.4)	5.1 (SD 3.8)	n/a	n/i	n/i	n/i
Number of participants with PWS in their families	8 (47.1%)	7 (43.8%)	n/a	n/i	n/i	n/i

Note. SP (stuttering patients); SC (stuttering controls); HC (healthy controls); p-value derived from group-pairwise statistical testing with the following methods: [a] (T-test); [b] (Fisher's exact test); [c] (Mann-Whitney-U-test); n/a (not applicable); n/i (not investigated); SD (standard deviation); * (significant result, $p<0.05$).

Apart from the stuttering in SP and SC, all participants had to meet the following criteria for inclusion into the study: 1) general MRI compatibility, 2) native German speakers, 3)

normal or corrected-to-normal vision, 4) no pregnancy, 5) no history of dementia or other CNS or psychiatric diseases and 6) no history of speech and language disorders. In the HC group, no family-history of stuttering was present.

All participants of the SP group were recruited at the therapy centre, via cover letters or information events directly hosted by the therapy centre. Regarding the SC group, recruitment was completed via stuttering support groups. Healthy, fluent speaking controls (HC) were recruited via advertisements at the University of Göttingen. Informed written consent for participating in the study was obtained from each subject. The study was approved by the local ethics committee of the University Medical Center Göttingen and conformed to the Declaration of Helsinki. Participants received 21 Euros for each measurement; travel costs were reimbursed.

3.2.2 Stuttering Therapy

Stuttering participants which took part in an intense stuttering therapy were recruited from the therapy centre of the Kasseler stuttering therapy (stuttering therapy located in Bad Emstal, Germany). At this therapy centre, therapists are providing intense group therapies for PWS (Iven & Hansen, 2017).

The Kasseler stuttering therapy (Euler et al., 2009) is a computer-assisted intensive biofeedback therapy and based on a fluency shaping therapy approach. Participants are training a specific soft and bound speech pattern which they have to use in a high frequency during speaking. The therapy starts with a 2 weeks intensive course in Kassel, followed by two refresher weekends in Kassel one months and approximately ten months later. In between these stationary therapy courses at the therapy centre, the patients have to accomplish a computer-assisted bio-feedback-training of 20 minutes daily. In addition; online-therapy sessions are conducted. Several studies and reviews confirmed the effectiveness of Kasseler stuttering therapy (e.g. Euler et al., 2009; Iven & Hansen, 2017; Kell et al., 2009; Neumann et al., 2017), showing there is a long-lasting

improvement of speech fluency as well as an reduction of stuttering-related negative emotions after therapy. The Kasseler stuttering therapy is finished by a therapy-closure-weekend after ten to 12 months of intense practice (depended on the patient's personal time schedule).

3.2.3 Research Design

A pre-post-test design was used to evaluate therapy-induced changes in SP as well as changes of brain function in SC and HC. In SP, the pre-test took place just before intense therapy started. All measurements were conducted at the University Medical Center, Göttingen; they contained an (f)MRI-Measurement as well as clinical speech analysis and behavioural examinations, to collect information about the stuttering severity and different attitudes and emotions towards stuttering and fluent speech. The post-test in SP took place after the therapy-closure-weekend. The pre-post-test interval of SC and HC was comparable to the pre- and post-test interval of the SP group (see Table 12). Pre- and post-test measurements consisted of identical clinical and (f)MRI examinations.

3.2.4 Clinical and behavioural examinations

At either measurement, the following tests were applied in the study:

Clinical speech analysis

Stuttering severity was assessed by using the *Stuttering Severity Instrument 4* (SSI-4; Riley, 2009). For obtaining the stuttering severity score for each participant and measurement; the frequency and duration of stuttered syllables as well as physical concomitants of stuttering have to be counted and rated. Therefore, we videotaped samples from reading aloud as well as spontaneous speech (participants were asked to describe their daily routine, hobbies and favourite TV series or books). 500 syllables of reading as well as 500 syllables of spontaneous speech were included into the analysis.

Subjective stuttering severity

To also explore the *subjective stuttering severity degree*, we asked the participants to rate the severity of their stuttering on a scale from 1 to 9, where 9 was representing a very high and 1 a very low degree of stuttering severity.

Behavioural questionnaires

The German version of *the Overall Assessment of the Speaker's Experience with Stuttering (*OASES; Yaruss & Quesal, 2006*)* was employed to evaluate the participants' experience of stuttering. It is also evaluating the impact of stuttering on communication and life quality and therefore able to measure stuttering treatment outcomes (Kohmäscher, 2017).

The *WHO-5 Well-Being Index* is a short self-report questionnaire consisting of 5 questions that reflect one's well-being. It has been applied as an outcome measure in diverse clinical trials (Topp et al., 2015; Wit et al., 2007).

The *Beck Depression Inventory* (BDI; Beck et al., 1961) is a self-report questionnaire measuring the severity of depression. Is has been used by clinicians and researchers in different settings (Richter et al., 1998). We applied it to control therapy-induced changes in terms of depressive behaviour.

To ensure that the communication behaviour of the healthy control participants was not associated with anxiety or social phobia and did not change during the study, we conducted the German *State-Trait Anxiety Inventory* (STAI; Laux et al., 1981) in this specific group.

3.2.5 Imaging Acquisition

We used a 3 Tesla MR system (Siemens Magnetom Tim Trio, Siemens Healthcare, Erlangen, Germany) as well as a standard 8-channel phased-array head coil. Participants were placed into the scanner in supine position. They wore headphones for

noise protection and MR-compatible LCD goggles (VisuaStim XGA, Resonance Technology Inc., Northridge, CA, USA).

First, a T1-weighted anatomical 3D turbo fast low angle shot FLASH sequence was accomplished (repetition time (TR) 2250 ms, inversion time 900 ms, echo time (TE) 3.26 ms, flip angle = 9°, voxel size 1x1x1 mm^3).

Second, a total of 434 volumes (voxel size 3x3x3 mm^3, field of view 192 mm, 33 slices, 20% gap) were acquired for blood oxygen level-dependent fMRI with a gradient-echo echo-planar imaging (EPI) sequence (TR 2000 ms, TE 30 ms, flip angle 70°) over two functional runs (18 trials per condition).

3.2.6 Experimental procedure of functional Magnetic Resonance Imaging (fMRI)

Figure 1 portrays the stimulus material and time course of the slow-event-related fMRI design. In the covert speaking condition, participants had to do a motor imagery task where they named the months of the year. A trial began with the active condition ,presenting the letter “J” which is a hint for “January” and a signal for participants to start imagine speaking the months’ names. After 6 s, a cross was appearing, indicating the subject to stop with imaginary speaking (rest condition; 18 s). Two functional runs were shown with 18 trials per condition, respectively. The task was adopted from Riecker and colleagues as well as from Neef et al. (Neef et al., 2016; Riecker, Ackermann, Wildgruber, Dogil, & Grodd, 2000). Onsets and durations of the experimental conditions “rest” and “covert speaking” were stored in the logfile that was created by the programme ‘Presentation’ (NeuroBehavioral Systems). This programme was used to run the previously described fMRI paradigm inside of the MRI scanner.

Before each MRI measurement started, the participants were introduced to the task by explaining the procedure and discussing open questions. After the structural measurements were finished, the participant was informed that the first functional run

will start and he has to covertly name the months of the year when the “J” is represented or to stop the imaginary speaking while the cross is appearing on the goggles’ screen.

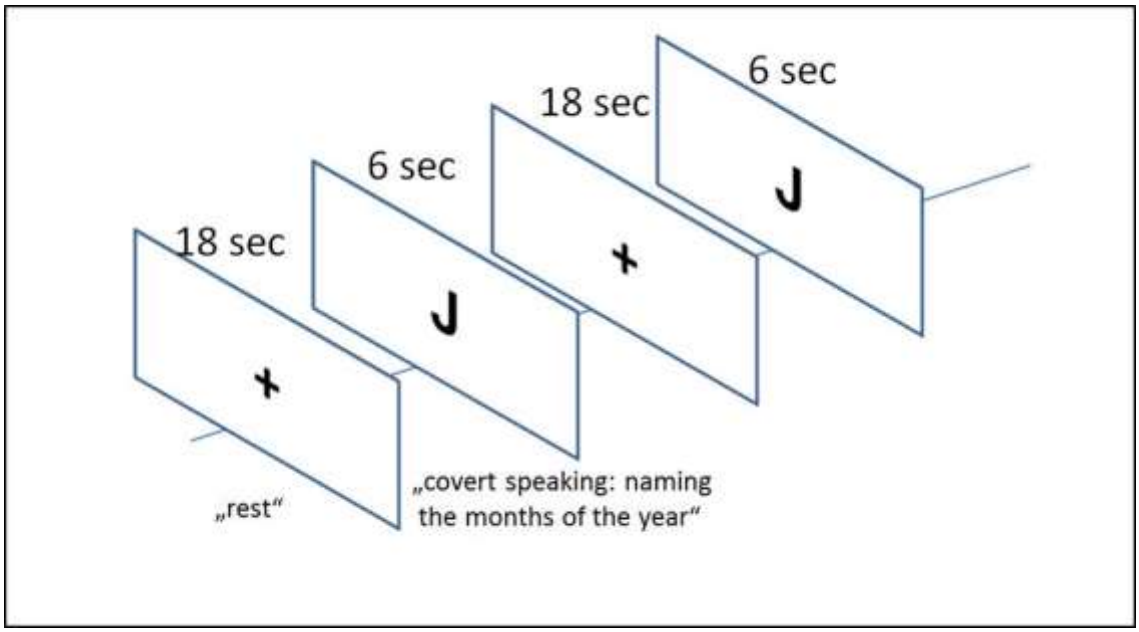


Figure 15. Covert speaking paradigm - experimental fMRI design.

3.2.7 Data analyses

3.2.7.1 Analysis of behavioural data

Interrater reliability calculation of the SSI-4 data

Each of two experienced speech and language pathologists (one of them was A.P.) analysed 50% of the full sample of SSI-4s according to the SSI-4’s manual instructions. The SSI-4s were distributed pseudorandomly to each rater; though every pathologist rated pre- and post-recordings of one participant and also rated an analogous amount of SPs’, SCs’ and HCs’ SSIs.

Before the interrater-reliability calculation and the main analysis started, an analysis-training with both raters (duration: 30 hours) took place. During this training, all SSI-4-manual guidelines were checked and inconsistencies were clarified. In addition, 3 different SSI-4 (subset of full sample) which were not part of the interrater-reliability

calculation were evaluated by both raters independently. Afterwards, the results were compared to extinguish still existing differences in analysis.

After this training was completed, we calculated the interrater-reliablity. Both speech and language pathologists analysed 9 SSI-4 (subset of the full sample; 3 of the SP group, 3 of the SC group and 3 of the HC group) independently. The results were statistically assessed with Krippendorf's Alpha Reliability Estimate (KALPHA) in SPSS, using a bootstrapping of 10000 and the ordinal data level (Hayes & Krippendorff, 2007).

Statistical analysis of behavioural questionnaires

We conducted the SSI-4, WHO-5 and BDI in all three groups of participants. To compare for behavioural differences between the three groups at one point of time, we used the Kruskal-Wallis-Test for ordinal scaled data and included also pairwise-comparisons tests, where we corrected p-values with the Dunn-Bonferroni method for multiple comparisons. To check for longitudinal changes in the behavioural questionnaires from pre- to post-measurement, the Wilcoxon signed-rank test was executed and corrected with the Holm-Bonferroni method for multiple comparisons.

The OASES and subjective stuttering severity score were assessed in the group of stuttering patients and stuttering controls. To compare for behavioural differences between both groups at one measurement, we applied the Mann-Whitney test for ordinally scaled data and corrected for multiple comparisons with Holm-Bonferroni. For evaluating changes over time from pre- to post-test, we calculated Wilcoxon signed-rank tests for the paired ordinal data in each group, respectively. Afterwards, we corrected the obtained p-values from the Wilcoxon signed-rank test for each calculation with the Holm-Bonferroni method for multiple comparisons.

The STAI was only applied in the group of healthy participants. Here, we used the Wilcoxon signed-rank test for finding significant differences in the score from pre- to post-test and also used the Holm-Bonferroni method.

For all statistical tests of behavioural data, the effect size estimate *r* was calculated as $r = \frac{z}{\sqrt{N}}$ (z = z-score that SPSS calculates; N = number of total observations on which z is based (Field, 2011, p. 295)).

3.2.7.2 Analyses of fMRI data

We extracted the onsets and durations of the experimental conditions “rest” and “covert speaking” out of the logfile that was created by the programme ‘Presentation’ (NeuroBehavioral Systems). Subsequently, the preprocessing of data started. All steps of preprocessing described below were conducted in reference to the manual of the Statistical Parametric Mapping software (SPM8; Wellcome Trust Centre for Neuroimaging, UK), running within Matlab 2012a (The MathWorks, Inc., Natick, MA, USA).

3.2.7.2.1 Preprocessing

Re-alignment

In the re-alignment step, head motion correction algorithms were used to align each functional image with the one measured before. In this way, it was reassured that voxels are always representing the same location in the brain and no motion bias interferes the data analysis.

Unwarping

After the correction of head movement, the unwarping function of SPM was applied to additionally correct for artefacts that are evoked by the movement-related magnetic field distortion.

Slice time correction

Due to the fact that the scanner acquires different slices within a single volume at various times, the slices of a volume contain functional activity from distinct timepoints.

Therefore, slice time correction was applied to assure a reference of all slices to the same point of time.

Normalisation

In the normalisation step, every individual brain of each study participant was aligned into standard space (EPI template of the Montreal Neurological Institute (MNI)). The normalisation algorithm creates spatial correspondence between all study participants and enables data averaging and group analysis.

Smoothing

The smoothing step during preprocessing applies spatial filtering – intensities of neighboured voxels are averaged to improve the signal-to-noise ratio. This averaging was conducted by using an isotropic Gaussian kernel with a full width at half maximum (FWHM) of 9x9x9 mm^3.

3.2.7.2.2 1st level analysis

After preprocessing, we checked all preprocessed data for movement artefacts and other abnormalities and excluded participants with images showing translational movements in at least one of the three axes > 3 mm (movements greater than one voxel size). In the 1st level analysis, individual onset and duration measures, the smoothed image files as well as movement parameters (three translation parameters and three rotation parameters, extracted during realignment) were loaded into the SPM batch system. After that, the GLM estimation started.

3.2.7.2.3 2nd level analysis

In the 2nd level analysis, estimated effects were tested statistically for significance. Hereby, all individually calculated statistical images from the 1st level analysis were included in the analysis and compared at group level. We explored group and time effects with an ANOVA (factor 1 = group (SP, SC, HC); factor 2 = time (PRE, POST).

Within this ANOVA, we used the SSI as a covariate and calculated two different contrasts:

First, we looked at the ANOVA-contrast ‘SP post > pre’ as well as ‘SP pre > post’ (paired T-tests) to evaluate an increase as well as a decrease of brain activation due to the intervention in the stuttering patients. Of interest were those regions with an interaction effect which showed a greater change of activation in stuttering patients compared to stuttering controls and healthy controls. To determine these regions, we evaluated the ANOVA contrast comparing the change of activation in SP with the change of activation in both control groups (paired T-tests).

We used the SSI score as a covariate of no interest to exclude activation effects due to higher stuttering severity score in stuttering patients and stuttering controls. Because of the different study groups, where stuttering was present in two groups but absent in one group, using the SSI score as a covariate of no interest was controlling for the obvious fact that PWS had a considerably higher SSI score than healthy controls. Using the SSI score as a covariate of no interest produced therefore a comparability between all groups by excluding the effects of stuttering severity.

Furthermore, we evaluated the existence of the correlation between stuttering severity and brain activation, especially to see if certain motor areas are more activated when the stuttering severity is high (positive correlation, SSI score as covariate of interest).

In a first step, based on our established a-priori hypotheses concerning the therapy-induced activation change of motor areas, we used a significance level of $p < 0.005$, uncorrected to test for activation changes in motor processing regions. In a second step, we applied a significance level of $p < 0.05$ using the family-wise error correction (FWE) to check if these regions which are the output of the first statistical test survive a more conservative correction.

3.3 Results[4]

3.3.1 Behavioural data

3.3.1.1 SSI-4

SSI-4 -interrater reliability

For the interrater reliability analysis, we obtained results >0.80 in each tested category of the SSI-4, pointing towards a good interrater reliability (Krippendorff, 2013). Especially the KALPHA result for the 'Total SSI score' interrater agreement was >0.95, so a high consensus and compliance between both raters became evident (see Table 13).

Table 13. Outcome of interrater-reliability calculation of the SSI-4.

(Sub-)scores of SSI-4	KALPHA
Reading Score	.8436
Spontaneous Speech Score	.9809
Duration Score	.8528
Concomitants Score	.8868
Total SSI Score	**.9578**

Note. KALPHA was calculated with the SPSS macro of Hayes (Hayes, 2017; Hayes & Krippendorff, 2007) using 10000 bootstrapping samples and the ordinal data level.

SSI-4 – differences between groups at pre- and post-test

A significant difference in the SSI-4 score between all participants groups was found at the *pre-test* (H(2) = 43.3, p = 0.000). Pairwise comparisons with Dunn-Bonferroni adjusted p-values for multiple comparisons showed that the scores differed significantly between stuttering patients and healthy controls (p = 0.000, r = 0.97, large effect) as well as stuttering controls and healthy controls (p = 0.000, r = 0.65, large effect). No

[4] Parts of the chapter 3.3.1. are copied from chapter 2 of the current thesis [Primaßin (2019)] or obtain similar phrasing due to the usage of the same behavioural measurements.

significant difference was present between stuttering controls and stuttering patients (p = 0.181, r = 0.33, medium effect; see Figure 16).

Testing for differences in the SSI-4 score between all participants groups at the *post-test*, we again obtained results for significant differences between all groups (H(2) = 33.7, p = 0.000): stuttering patients and healthy controls (p = 0.000, r = 0.64, large effect) as well as stuttering controls and healthy controls (p = 0.000, r = 0.84, large effect) differed significantly, while there was no significant difference between stuttering controls and stuttering patients (p = 0.181, r = -0.20, small effect; see Figure 16).

SSI-4 – longitudinal changes in each group

Comparing the *changes of SSI-Scores from pre- to post-test in each group, respectively,* we saw a significant decrease of the SSI-Score in the group of stuttering patients (median_pre = 27, median_post = 9, z = -3.624, p = 0.000, r = -0.87, large effect; see Figure 16). No significant changes in the SSI-4 from pre- to post-test were detectable in the group of stuttering controls (median_pre = 14, median_post = 12.5, z = -0.666, p = 0.505, r = -0.17, small effect) as well as healthy controls (median_pre = 0, median_post = 0, z = -1.474, p = 0.140, r = -0.29, small effect; see Figure 16).

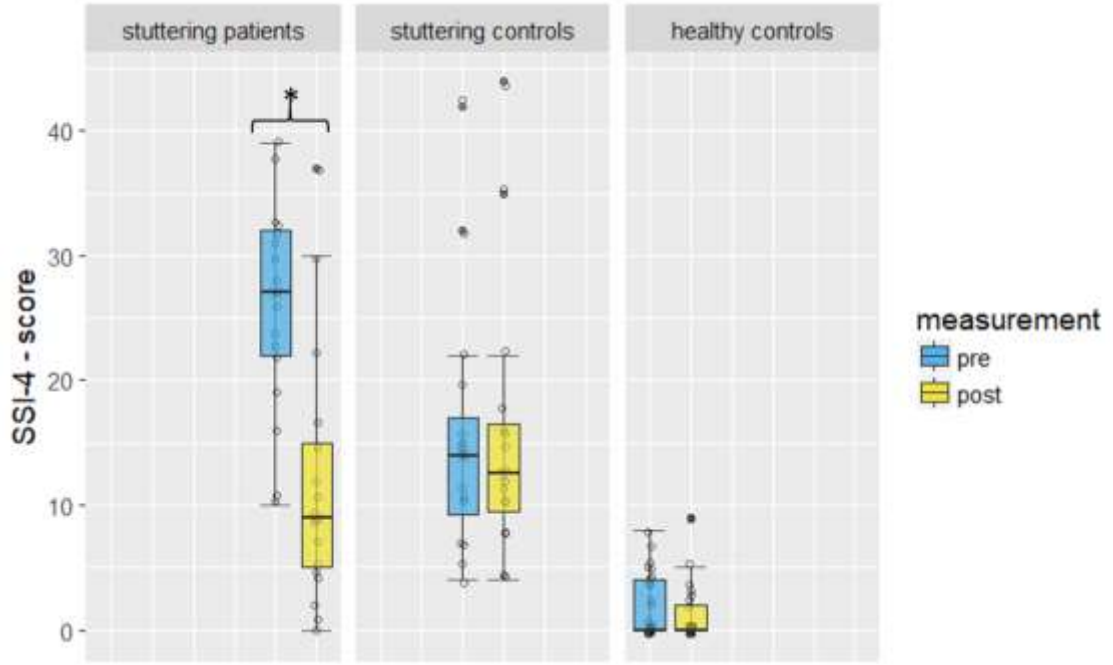


Figure 16. SSI-4 score of all study groups and measurements.*(Significant difference between post- and pre-measurement, p = 0.000, r = -0.87).

3.3.1.2 Subjective stuttering severity

Subjective stuttering severity – differences between groups at pre- and post-test

At pre-test, the subjective stuttering severity score in the SP group (median_pre = 5) was significantly higher compared to the group of stuttering controls (median_pre = 3) (U = 67, z = -2.535, p = 0.011, r = -0.44, medium effect). At the post-measurement, no significant difference in the subjective stuttering severity score could be shown between stuttering patients (median_post = 2) and stuttering controls (median_post = 3) (U = 98, z = -1.405, p = 0.160, r = -0.24, small effect).

Subjective stuttering severity – longitudinal changes in each group

For the subjective stuttering severity, we found a significant decrease of severity in the group of stuttering patients from pre- to post-test (median_pre = 5, median_post = 2, z = -3.325, p = 0.000, r = -0.81, large effect; see Figure 17). For the stuttering controls, no significant change was detected (median_pre = 3, median_post = 3, z = -1.155, p = 0.248, r = -0.29, small effect).

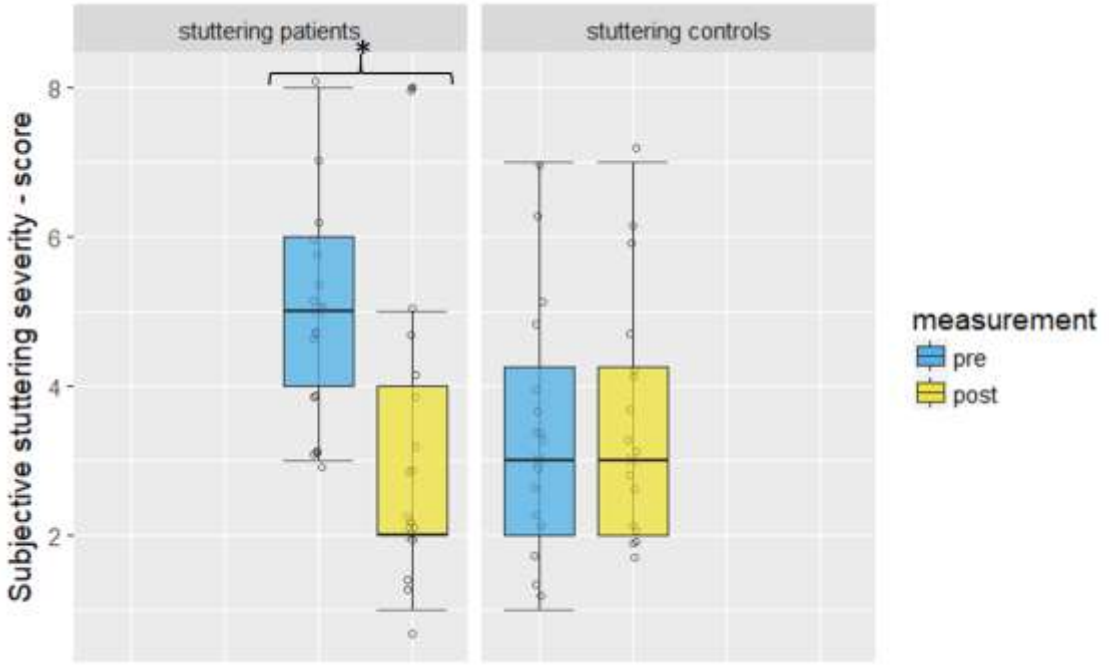


Figure 17. Subjective stuttering severity score of stuttering patients and stuttering controls. *(Significant difference between post- and pre-measurement, p = 0.000, r = -0.81).

3.3.1.3 OASES

OASES – differences between groups at pre- and post-test

At pre-test, the OASES score in the SP group (median_pre = 3) was significantly higher compared to the group of stuttering controls (median_pre = 2.06) (U = 12, z = -4.467, p = 0.000, r = -0.78, large effect). At the post-measurement, no significant difference in the OASES score could be shown between stuttering patients (median_post = 1.91) and stuttering controls (median_post = 1.95) (U = 130, z = -0.216, p = 0.829, r = -0.04, small effect; illustrated in Figure 18).

OASES – longitudinal changes in each group

In the OASES, a significant decrease was found in the group of stuttering patients from pre- to post-test (median_pre = 3, median_post = 1.91, z = -3.621, p = 0.000, r = -0.87, large effect). No significant difference between both measurements could be shown in

the stuttering controls (median_pre = 2.06, median_post = 1.95, z = -0.621, p = 0.535, r = -0.16, small effect; see Figure 18).

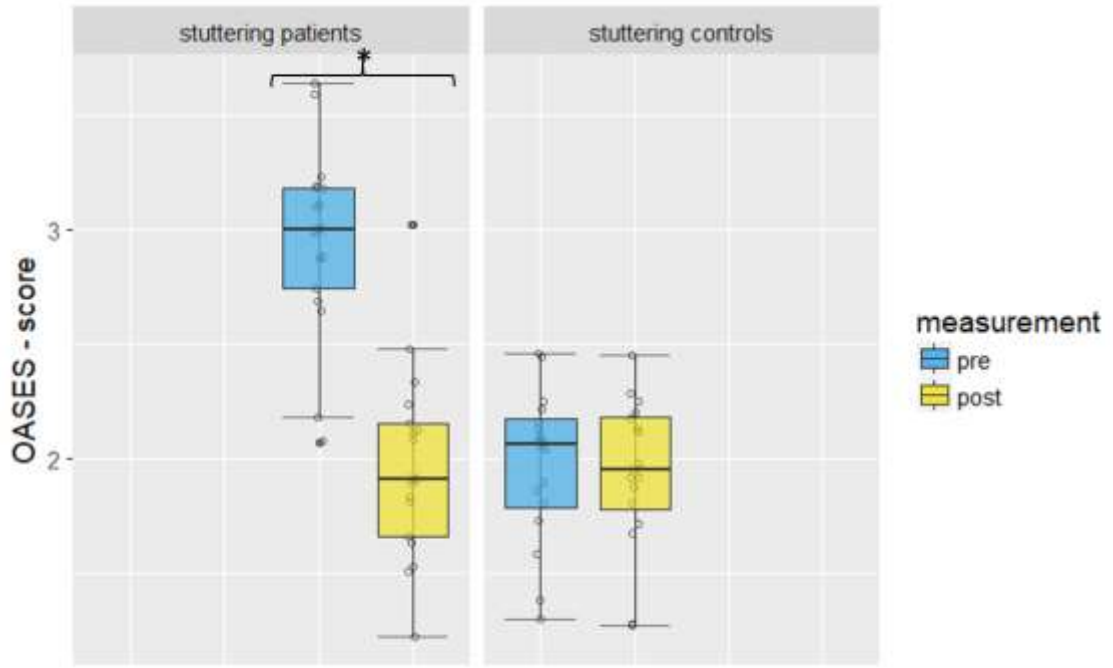


Figure 18. OASES scores of stuttering patients and stuttering controls. *(Significant difference between post- and pre-measurement, p = 0.000, r = -0.87).

3.3.1.4 WHO-5

WHO-5– differences between groups at pre- and post-test

Testing for differences between the three groups at the *pre-test* (H(2) = 0.65, p = 0.723; see Figure 3) and post-test (H(2) = 4.455, p = 0.108), we found no significant difference in the WHO-5-score.

WHO-5 – longitudinal changes in each group

Elucidating the *changes of WHO-5 scores from pre- to post-test in each group, respectively,* there was no significant change detectable (see Table 14, Figure 19).

Table 14. Statistical testing of longitudinal changes in the WHO-5-score in each group.

Group	median pre	median post	z	p	r
SP	17	18	-1.061	0.289	-0.26
SC	17.5	16	-1.302	0.193	-0.33
HC	17	18	-1.362	0.173	-0.27

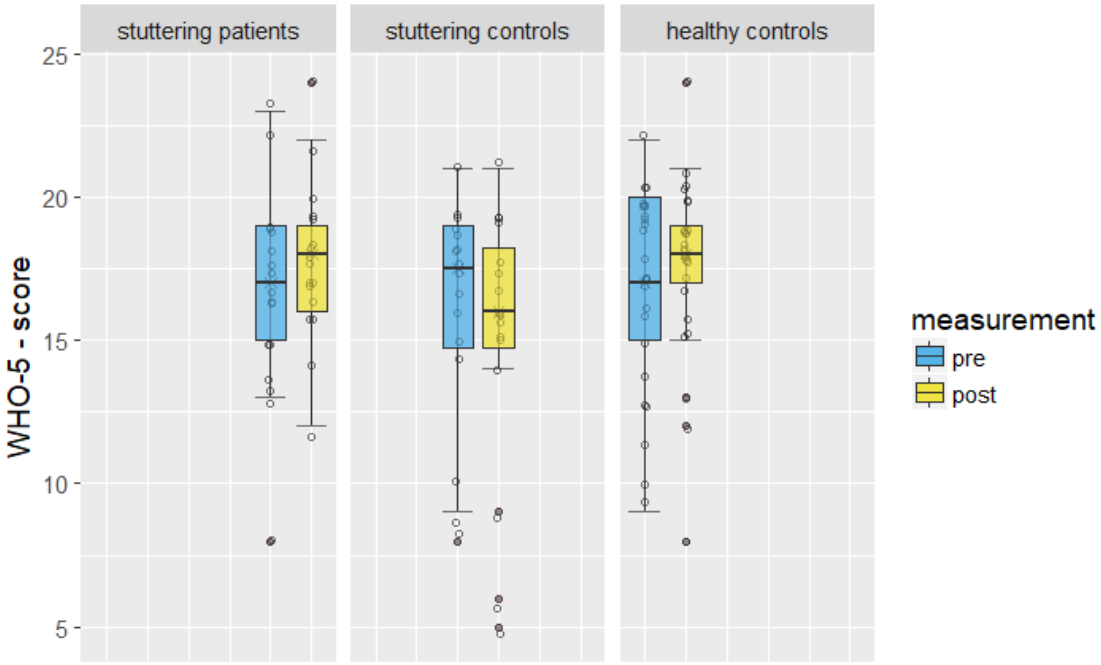


Figure 19. WHO-5 scores of all study groups and measurements.

3.3.1.5 BDI

BDI – differences between groups at pre- and post-test

Testing for differences between the three groups at *pre*-test (H(2) = 1.385, p = 0.500) as well as *post-test* (H(2) = 0.459, p = 0.795), we found no significant difference.

BDI – longitudinal changes in each group

Comparing the changes of BDI scores from pre- to post-test in each group, respectively, Table 15 reveals a significant difference solely in the group of stuttering patients (p = 0.007, r = -0.65, large effect; see also Figure 20).

Table 15. Statistical testing of longitudinal changes in the BDI-score in each group.

Group	median pre	median post	z	p	r
SP	3	2	-2.687	0.007	-0.65
SC	3	1	-1.623	0.105	-0.41
HC	2	1	-0.078	0.938	-0.02

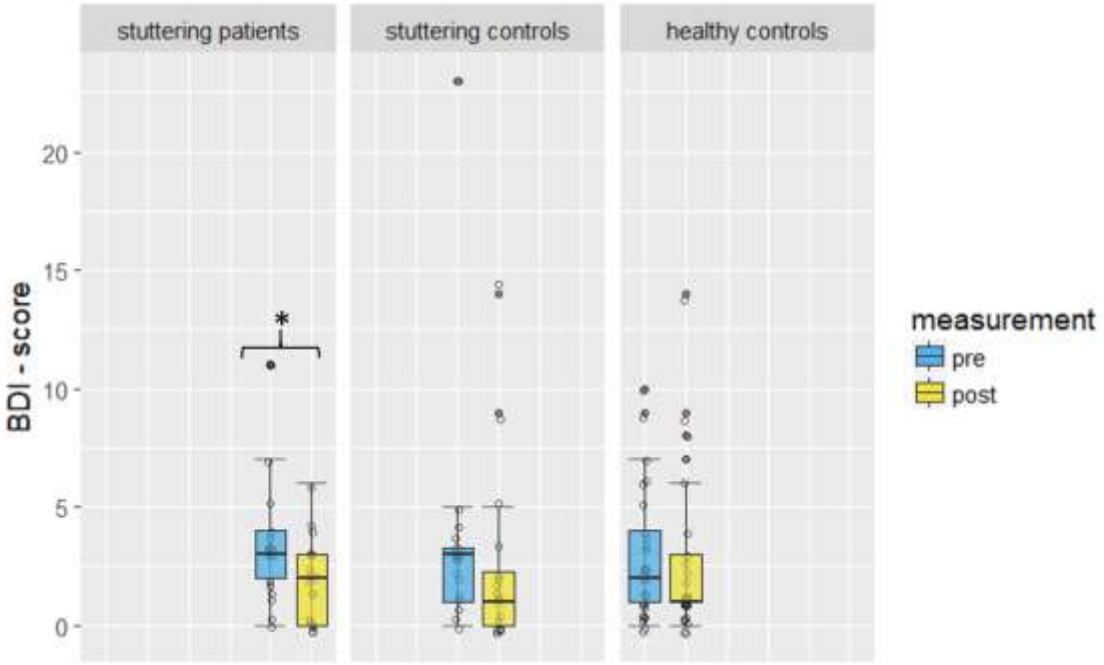


Figure 20. BDI scores of all study groups and measurements. *(Significant difference between post- and pre-measurement, p = 0.007, r = -0.65).

3.3.1.6 State-Trait Anxiety Inventory

For both STAI subtests X1 and X2, there was no significant change between the two measurements in the group of healthy participants (see Table 16, Figure 21).

Table 16. Statistical testing of longitudinal changes in the STAI-score in the healthy controls. X1 (state), X2 (trait).

Group	subtest	median pre	median post	z	p	r
HC	X1	29	29	-0.503	0.615	-0.10
	X2	29	28	-0.412	0.681	-0.08

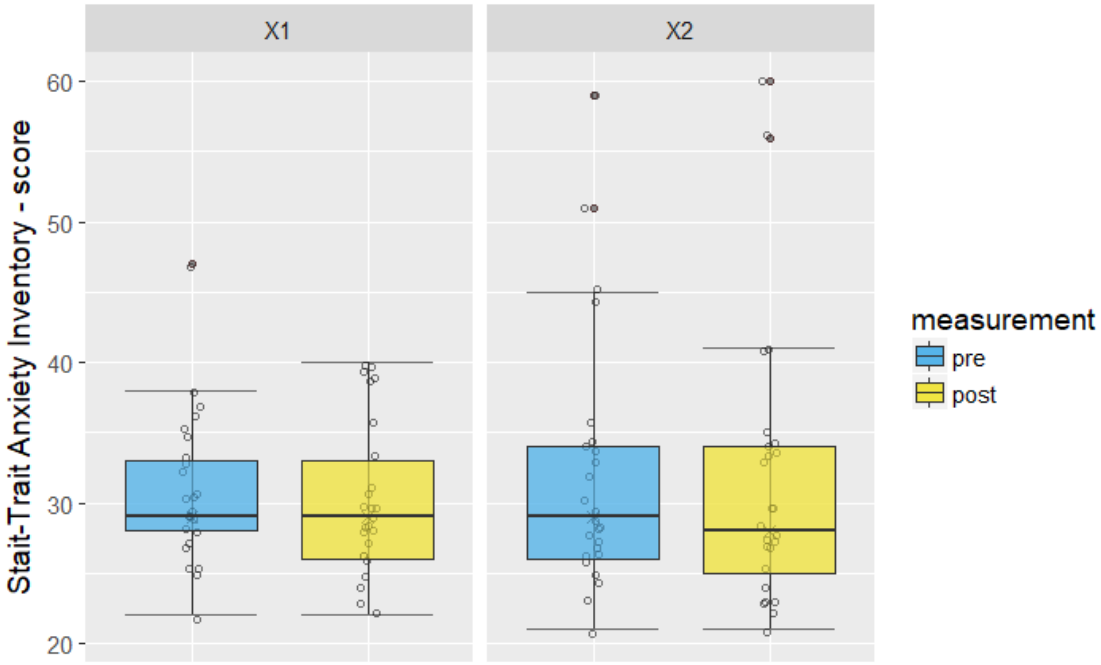


Figure 21. STAI scores of healthy controls. X1 (state), X2 (trait).

3.3.2 Outcome of fMRI analysis

3.3.2.1 Outcome of fMRI analysis – evaluation of therapy effects on brain activation

Using the ANOVA, it is possible to identify differences in activation across all groups and times. First, we wanted to analyse the effect of stuttering therapy on brain activation and included the total SSI score as a covariate of no interest in the ANOVA. By doing this we reassured that the different stuttering severity levels of all participants in the distinct study groups (stuttering patients with intervention, stuttering controls, healthy controls) would not influence therapy-induced effects on brain activity.

In the t-contrast ‘SP pre>post’, which would indicate a therapy-induced decrease of activation, we did not find any significant decrease of activity which was more pronounced in the stuttering patients compared to the control groups.

However, in the t-contrast 'SP post>pre' which is indicating an increase of brain activation in the SP group (comparison of pre- and post-measurement via pairwise T-test), we found several anatomical regions showing a stronger increase of brain activity in the stuttering patients compared to either control groups. All of these regions showed a higher increase of activation compared to the control groups. Among all of these regions, the motor regions showing this stronger increase are presented in Figure 22.

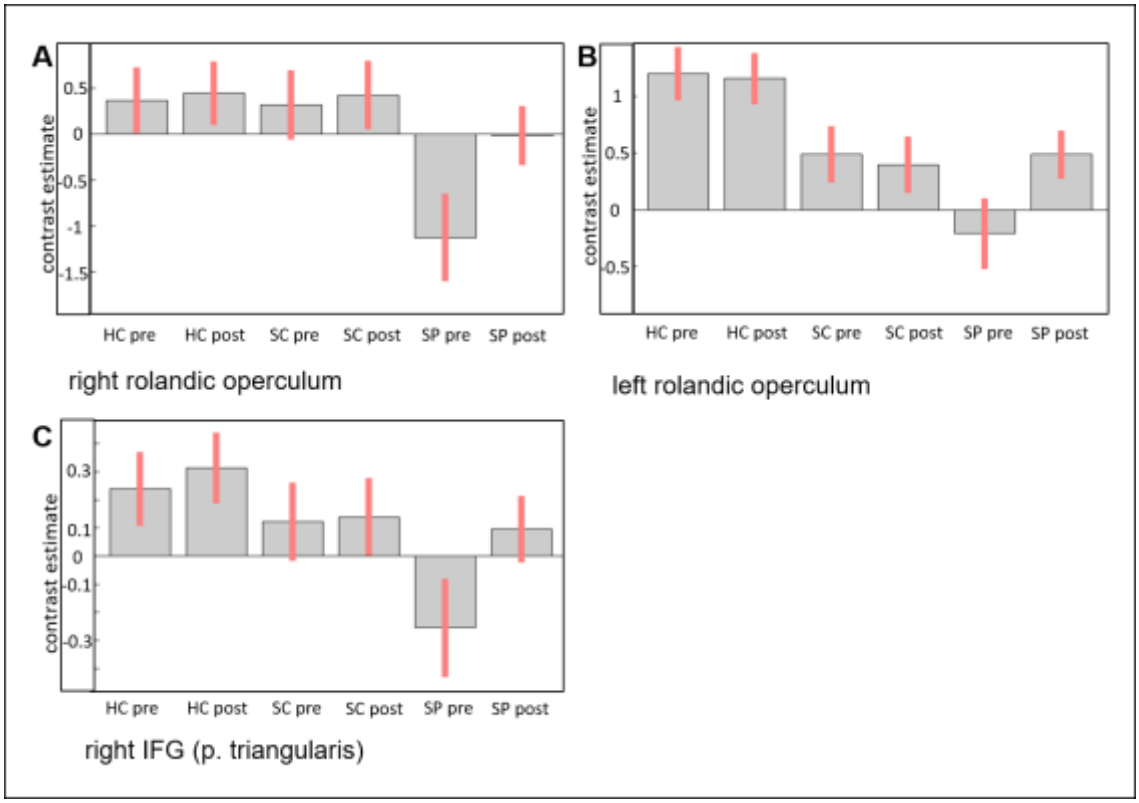


Figure 22. Contrast estimates overview. Contrast estimates at the peak coordinates from the contrast SP post>pre in motor regions, SSI-score was used as a covariate of no interest (uncorr., p <0.005). From the left to the right. bars represent healthy controls (HC) pre and post, stuttering controls (SC) pre and post and stuttering patients (SP) pre and post. A (right rolandic operculum, coordinate 60 -4 13), B (left rolandic operculum, coordinate -63 -1 13), C (right IFG, coordinate 63 20 25). An overview of all coordinates is represented in **Table 17**.

Not only in these motor regions, but also in other anatomical brain regions like the left amygdala, the right supermarginal gyrus as well as left superior temporal gyrus and left temporal pole, an increase of activation was present in the group of stuttering patients after therapy (see Figure 23, Table 17). Striking is that in all those areas, the stuttering patients showed a hypo-activity at the pre-test, which was reduced or not traceable at the second measurement. Neither the stuttering controls, nor the healthy controls

showed this hypoactivation at the pre-measurement. Therefore, we could assess that the ANOVA was predominantly driven by the “SP pre” data.

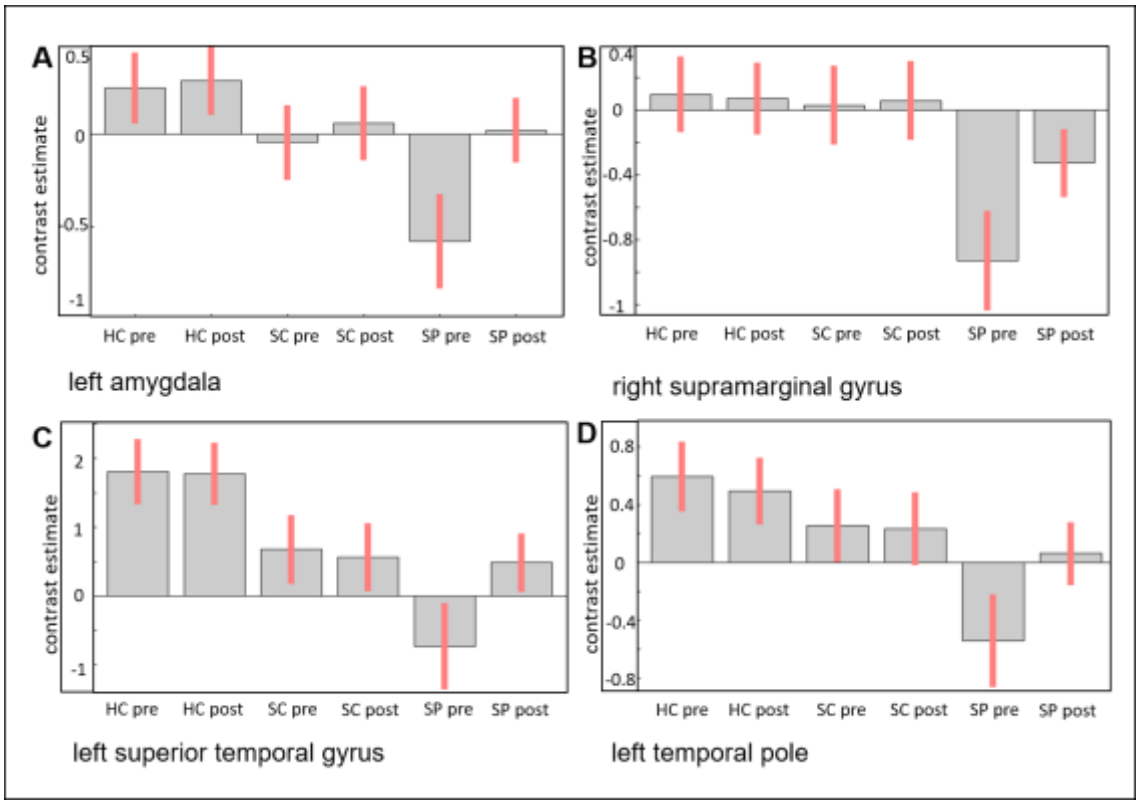


Figure 23. Contrast estimates overview. Contrast estimates at the peak coordinates from the contrast SP post>pre in non-motor regions, SSI-score was used as a covariate of no interest (uncorr., p<0.005). From the left to the right, bars represent healthy controls (HC) pre and post, stuttering controls (SC) pre and post and stuttering patients (SP) pre and post. A (left amygdala, coordinate -21 -4 -20), B (right supramarginal gyrus, coordinate 69 -19 22), C (left superior temporal gyrus, coordinate -60 -34 19), D (left temporal pole, coordinate -57 8 -14). An overview of all coordinates is represented in **Table 17.**

An overview of all the regions showing an increase of activity in the stuttering group is also given in Table 17 and Figure 24.

Table 17. Significant clusters of fMRI Analysis, SSI integrated as a covariate of no interest. Contrast SP post > pre, p < 0.005, uncorr.

Brain area	**coordinates**			**T-value**	**Z-value**	**K**	**P < 0.005 uncorr.**
	X	Y	Z				
L Amygdala	-21	-4	-20	3.98	3.84	63	0.000
R Rolandic Operculum	60	-4	13	3.97	3.83	82	0.000
L Rolandic Operculum	-63	-1	13	3.81	3.69	54	0.000
R IFG (p. triangularis)	63	20	25	3.42	3.33	15	0.000
R SupraMarginal Gyrus	69	-19	22	3.34	3.25	46	0.001
L Superior Temporal Gyrus	-60	-34	19	3.29	3.20	64	0.001
L Temporal Pole	-57	8	-14	3.21	3.13	10	0.001

Note. Outcome of the 2nd level ANOVA with SSI serving as a covariate of no interest. Coordinates (MNI coordinates (mm)), T (Height threshold), K (cluster size) FSL JHA (Jülich Histological Atlas included into FSL; Eickhoff et al., 2005). Significant regions are presented with decreasing T-Value.

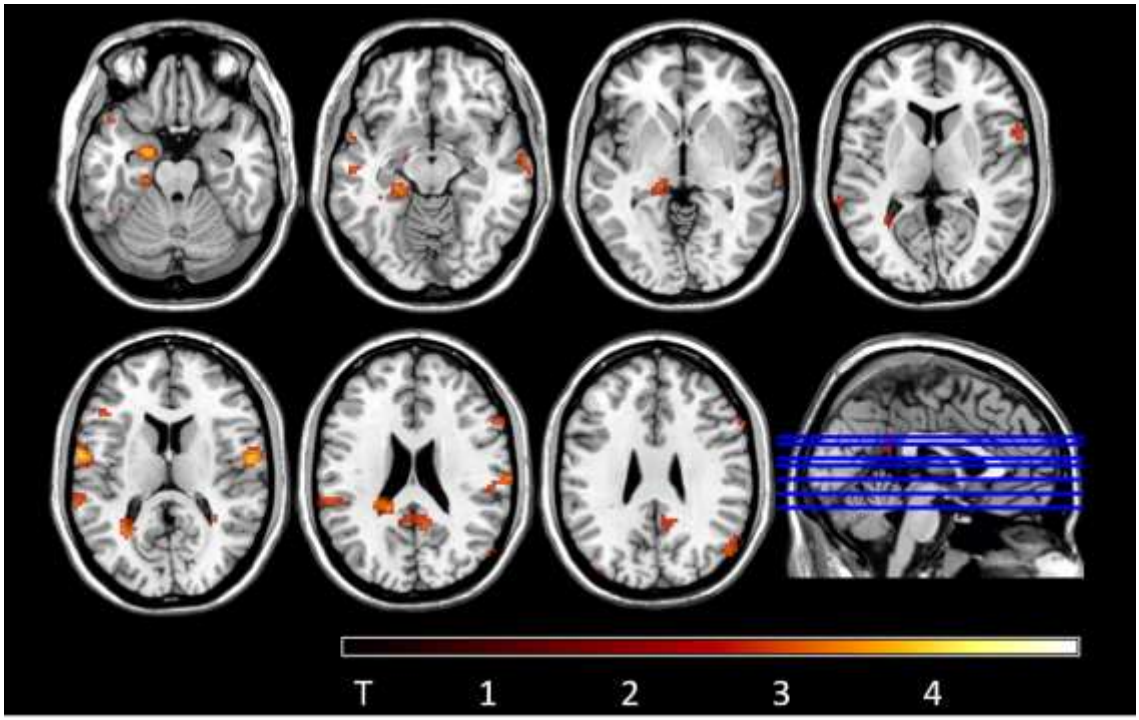


Figure 24. Whole-brain analysis with SSI as a covariate of no interest. Overlay of significant activations in the contrast SP post>pre on a standard T1-weighted MNI brain, presented in axial multi-slice format. The T-value is illustrated in the coloured bar at the bottom – orange-yellow indicates the brain parts where SP show a significant increase of activation (uncorr., p <0.005) from pre- to post-measurement compared to both control groups during covert speaking.

3.3.2.2 Outcome of fMRI analysis – Correlation between brain activity and stuttering severity

The SSI score was also added to our ANOVA as a covariate of interest – the approach was chosen to test if brain activation in specific brain areas correlates with different levels of stuttering severity, including all participant groups and points of time. In particular, we assumed to see a positive linear correlation between the intensity of SSI score and brain activity.

Calculating the SSI + contrast (positive correlation between changes in brain activity and SSI score) and SSI – contrast (negative correlation between changes in brain activity and SSI score), we found no negative correlation, but obtained results of a highly significant positive correlation for both tested significance levels ($p < 0.005$ uncorr., as

well as $p < 0.05$, FWE, see Table 18, Figure 25). This means that the higher the SSI-total-score, the higher the brain activation.

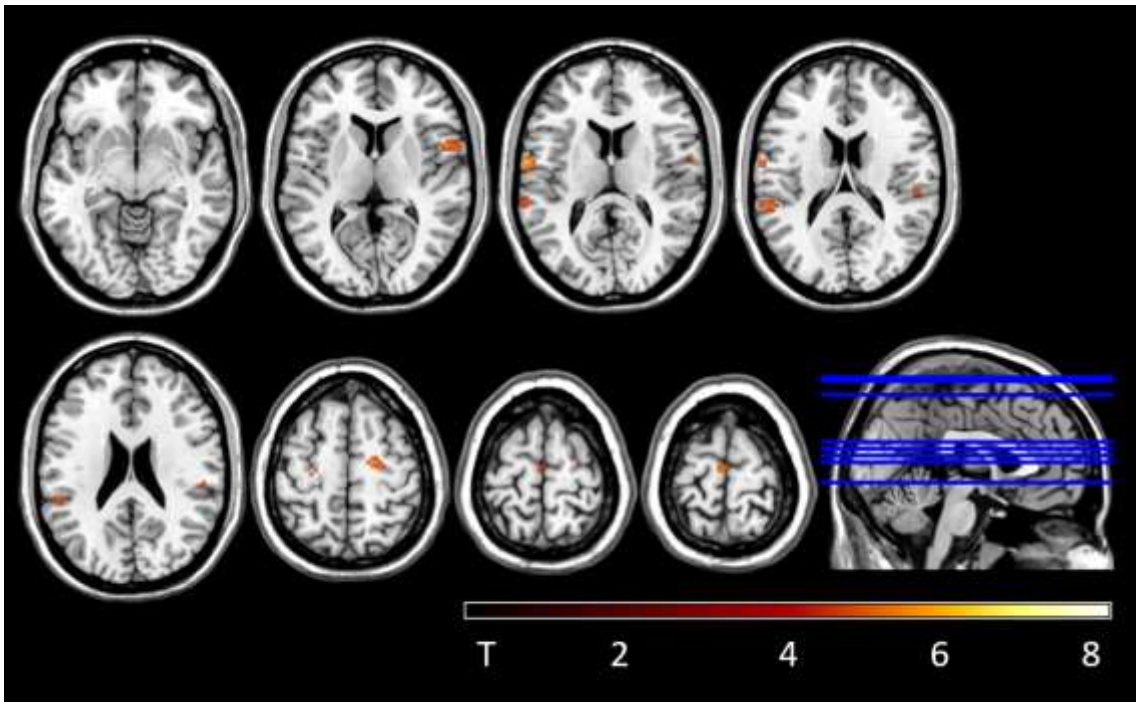


Figure 25. Whole-brain analysis with SSI as a covariate of interest. Overlay of significant activations in the contrast "SSI +" on a standard T1-weighted MNI brain, presented in axial multi-slice format. The T-value is illustrated in the coloured bar at the bottom – orange-yellow indicates the brain regions presenting with a high positive correlation (FWE, $p < 0.05$) between stuttering severity and brain activity, calculated across all participant groups and points of time during covert speaking.

The FWE-corrected, positive correlation is evident in diverse brain regions (see Table 18). Prominent is that regions well-known for motor (speech) processing show this correlation (e.g. right BA 44 (Broca's area), left and right rolandic operculum, left precentral gyrus, left premotor cortex BA 46). In addition, areas having an impact on cognitive functions and emotional processing like right amygdala, left subiculum and right middle temporal gyrus (see Table 18) are also outcome of this positive correlation.

Table 18. Significant clusters of fMRI Analysis, SSI integrated as a covariate of interest. Contrast SSI +, p <0.05, FWE corrected.

Brain area	coordinates			T-value	Z-value	K	p < 0.05 FWE
	X	Y	Z				
R Superior Frontal	18	-10	61	6.09	5.64	32	0.000
L Rolandic Operculum	-63	-4	13	5.95	5.53	29	0.000
Area 44 / R rolandic Operculum	54	8	7	5.23	4.93	18	0.005
R Amygdala	18	-10	14	5.19	4.90	12	0.006
L Subiculum (L ParaHippocampal Gyrus)	-24	-25	17	5.18	4.88	10	0.007
L posterior-medial FSL JHA: GM Premotor cortex BA46 L	-3	-13	70	5.17	4.88	9	0.007
L Area PFcm (IPL)	-57	-37	19	5.16	4.87	32	0.007
Area OP1, R Rolandic Operculum	54	-25	19	4.90	4.64	7	0.018
SPM: not found, FSL JHA: 48% WM CST L	-24	-19	55	4.86	4.61	11	0.021
R Middle Temporal Gyrus	69	-25	-8	4.74	4.51	2	0.032
R Rolandic Operculum	60	-4	13	4.70	4.47	3	0.037
L Precentral Gyrus	-42	-7	34	4.67	4.45	1	0.040

Note. Outcome of the 2nd level ANOVA with SSI serving as a covariate of interest. Coordinates (MNI coordinates (mm)), T (height threshold), K (cluster size), FSL JHA (Jülich Histological Atlas included into FSL; (Eickhoff et al., 2005). Significant regions are presented with decreasing T-Value.

3.4 Discussion

The purpose of our study was to investigate the long-term influence of an intense stuttering therapy on brain activation. Previously, functional imaging studies in this area mainly used a short follow-up period < 6 months, therefore likely missing effects of the maintenance phase including self-training in the every-day life of patients as well as relapses. In our study, we provided a follow-up measurement of approximately 11 months after therapy start and were therefore able to elucidate long-term functional activity changes evoked by the intervention and its maintenance phase.

In addition, we wanted to assess the existence of a positive correlation between stuttering severity and brain activation in our study population. We assumed that a high stuttering severity score correlates with a hyperactivation in prominent motor processing brain regions, because the parameter of healthy controls (normal activation in motor areas, low stuttering severity score) might significantly differentiate from PWS' parameters (hyperactivation of motor regions, higher stuttering severity score).

There are manifold outcomes of this study:

Concerning the behavioural measurements, a significantly decreased amount of stuttering symptoms in the intervention group was characterised by the significant decline of the total SSI-score. Furthermore, significant reductions of the subjective stuttering severity and the OASES score were found. The findings indicate a subjectively perceived increase in speech fluency and a decrease of the impact of stuttering on the quality of life. These behavioural changes seem to be related to the stuttering therapy, because comparable changes were absent in the stuttering control group.

Although the WHO-5 provided no significant longitudinal changes in all study groups, respectively, there was a significant reduction of the BDI score from pre- to post-measurement in the intervention group. This can be regarded as a therapy effect – the better the stuttering patients could implement their newly-learned speech pattern, the

less adverse attitudes and feelings they had to deal with (e.g. Euler et al., 2016). Between pre- and post-measurement, the healthy, fluently speaking participants did not show any significant changes in the STAI. This is pointing to their permanent and stable psychological condition concerning general anxiety and anxiety towards various communication settings.

Concerning the influence of an intense stuttering therapy on brain activation, an increase of activity in the intervention group was found after therapy in several brain regions. Interestingly, at pre-test, the stuttering patients showed a hypoactivation in these regions at post-test which approached the values observed in fluent and stuttering controls. As expected, the regions where an increase of activation was traceable were prominent motor-processing brain areas like the bilateral rolandic operculum and right IFG (pars triangularis). However, the increase of activity was also detectable in regions which are involved in cognitive and emotional and cognitive processing, e.g. in the left amygdala (Javanbakht et al., 2015).

Concerning the correlation between brain activity and stuttering severity, we were able to confirm a positive correlation calculated over all study groups and measurements – the higher the stuttering severity score has been, the higher the functional activity in specific motor regions was. These regions included prominent motor processing areas like the left and right rolandic operculum, the right BA 44 and the left precentral gyrus. Beyond that, the positive correlation was also evident in non-motor regions like the left subiculum and right middle temporal gyrus.

In the following, we discuss the observed treatment-induced change of brain activation in reference to previous studies evaluating functional effects of stuttering therapies. In addition, we examine the brain regions in which activation changed after therapy and debate their role within the pathomechanisms of stuttering.

3.4.1 Increased activity after stuttering therapy – changes of activation patterns as an intervention effect

A striking result of the current study is the increase of activation stuttering patients show in the long-term follow-up measurement. The motor regions right and left rolandic operculum as well as the right IFG (p. triangularis, see Figure 22) and in addition regions involved in cognitive and emotional processing like the left amygdala and the right supramarginal gyrus (see Figure 23) provided this growth of activity from pre- to post-test.

At pre-test, patients showed a hypoactivation compared to stuttering and healthy controls in all the regions where they exhibited the rise of functional activity after therapy. Comparing the contrast estimates of SP pre and post with the controls' contrast estimates pre and post (see Figure 22, Figure 23, Table 17), the patients' functional activity at post-test shows a trend for adjustment to the intensity levels that both control groups exhibit.

In past studies where therapy-induced activation changes were investigated, stuttering patients also showed an increase of activation after therapy. The region and also hemisphere where this increase was detectable were distinct, though. A shift of brain activity that was often found in patients taking part in a stuttering intervention was a shift of overactivity from bilateral and right-hemispheric motor areas to left-hemispheric motor regions (Belyk et al., 2015). Following, right-hemispheric activity in motor processing areas was reduced, while an increase of left hemispheric activation intensity was reported.

Referring to the studies that chose a long-term follow-up measurement > 6 months after the therapy start, De Nil and collaborators (2003) reported this shift of activity to the left hemisphere. Before the stuttering therapy started, stuttering patients showed an overactivation in the bilateral superior temporal gyrus, pre- and post-central gyrus, insula

and cerebellum as well as in the right-hemispheric medial frontal gyrus, the anterior cingulate and putamen. One year after therapy, the right and bilateral hemispheric activation was reduced except the activation of the right middle temporal gyrus as well as the bilateral frontal gyrus. The left-hemispheric activation increased; a growth of activation intensity was reported mostly in speech motor regions like the left precentral gyrus, globus pallidus, middle frontal gyrus and insula, but also in the left superior and middle temporal gyrus. These results are similar to the outcome of our study – we are able to report a left-hemispheric increase of activity in the rolandic operculum. In addition, we detected an increase in right-hemispheric speech motor areas like the right rolandic operculum as well as the right IFG. Our study was also able to confirm an increase of activity in the same area where De Nil et al. (2003) had discovered it before; it was located in the left superior temporal gyrus. Furthermore, stuttering patients also exhibited an increase of activity in the left temporal pole in the current investigation. The increase of activation we found in the right-hemispheric regions of the right supramarginal gyrus, right IFG and right rolandic operculum as well as the left amygdala was not affirmed by De Nil et al (2003).

Neumann and colleagues (2004) reported a hypoactivation in left frontal brain regions of PWS as well as a larger and more widespread activation pattern in PWS compared to healthy control participants before therapy. Two years after the therapy start, the hypoactivation of left-frontal speech motor regions was still detectable in the group of stuttering patients. Neumann et al. concluded that this hypoactivity might be a hallmark of stuttering. In our long-term-follow-up study, we were also able to find evidence for a hypoactivity in stuttering patients before therapy. Contrary to the results of Neumann et al. (2004) we detected this hypoactivation not only in left, but also right-hemispheric motor regions. The participants of the current intervention group show a trend towards normalisation of activity, although their contrast estimates do not reach the level of healthy participants. One could argue that this underlines Neumann and collaborator's

interpretation of the hypoactivation being a sign of the dysfunctional physiology in the stuttering brain.

In their newest study, Neumann and collaborators (2018) were able to show that dysprosody in stuttering patients was associated with a hypoactivity of the left anterior insula and the caudal part of the inferior frontal gyrus (pars orbitalis) compared to healthy controls. After one year, prosodic speech elements of the stuttering patients improved. Also the hypoactivation in left inferior frontal regions as well as in the anterior insula was normalised. In addition, a newly-found hypoactivation in the bilateral dorsal striatum as well as an additional activation of limbic regions (e.g. bilateral amgydala) were observed one year after therapy.

These results seem to have the biggest correspondence to the current study: Like Neumann and colleagues (2018), we were also able to find a hypoactivation in left motor areas like the left rolandic operculum before therapy. In the second measurement this left-hemispheric hypoactivation was also diminished in our study. Like Neumann et al., we were furthermore able to show an increased activity in the amygdala, which was in our case only left-sided. Beyond the newest results of Neumann et al., we also found hypoactivations in right-hemispheric motor regions as well as in left temporal areas which were also reduced after therapy.

In relation to the studies investigating the influence of stuttering therapy on brain activations with a short follow up < 6 months, we also found interesting parallels towards the current study outcome:

Neumann et al. (2005) reported an hypoactivation in the left rolandic operculum in the pre-measurement of patients taking part in the Kasseler stuttering therapy. Directly after the intense therapy course, this hypoactivation normalised and the activity in the left rolandic operculum increased – this increase was also observable in our currently investigated group of stuttering patients.

In 2018, Kell and colleagues evaluated changes in functional connectivity after an intense stuttering therapy course – interestingly, after therapy the connection between the supramarginal gyrus and prefrontal cortex intensified (Kell et al., 2018). Although we did not conduct functional connectivity analysis in this paper, it is striking that the activation of the right supramarginal gyrus as well as right (speech) motor regions like the rolandic operculum and IFG increased in our study.

To conclude, in studies with a short-time as well as long-time-follow up, a hypoactivation of left-hemispheric motor regions in untreated PWS is a result that was described and replicated several times (e.g. De Nil, Luc F. et al., 2003; Neumann et al., 2005). Our additional result that this hypoactivation can also be found in right-hemispheric motor regions and beyond that in temporal regions (also found in De Nil et al., 2003) as well as in limbic regions (solely found by Neumann et al., 2018) is a rather rare outcome. Also the finding that a therapy-induced increase of activation was found in both left and right motor and emotion processing brain regions is notable.

3.4.2 Brain regions with activation changes after therapy and their relevance towards stuttering

Not only the change of activation patterns that is influenced by the intervention, but also the regions themselves showing a changed activation intensity are of interest. As stated above, the hypoactivation in left hemispheric frontal areas like the left rolandic operculum is an often replicated pre-treatment result in studies investigating the effects of stuttering therapy on brain activation (for a review, see Etchell et al., 2017). Furthermore, these frontal regions also revealed less activation in PWS compared to healthy controls in studies investigating the neural correlates of stuttering by comparing functional activity of fluent controls and PWS (also called “trait” of stuttering, see Belyk et al., 2015). Especially a hypoactivity in the left primary motor cortex including the larynx area was reported in the meta-analysis of Budde et al. as well as Belyk and colleagues (Budde et al., 2014; Belyk et al., 2015). Due to these several replications, hypoactivity in left frontal

motor regions like the left rolandic operculum which we found in our current study as well as hypoactivity in the left M1, left IFG and further regions in close vicinity (Belyk et al., 2015) are likely to represent a neural hallmark of stuttering. Additionally, the decline of white matter integrity in these regions which was found in several diffusion MRI studies (for a meta-analysis, see Neef et al., 2015; in addition Sommer et al., 2002) affirms the role of these areas as neural hallmarks of stuttering.

We here present the result of hypoactivity in right hemispheric motor regions (right rolandic operculum, right IFG) in PWS compared to fluent controls before the treatment starts and which approached the values of control participants after therapy. In past studies, a hyperactivation in right hemispheric motor areas like the primary motor cortex, pre-SMA and SMA as well as IFG was reported (see e.g. Budde et al., 2014), which often diminished after a stuttering intervention (e.g. De Nil, Luc F. et al., 2003). It is unclear why in our case PWS in the treatment group, but not in the control group, showed a hypoactivation in adjacent regions before the treatment started. One reason for this interesting finding could be the significant difference in the OASES score between the intervention and stuttering control group at pre-test. Due to a higher subjectively perceived psychological strain as well as a more impaired quality of life, patients in the intervention group might have manifested different dysfunctional (or compensational) activation patterns compared to the stuttering controls, resulting in a right hypoactivity of motor regions. It is still unclear why this result was not replicated by other studies. Future fMRI studies exploring therapy effects in stuttering populations should further investigate if this hypoactivity in right motor regions can be replicated and how it might be interpreted on a neural level.

Surprisingly, not only motor regions showed a hypoactivation before and a normalised activation after stuttering therapy. This activation pattern was also present in regions that are not associated with speech production. First, an increase of activation in the left amygdala became evident. The amygdala is a core part of the limbic system – it is

involved in emotional responses as fear, anxiety and aggression as well as in memory and decision making (Javanbakht et al., 2015; Neef et al., 2017). Toyomura and colleagues investigated the relation between amygdala activity and speech disfluency in PWS (Toyomura, Fujii, Yokosawa, & Kuriki, 2018). Stuttering participants presented with a significant correlation between the discomfort level during talking to a stranger and activity in the right amygdala. Furthermore, the activity of the PWS' prefrontal cortex which is involved into emotional regulation was decreased in PWS compared to fluent controls. Toyomura et al. therefore concluded that amygdala activity during interpersonal communication is involved in the speech of PWS. Also Neumann et al. (2018) observed that PWS exhibited an increase of left prefrontal activity as well as a new, additional bilateral amygdala activation one year after the patients had started the stuttering therapy. Therefore, the boosted amygdala activation in the intervention group of this study which was hypoactive in the pre-measurement could represent a change in PWS' emotional regulation. Considering the significantly decreased OASES score as well as the reduction of the BDI score in the group of stuttering patients, one could argue that the rise of amygdala activity might be a sign for a more self-reliant, successful coping strategy towards the daily difficulties that arise with stuttering.

In addition to an activation increase in the left amygdala, also an increase of activation in the right supramarginal gyrus was found in stuttering patients from pre- to post-test. Though the intensity of activation rose in the group of stuttering patients, the contrast estimates were, at both points of measurement, still lower and hypoactivated compared to fluent controls (see Figure 23). Remarkably, also Yang et al. (2016) who conducted a functional connectivity study reported that the activation of the right supramarginal gyrus was decreased in PWS during the production of dysfluent speech. The supramarginal gyrus is relevant for the acoustic-phonological processing during speech planning (Démonet, Price, Wise, & Frackowiak, 1994; Hartwigsen et al., 2010). Silani and colleagues even reported that the right supramarginal gyrus is necessary for emotional

and social judgement and for empathy (Silani, Lamm, Ruff, & Singer, 2013). Therefore, the treatment-induced increase of activity in this brain region might have been emerged through the intense training of a new motor speech pattern (where acoustic-phonological processing plays a major part). The treatment could also have enhanced empathic and social abilities, probably conditioned by the group-therapy setting and the in-vivo training. Thus, our result underlines the right supramarginal gyrus might be a neural hallmark of stuttering.

Also the left superior temporal gyrus and the left temporal pole were brain regions where a hypoactivation at pre-test and an increased activity at post-test were evident in the current intervention group. Especially the increase of activation intensity in the superior temporal gyrus is of interest: the bilateral superior temporal gyrus facilitates the human voice perception, it builds a self-monitoring mechanism for phonological feedback (Hashimoto & Sakai, 2003). Yang and colleagues were able to show changes in functional connectivity between putamen and superior temporal gyrus in PWS. They concluded that this affected connectivity might disturb the sensorimotor integration between auditory feedback and motor control during speaking and could play a role in the aetiology of stuttering symptoms (Yang et al., 2016). De Nil et al. (2003) also observed an increase of activation in the left superior temporal gyrus one year after PWS had started an intense stuttering therapy. They infer that this increase might mirror an improved awareness as well as a better control of articulation processes. De Nil's interpretation also seems valid for our observed change of activity in this area: in the Kasseler stuttering therapy, patients intensively learn the implementation of a new motor speech pattern which demands awareness of and attention towards phonetic knowledge and articulatory proprioception. It seems plausible that this newly-learned motor control and the focus on perfectly executed motor production is in need of a higher activation of the superior temporal gyrus.

Functional or resting state connectivity studies revealed the previously described regions as components of different networks. The superior temporal gyrus for example is part of the somatomotor network, while the supramarginal gyrus is a constituent of the ventral as well as dorsal attention network (Chang et al., 2017). Chang and colleagues (2017) reported that the trait of stuttering was associated with atypical network connectivity involving the default mode network and its connectivity with attention, somatomotor and frontoparietal networks. This finding also fits to our current results: the pathomechanims of stuttering are not only detectable in aberrant motor functional activity, but also in changed activity patterns in other brain regions and probably networks not associated with articulation.

3.4.3 Positive correlation between brain activity and stuttering severity

We confirmed a positive correlation calculated over all study groups and measurements – the higher the stuttering severity was, the higher was the functional activity in certain motor regions. This is a plausible outcome due to the fact that healthy controls show low SSI scores and, compared to PWS, high contrast estimates pre and post (see Figure 22, Figure 23). Stuttering patients, on the other hand, present with higher SSI scores pre, which are reduced in the stuttering patient group after intervention. Concerning brain activity, stuttering patients show a hypoactivation at pre-test with a trend to normalisation at post-test (Figure 23, Figure 24). Due to these associations, a positive, FWE corrected correlation calculated across all groups and points of time, became evident.

The obtained positive correlation between stuttering severity and brain activity across fluent and stuttering groups of participant might help to predict if an individual measured with a similar fMRI paradigm is stuttering. If a high activation in these brain regions which were the outcome of our correlation (see Table 18) is evident, it is more likely that this person belongs to the population of PWS. This approach could be interesting for several purposes, e.g. 1) separating stuttering from healthy individuals as a preparation for the recruitment of an (fMRI) research study 2) securing the diagnosis of stuttering when the

examined participant shows an efficient avoidance behaviour (rephrasing sentences in a way that no stuttering symptoms occur), and 3) gaining a better understanding of the aetiology of stuttering as well as developing new therapeutic approaches to treat it.

Concerning the last-mentioned purpose, it is interesting to see which brain regions show the significant correlation between stuttering severity and intensity of brain activation: included are prominent motor processing areas like the left and right rolandic operculum, the right BA 44, the left precentral and premotor gyrus. Beyond that, the positive correlation was also evident in non-motor regions like the right superior frontal cortex right amygdala, left subiculum and right middle temporal gyrus (see Table 18).

The superior frontal cortex is meaningful for the processing of self-awareness (Goldberg, Harel, & Malach, 2006) and the down-regulation of emotional arousal (Falquez et al., 2014). The amygdala, as previously described, plays a role in emotional regulation and emotional responses (Javanbakht et al., 2015; Neef et al., 2017). The subiculum is a multifunctional structure, responsible among others for learning and memory (Aggleton & Christiansen, 2015), though its functionality seems to be influenced by stress (Howland & Davies, 2014). The middle temporal gyrus is a component of the dorsal attention network (Chang et al., 2017) and meaningful for assessing conceptual information (Saur et al., 2008).

All the functional purposes of these regions are largely related to characteristics of stuttering: PWS often experience emotions like anxiety and fear due to the loss of motor control during speaking – this emotional arousal might be processed by amygdala activity. They also have to treat negative reactions towards stuttering symptoms from their listeners and peer groups, which produces a high stress level and might impair the function of the subiculum (Howland & Davies, 2014). Furthermore, secondary symptoms of stuttering like avoidance behaviour are highly learned in the patients' conversational history – that might be the reason why the activation of the subiculum might play a

meaningful role in the aetiology of stuttering. In addition, attention networks (where the middle temporal gyrus is a constituent of) as well as the default mode network showed abnormalities and abberent network connections among themselves in PWS (Chang et al., 2017; Xuan et al., 2012). We conclude that a stuttering severity positively correlated with a range of brain activity involved in attention, emotional and motor networks is likely reflecting the multifactorial nature of the disorder.

Limitations

There are certain limitations of this study to be discussed here. First, in reference to the study groups, an optimised matching of participants would have enhanced the quality of the study. Indeed, the stuttering patient group was adequately matched to the healthy control group, but stuttering controls were older and, likely in connection to that higher age, better educated. There is a possibility that differences in age might have influenced the results reported here.

In addition, it was not feasible to exclusively include right handers into the study, although we were able to match the laterality quotient of all study participants. In contrast to language disorders like aphasia or specific language impairment where the language system and its left-hemispheric dominance are essential for the resulting symptoms, stuttering is a speech disorder and therefore not dependent as much on this hemispheric influence. This is why we deemed slightly different handedness lateralities acceptable here.

In our study, we concentrated on the investigation of changed patterns of functional activity due to the influence of an intense stuttering therapy. Therefore, we used an established covert speaking paradigm. Even though the method of examining differences in localized activations with a specific fMRI paradigm is widely used (Etchell et al., 2017), the results of the current study indicate that the additional application of a functional connectivity analysis would have been a significant benefit. Regions where

brain activation changed after therapy in the patient group are well-known constituents of previously determined functional networks. For example, the supramarginal gyrus is a component of the ventral attention network that also connects premotor and motor regions in the vicinity of the motor regions showing a substantial change of activity in the recent study. By detecting changes in whole networks instead of single regions, a deeper neurophysiological understanding of the effect of stuttering therapy on functional plasticity could have been enabled.

Since motor speech production is a demanding process, it seems plausible that whole-network changes after an intense stuttering therapy and its maintenance phase are observable. These network changes could become evident in attention networks (online application of new-learned speech technique) and motor networks. Future studies that also want to add further knowledge on how stuttering interventions change brain activity within a long-term follow-up period should therefore use functional connectivity analysis to detect network changes. Still, our approach to locate different regions where the participants of the intervention group exhibit significant changes compared to both control groups offers new insights on long-term recovery after stuttering therapy.

A critical point in conducting fMRI studies in a stuttering population is the loudness and rhythm of the scanner noise. Rhythm as well as noise masking induce fluency in PWS (Guitar & McCauley, 2010). Because we used a covert speaking paradigm, it is questionable if and how the PWS were influenced by the scanner noise during the imagery speaking. Future studies should develop advanced methods to avoid a bias due to the scanner noise and rhythm. In addition, future studies investigating therapy effects should also implement the evaluation of RSFC activity. Because first studies were able to report anomalous associations between default mode networks and intrinsic connectivity networks in PWS (Chang et al., 2017), therapy-induced changes of these networks should be explored for a deeper understanding of the treatment mechanisms of stuttering.

Further research investigating the long-term effects of stuttering therapies including the maintenance phase after the first intense therapy course will support the understanding of how stuttering interventions work on a neuronal level. Hereby, it would be interesting not only to investigate the effects of one therapy approach, but also to explore therapy effects of other intense, evidence-based stuttering therapies. In Germany, neuroimaging studies evaluating changes in brain activation and intrinsic connectivity networks evoked by stutter modification therapy are still missing, although this is one of the most applied therapy approaches in Germany (Euler et al., 2014). It would be highly interesting to compare activation changes of a stuttering modification therapy with these seen in the current study, where we investigated stuttering patients taking part in the evidence-based Kasseler stuttering therapy (fluency shaping approach).

If further evidence of long-term follow up studies tells us which brain areas or networks are most likely to change their activation pattern due to the intervention, it might be possible to support this neural activity change by the accompanying use of non-invasive brain stimulation techniques (first evidence is provided by Chesters, Möttönen, & Watkins, 2018).

Finally, it is important to mention that PWS show large inter-individual varieties concerning personal traits, the amount and quality of primariy and secondary symptoms, the application of coping strategies and the capability to realise trained speech techniques. These differences are difficult to control in research studies and could have affected the outcomes of the current study, too.

Conclusion

The data we present here contains new knowledge about the long-term therapy-induced brain activity changes of an intense stuttering therapy. Our results suggest that stuttering patients taking part in the intense Kasseler stuttering therapy show an increase of activation in motor as well as emotion and attention regulating regions that have been

hypoactivated before therapy. A normalisation of activation intensity towards the level of fluent controls is evident. In the regions where stuttering patients displayed the growth of activation after therapy, stuttering controls as well as healthy peers did not show significant changes. Therefore, the slope of functional activity is therapy-related. The current study underlines the importance to consider that not only motor regions like left and right operculum are neural correlates of stuttering and of therapy improvements. Indeed, also areas like the amgydala and the superior temporal gyrus involved in attentional and emotional processing showed this trend to normalisation and might also be of importance for the confirmed behavioural improvements after therapy. Future studies should include the investigation of complex brain networks involving different modalities and their long-term changes after an intense stuttering therapy to meet the requirements of this complex speech disorder.

4 Cessation of stuttering after left cerebellar haemorrhage – a case report[5]

4.1 Introduction

Stuttering is a disorder of speech fluency characterised by repetitions and prolongations of speech elements, tense speech blocks, and other motor and behavioural features (Bloodstein & Ratner, 2008). The most prevalent form occurs in childhood, usually between 3-6 years of age, for unknown reasons. It persists into adulthood in a minority of affected individuals (Yairi & Ambrose, 2013). A neurophysiological basis of this persistent developmental stuttering (PDS) has increasingly been uncovered over the last two decades. A reduction of white matter integrity in mostly left frontal brain regions, e.g. in the left frontal operculum and its surrounding areas (Brown et al., 2005; Cykowski et al., 2010; Sommer et al., 2002) is characteristic for PDS.

In contrast, an acquired “neurogenic” form of stuttering has long been known to occur after brain lesions in a variety of subcortical and cerebellar regions, particularly in the left hemisphere (Alm, 2004; Ludlow & Loucks, 2003; Theys, Nil, Thijs, van Wieringen, & Sunaert, 2013). Consistent with this, previous case studies reported occurrence of stuttering after cerebellar (Tani & Sakai, 2010) and subcortical infarcts (Craig-McQuaide, Akram, Zrinzo, & Tripoliti, 2014; Ludlow & Loucks, 2003). In reference to the latter, neurogenic stuttering often appeared in case of lesions in the thalamus (Abe et al., 1993; Levine & MacDougall, 2016) and lesions (as well as microlesions through deep brain stimulation (DBS)) in the basal ganglia (Nebel et al., 2009; van Borsel et al., 1998).

The cessation of PDS after neurological lesions has more rarely been described. An early study reported an alleviation of acquired stuttering after mesothalamic stimulation in a 61 year old male with a history of chronic trigeminal pain (Bhatnagar & Andy, 1989).

[5] Manuscript in preparation together with Sarah Wolter, Christoph Bütfering, Nicole Neef, Claudia Lange, Peter Dechent, Roberto Goya-Maldonado and Martin Sommer

Furthermore, an alleviation of PDS was described after left ventralis intermedius thalami DBS for intractable essential tremor in a 64 year old woman (Maguire et al., 2012) and after bilateral thalamic ischemia in a 66 year old man (Muroi et al., 1999).

Here we had the unique chance to study in detail and to follow-up a case of cessation of stuttering after a left cerebellar infarction. To our knowledge a similar case was only reported once: Miller (1985) observed two patients with developmental stuttering; their stuttering disappeared as signs of progressive multiple sclerosis increased. In accordance with clinical examinations, both patients showed a bilateral cerebellar dysfunction. However, there is no further evidence for a cerebellar lesion location because no imaging was applied (Ludlow & Loucks, 2003; Miller, 1985).

Therefore, the case study we present is unique due to its novelty in literature and also due to its MRI and clinical follow up. Beside the case description and study outcome, we want to associate the findings with cerebellar anatomic knowledge and previous research evaluating the role of the cerebellum as a possible neural correlate of stuttering.

The cerebellum plays a prominent role in diverse motor, cognitive and emotional activities (Callan & Manto, 2013; Grimaldi & Manto, 2012; Stoodley & Schmahmann, 2010). It is part of a network providing pathways to other centres in the brain and to the spinal cord. One prominent pathway originating at the cerebellum is the cerebello-thalamo-cortical pathway (also called dentato-thalamocortical pathway). It connects the dentate nuclei of the cerebellum via the contralateral thalamus with the supplementary motor area, M1 and associative areas in the cortex (Callan & Manto, 2013; Jürgens, 2002; Mariën et al., 2014). The planning of a specific motor movement has its origin in the limbic system and is subsequently transported to associative areas and M1, then reaching the pons and via pons the cerebellum (Trepel, 2004). From the cerebellum, the cerebello-thalamo-cortical pathway leads the afferent projections (e.g. corrections of the planned specific movement) to the motor cortex and afterwards to the spinal cord where

the controlled movement is executed (Trepel, 2004). In parallel, the basal ganglia pathway is controlling the extent of the movement, so that all of these pathways and loops provide a secure control of planned motor movements in humans. This automated cerebello-thalamo-cortical pathway of information processing has the advantage that motor and cognitive controlling can be executed more precisely with less effort (Callan & Manto, 2013).

Different conditions of speech like the preparation, initiation and coordination of articulation are also controlled via the cerebello-thalamo-cortical pathway (Callan & Manto, 2013). The clarity of speech is dependent on the cerebellar control of the sensorimotor functions of the vocal tract (Mariën et al., 2014). Retrospectively, the existence of a cerebellar dysfunction due to haemorrhage and cessation of stuttering in a person who stuttered before the haemorrhage seems plausible.

In previous studies investigating PDS, the cerebellum presented anomalous neuronal activities or divergent white matter integrities, pointing towards its possible character as a neural correlate of stuttering. In fMRI studies, persons who stutter (PWS) showed an abnormal activation of the bilateral cerebellum which was not present in healthy controls but was normalised after stuttering therapy (De Nil, Luc F. et al., 2003). In addition, Lu and colleagues (2012) found that PWS showed an increased RSFC in the cerebellar vermis that was also normalised following therapy. Yang and collaborates were able to proof a lower RSFC between left and right cerebellar regions in PWS – they hypothesised that PWS might therefore present less integration of cerebellar motor control and high-level execution functions (Yang et al., 2016). A reduced RSFC between the left cerebellar lobule VI and right BA 4/6 was also found in PWS in comparison to fluent control participants. Additionally, a reduced RSFC was reported between the right cerebellar lobule VI and the bilateral middle frontal gyrus in PWS (Yang et al., 2016), pointing towards a changed cerebello-thalamo-cortical pathway in PWS compared to healthy controls. Sitek and colleagues (2016) were able to show an alteration of the

cerebello-thalamo-cortical pathway – PWS showed a stronger RSFC between the cerebellum and thalamus than healthy control participants. Concerning white matter integrity in the cerebellum, Chang and colleagues (Chang et al., 2015) provided evidence that CWS (CWS) have an increased fractional anisotropy (FA) in the cerebellum that is associated with the organisation of sequential movements – the authors interpreted this FA increase as a compensation process which had its origin in the onset of stuttering. In contrast, Lu et al. (2010) found a reduction of white matter in the bilateral cerebellum in PWS. Also Connally and colleagues (2014) revealed a decreased white matter integrity in PWS that was located in the three pairs of cerebellar peduncles. The previously described outcomes underline the possibility that the cerebellum might be influential on speech fluency and therefore present a prominent factor in the pathogenesis of stuttering.

The central aim of this study is to elucidate neurophysiological processes which are responsible for the cessation of stuttering in a case of haemorrhage in the left cerebellum. To integrate the previously described knowledge of cerebellar anomalies in PWS and to compare the changes in brain activity and white matter integrity in our single case with a reference group, we added one control group of fluent speaking participants and another control group of stuttering participants to our case study. We conducted functional and diffusion MRI to investigate brain activation as well as white matter structure and plasticity. For functional MRI, we evaluate changes between PRE (before haemorrhage) and POST (after haemorrhage) measurement in the single case and the control groups globally as well as in our region of interest (ROI) BA 44. BA 44 represents Broca's area and is highly involved in motor speech processing. As it is therefore considered to be a neural hallmark of stuttering (Neef et al., 2016), this ROI is integrated into our fMRI analysis. Concerning the DTI analysis, we were specifically interested in which regions the integrity of white matter changed in the single case patient in comparison to the healthy control group.

Four research questions were determined:

1. Which anatomical structures were damaged by the lesion and might have led to the cessation of stuttering?

2. How do speech-related brain activations of the patient differ

a. before and after haemorrhage?

b. in comparison to healthy and stuttering control groups?

3. How does Fractional Anisotropy (FA) of the patient differ before and after haemorrhage in comparison to the healthy controls?

4. Which neurophysiological mechanisms can be responsible for the cessation of stuttering after cerebellar haemorrhage?

4.2 Material and methods

4.2.1 Case presentation

Five months after the patient had participated in a brain imaging study in our centre, the 52 years old female office clerk with PDS since childhood developed progressive hearing difficulties on the left side, and intermittent left temporal headache. At presentation in an external hospital, hypoacusis on the left side, and an unsteadiness of tandem gait was observed. A left acoustic neuroma was detected using MRI, and subsequently operated. Immediately postoperatively, she presented with a left facial paresis, and a secondary clinical worsening related to a left cerebellar haemorrhage. This worsening included the compression of the IVth ventricle and a hydrocephalus, requiring emergency surgical revision and implantation of an external ventricular drain with an intracranial pressure monitoring device. The drain and the monitoring device were removed 20 days later. Finally, she was discharged to a rehabilitation unit wheel-chair bound with a flaccid, left dominant tetraparesis, with permanent nausea and repeated vomiting. Because of

intermittent episodes of hallucinosis she was transiently on olanzapine 5 mg once a day. In the long term outcome, she was able to walk independently with a stick, some mild imbalance, hypakusis on the left side, and a remaining dysarthria, normal swallowing, normal coughing. A follow-up MRI confirmed a left cerebellar lesion (see Figure 26) and pontine microlesions bilaterally, presumably of vascular origin. Surprisingly, the patient's long-term childhood stuttering had ceased after surgery, and never reappeared to this day.

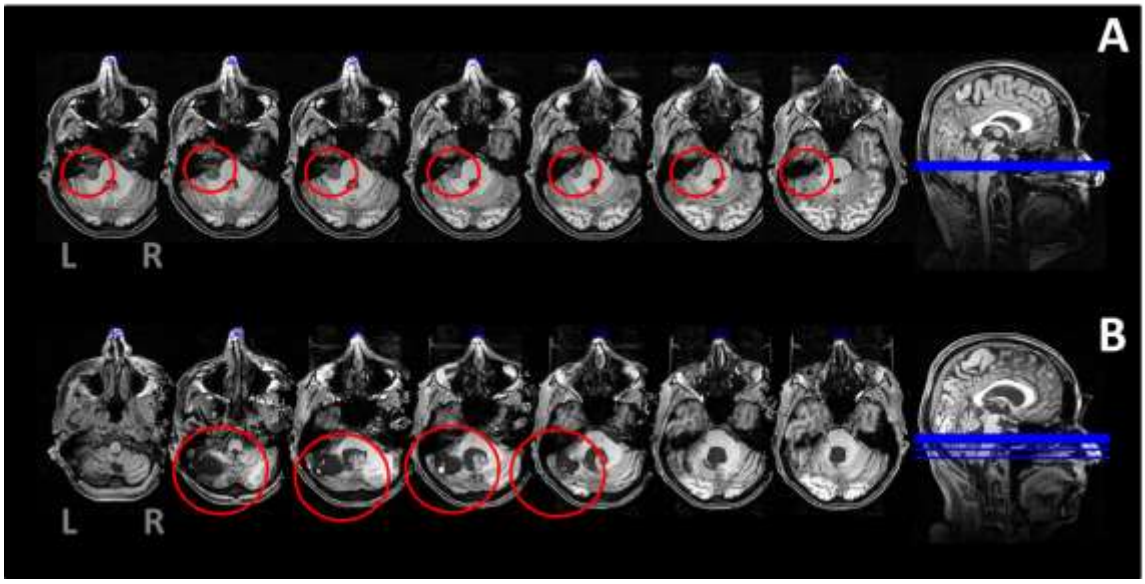


Figure 26. Case presentation. A (acoustic neuroma before surgery); B (cerebellar lesion after haemorrhage).

4.2.2 Study design and participants

The patient had taken part in one of our ongoing brain imaging studies on PDS 5 months before surgery (PRE). When she described her cessation of stuttering in the magazine of the German Stuttering Association, we became aware of her clinical course and invited her to repeat the entire examinations once again. This enabled a pre-post comparison and allowed a better insight into mechanisms of her recovery from stuttering. 36 months after the initial examination, i.e. 31 months after surgery and 27 months after discharge from a rehabilitation unit, we repeated the entire examination (POST). Written

informed consent was obtained from the patient for conduction of the second measurement (POST) in addition to the previously obtained informed consent (PRE).

Both PRE- and POST-measurement comprised a speech-fluency assessment with the Stuttering Severity Instrument 4 (SSI-4; Riley, 2009) the Edinburgh Inventory of Handedness (Oldfield, 1971) and the German version of the Overall Assessment of the Speaker's Experience of Stuttering (OASES; Yaruss & Quesal, 2006) as well as structural and functional magnetic resonance imaging.

For POST, additional measures included a dysarthria examination (Frenchay Dysarthrie Assessment – 2 (FDA-2); Enderby & Palmer, 2012), a medical neurological screening, a neuropsychological assessment probing the cognitive performance, the Beck Depression Inventory (BDI; Beck et al., 1961) and the WHO's Well-Being Index (WHO-5; Wit et al., 2007).

In order to investigate the effects of the cerebellar lesion on *brain activity*, we compared the patient's fMRI data with a group of native German speaking healthy, fluent-speaking controls and stuttering control participants. We included the imaging data of 22 healthy controls measured once (15 females; 20-57 years, mean age 35,4 years) and 7 stuttering controls (2 females; 19-51 years, mean age 32.9 years); they were measured twice with a mean time interval of 36.2 months between pre- and post-measurement (SD = 7.6) with the same fMRI paradigm used in this single case study. This data was obtained in an earlier study (see Bütfering, 2015; Neef et al., 2016); the seven stuttering controls took voluntarily part in the second measurement.

To explore the effects of the patient's lesion on *brain structure*, we compared the patient's fibre tracts with a group of native German speaking healthy controls. We therefore included 21 healthy controls (2 females; 18-34 years, mean age 25.5 years), measured twice with a mean interval of 11.5 months between pre- and post-measurement (SD = 0.81). This control group was taken from a recent study from our

research group (Primaßin, 2019, chapters 2 and 3; Sommer & Primaßin, 2017, 2018). None of the control groups' participants showed any neurological or psychiatric disorders beside of fluency disorder stuttering in the stuttering control group; all participants provided general MRI compatibility and gave their written informed consent prior to participation in the studies. The ethical committee of the University Medical Center Göttingen approved these mentioned studies in accordance with the Declaration of Helsinki.

4.2.3 fMRI experimental procedure

The experimental procedure is described in detail elsewhere (Neef et al., 2016). In brief, we used a speech motor imagery as well as melody motor imagery paradigm in which the patient had to name the month of the year or to hum the melody from a serenade covertly. The paradigm was chosen to avoid an activation bias seen in overtly speaking paradigms. During overt speaking, a major part of activity is found in motor speech processing brain areas – this might complicate imaging analysis. To circumvent these straits, the covert speaking paradigm was applied.

4.2.4 MRI acquisition

We used a 3 Tesla MR system (Siemens Magnetom Tim Trio, Siemens Healthcare, Erlangen, Germany) as well as a standard 8-channel phased-array head coil. Participants were placed into the scanner in supine position. They wore headphones for noise protection and MR-compatible LCD goggles (VisuaStim XGA, Resonance Technology Inc., Northridge, CA, USA).

First, a T1-weighted anatomical 3D turbo fast low angle shot FLASH sequence was accomplished (repetition time (TR) 2250 ms, inversion time (TI) 900 ms, echo time (TE) 3.26 ms, flip angle = 9°, voxel size 1x1x1 mm^3).

Second, we applied a fluid-attenuated inversion recovery (FLAIR) sequence (TR 5000 ms, TI 1800 ms, TE 294 ms, voxel size 1x1x1 mm^3, sagittal orientation).

Third, a total of 578 volumes (voxel size 3x3x3 mm^3, field of view 192 mm, 33 slices, 20% gap) were acquired for blood oxygen level-dependent fMRI with a gradient-echo echo-planar imaging (EPI) sequence (TR 2000 ms, TE 30 ms, flip angle 70°) over two functional runs (24 trials per condition). Originally, three functional runs were recorded in every study participant. Due to the fact that the single case patient showed artefacts in the 1st functional run at PRE measurement, we excluded this run from the analysis in all participants.

Furthermore, a gradient echo sequence for field mapping was acquired (36 slices, voxel size 3x3x3 mm^3, field of view (FOV) 192 mm, TR 488 ms, TE 4.92/7.38 ms, flip angle 60°).

Diffusion-weighted images were carried out using spin-echo EPI with 64 diffusion sensitized gradient directions (TR interval 10100 ms, TE 93 ms, b-values 0,1000 s/mm^2, 74 axial slices, voxel size 1.9x1.9x1.9 mm^3, phase-encoding in anterior-to-posterior direction). In the group of healthy controls, we additionally acquired one volume without diffusion-weighting and opposite phase encoding direction (posterior-to-anterior) to conduct a TOPUP correction (correction of the distortion caused by magnetic field inhomogeneities; Andersson et al., 2003).

4.2.5 Data analyses

Structural Analyses - Lesion analysis of the FLAIR sequence

For the lesion analysis, the lesion was marked within the FLAIR image using FSLview (Smith et al., 2004). Then, the lesion map as well as the FLAIR image were normalised and transformed into MNI standard space with FNIRT, using the patient's T1 image and a linear (FLIRT) T1-to-MNI transformation matrix (Andersson, Jenkinson, & Smith, 2010; Jenkinson & Smith, 2001). The anatomical masks of interest which were provided by FSL's atlases were extracted and binarised, no thresholding was applied. Afterwards, the size of all atlas masks as well as the lesion mask were defined by counting their non-

zero-voxels. Then, an intersection of each atlas mask of interest with the patient's lesion mask in standard space was produced by multiplying the masks with each other via fsmlaths (FSL command line tools; Jenkinson & Smith, 2001). The outcome was a map representing the impairment of the specific atlas structure caused by the patient's lesion. Again, the size of this map was defined by counting all non-zero voxels inside. Following, we calculated the percentage of the anatomical structure damaged by the lesion: we divided the voxel count of the intersection by the voxel count of the anatomical map.

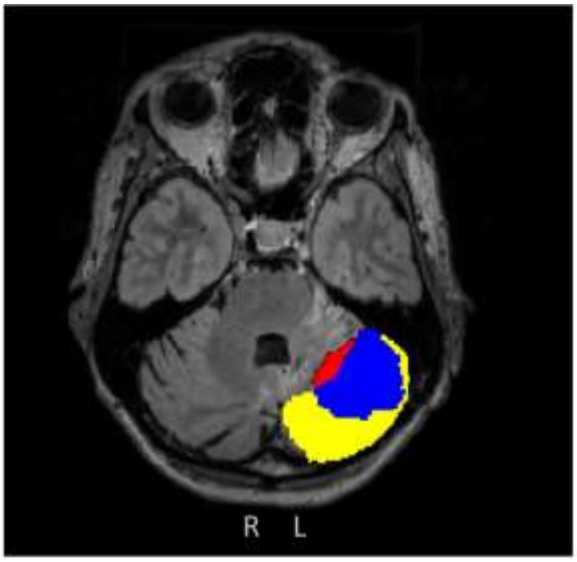


Figure 27. Procedure of lesion analysis. Yellow (Atlas structure ‚left crus I', Probabilistic cerebellar atlas with non-linear registration; (Diedrichsen, Balsters, Flavell, Cussans, & Ramnani, 2009); red (lesion mask); blue (intersection of lesion and atlas structure ‚left crus I').

The structural map of the dentate nucleus was not provided by FSL's atlases. To check if this structure was also damaged by the lesion, we used the dentate nucleus coordinates of Dimitrova and colleagues (2006). They studied the MRI anatomy of the dentate nucleus and interposed nuclei in a group of healthy participants by creating ROI masks for every individual participant's dentate nucleus, transforming these ROI masks to standard space and overlapping them. Afterwards, they present different percentage maximum ROI overlaps and the corresponding MNI coordinates for maximal ROI extension of the dentate nucleus. We took the maximal x, y and z coordinates in MNI

space for the 61% - 70% ROI overlap for the left dentate nucleus (maximum overlap percentage in the study of Dimitrova et al., 2006). With the FSL command line tool fslmaths we created a sphere of 1 mm around these MNI coordinates of maximal ROI overlap (Coordinate 1: x -18 y -63 z -40; Coordinate 2: x 19 y -55 z -36) and checked if these coordinates are within the lesion of our patient.

Structural Analyses - Analysis of diffusion data

After checking for artefacts in the DTI data, we used the FMRIB's Diffusion Toolbox (Smith et al., 2004; Smith et al., 2006; Woolrich et al., 2009; www.fmrib.ox.ac.uk/fsl) for all steps of analysis.

In the single case patient, we created a fieldmap and inserted it into FUGUE (Jenkinson & Smith, 2001) to correct for distortion due to magnetic field inhomogenities. Afterwards, we applied EDDY to correct for eddy current distortions and movement artefacts.

Concerning the group of healthy controls, the distortion due to magnetic field inhomogeneities in the DTI data was corrected with TOPUP (Andersson et al., 2003). Here, an additional dataset without diffusion-weighting and opposite phase encoding direction is used, resulting in pairs of images with distortions going in reverse directions. From these pairs the susceptibility-induced off-resonance field was estimated and applied to correct the magnetic field inhomogeneities of the DTI dataset. In TOPUP, we also created a brain extracted, undistorted mask for the performance of EDDY, where we corrected for head motion and eddy current artefacts.

Next, we ran DTIFIT for the single case patient as well as for the healthy controls to fit diffusion tensors to the data, resulting in the FA images for each participant.

In the following, Tract-Based Spatial Statistics (TBSS; Smith et al., 2006; Smith et al., 2007) of the patient as well as the control group were conducted as recommended in the FSL guidelines. In brief, FA images were preprocessed and registered to the

FMRIB58_FA template provided by the FMRIB's Diffusion Toolbox to produce a mean FA skeleton in the MNI152 standard space. Pre and post-specific FA values were projected onto this mean FA skeleton to explore variations in FA at both points of time. Here, a FA threshold of 0.2 was used to exclude non-white matter from analysis. We checked all transformed FA images for correctness because the lesion of the patient could cause non-reliable warping-effects by transforming the patient's brain to the standard template. For statistical analysis, we applied permutation based statistics within the white-matter skeleton using FSL's RANDOMISE command. In RANDOMISE, we performed a general linear model (GLM) along the WM skeleton, where we compared the patient against the group of controls (contrast 1: patient > group mean, contrast 2: patient < group mean). Threshold-free cluster enhancement (TFCE) and a variance smoothing of 2 mm were used. If voxel in white-matter survived the family-wise error (FWE) correction for multiple comparisons, they are reported with $p<0.05$. If no clusters survived, we reported results uncorrected at the described significance level.

Functional Analyses

Before starting the analyses procedure, we checked all data for movement artefacts and other abnormalities and removed images with translational movements > 3 mm. Then, we used the Statistical Parametric Mapping software (SPM8; Wellcome Trust Centre for Neuroimaging, UK), running within Matlab 2012a (The MathWorks, Inc., Natick, MA, USA). Preprocessing consisted of realignment and unwarping, corrections for the differences in slice time acquisition, normalisation into standard space (EPI template of the Montreal Neurological Institute (MNI)) as well as spatial smoothing with an isotropic Gaussian kernel of 9 mm full-width half-maximum. For the 1st level statistical analysis of the functional images, the onsets and durations of the experimental conditions were modelled by the convolution with a hemodynamic response function accounting for the delay of the blood oxygenation level dependent (BOLD) response. Additionally, movement parameters (three translation parameters and three rotation parameters),

extracted during realignment, served as covariates of no-interest. For each subject, statistical images were computed for the contrasts “covert humming vs. rest” and “covert speaking vs. rest”.

For comparing the activation of the single case patient with both control groups in the chosen ROI BA 44, we extracted beta values of the contrasts “covert humming vs. rest” and “covert speaking vs. rest” to evaluate changes of functional activity over time in our ROI. The MarsBaR Toolbox (Brett, Anton, J. L., Valabregue, & Poline, 2002) was applied for that purpose. We fed the ROI of BA 44 (Anatomy Toolbox; Eickhoff et al., 2005) into MarsBaR and extracted mean beta-values in this ROI for the single case patient and both controls groups in each contrast.

To elucidate changes of functional activity globally throughout the whole brain, we created difference images (POST-PRE) in the group of the 7 stuttering participants. Therefore, we calculated a paired T-Test within the contrasts “covert humming vs. rest” and “covert speaking vs. rest” ($p<0.05$, uncorrected for explorative purposes). A similar procedure was used for the single case patient: we created difference images for each contrast “covert humming vs. rest” and “covert speaking vs. rest”. But here we subtracted the voxelwise T-statistic maps automatically created in SPM8 in the 1st level analysis to investigate changes from PRE to POST measurement in each contrast using the ImCalc Toolbox. Then we applied the same corresponding height threshold of p_uncorr <0.005 from the contrast “covert speaking > rest” (T-Value of 2.58) as a significance threshold. The binarised maps (POST-PRE covert humming, POST-PRE covert speaking) were afterwards plotted on an MNI152 standard brain with MRIcron (Rorden & Brett, 2000) for the single case patient and the stuttering controls, respectively.

4.3 Results

Speech Fluency

In the single case, the SSI-4 overall score (Riley, 2009) was 14 at PRE and dropped to 6 POST (see Table 2). At the second assessment, no stuttering could be diagnosed according to the percentile ranks and severity equivalents of the SSI-4. The remission was also confirmed by the patient's hometown, long-standing speech and language therapist.

Table 19. Overview on the results in the SSI-4 (Riley, 2009).

SSI-4 results	PRE	POST
Frequency of stuttering (score)	5	4
Duration (score)	6	2
Physical Concomitants (Score)	3	0
Overall Score	14	6
Percentile	5-11	<1
Severity	very mild stuttering	no stuttering

Handedness

At PRE, the outcome of the Edinburgh Handedness Inventory (Oldfield, 1971) was +100, which means 100% right handed. At POST, the handedness score was +80.

OASES

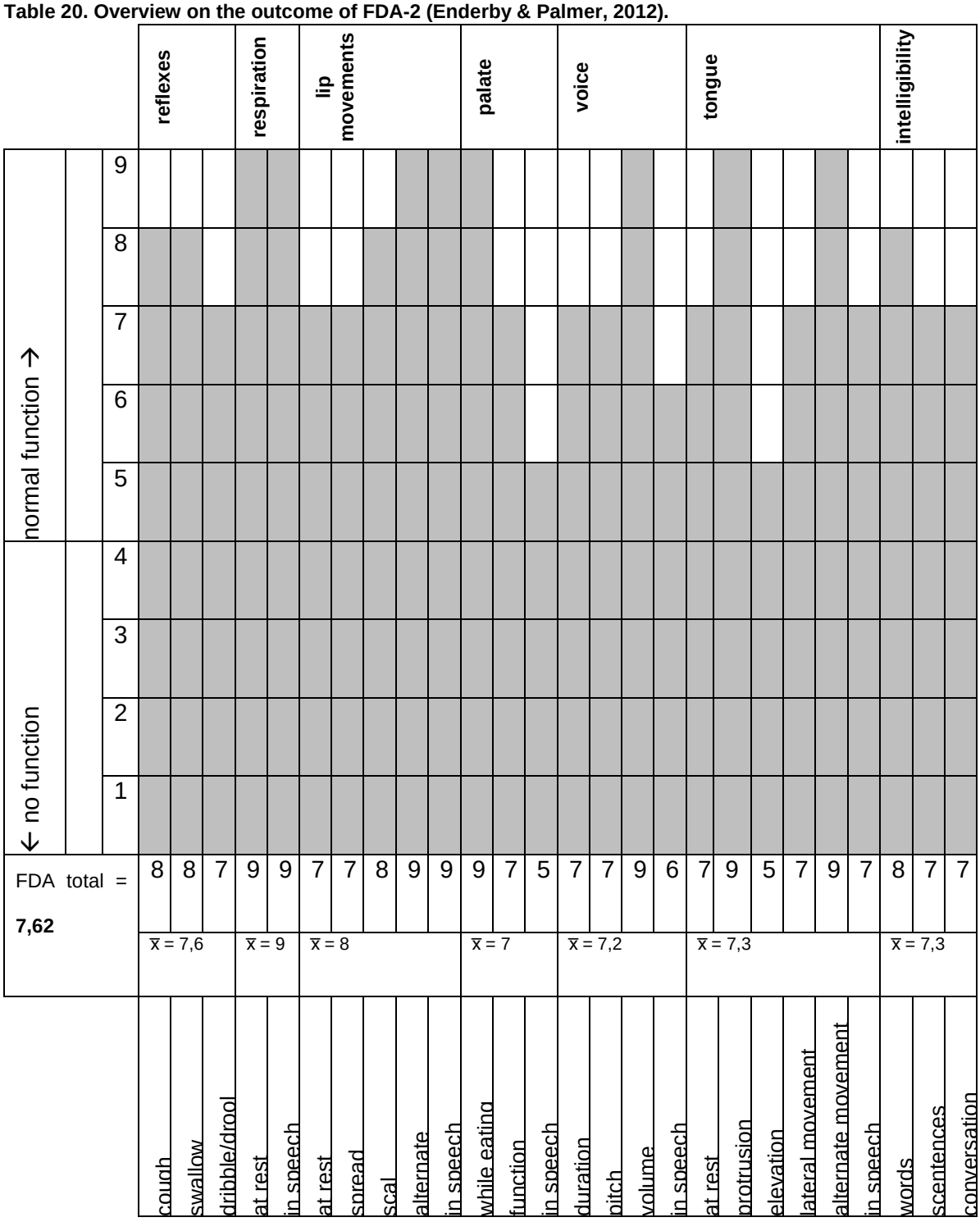
The OASES (Yaruss & Quesal, 2006) Overall Impact Score at PRE-measurement was 3.26, which represents a moderate to severe impact of stuttering. At POST, the OASES was not evaluable due to the fact that the patient was not capable of answering many questions related to attitudes and habits towards stuttering. The patient stated that she did not feel as a stuttering person any longer and had no experience with stuttering anymore because it had disappeared after the cerebellar haemorrhage.

Dysarthria

The scores the patient obtained in the FDA-2 (Enderby & Palmer, 2012) at POST (for an detailed overview, see Table 3) represented some typical symptoms of an ataxic dysarthria.

During spontaneous speech, *phonation* was hoarse and pressed; it faded sometimes, so the quality of voice was inconsistent. In addition, the intonation was monotonous. Concerning *respiration*, the outcome of the applied examinations was nearly physiological; an exception was the respiration during fast speaking and reading; here, the coordination between respiration and phonation was not working properly and the patient showed an increased rate of inspiration. In reference to *articulation*, movements of speech were chanted and slower, the diadochokinesis of complex syllables was impaired. The *intelligibility* of the patient was quite good in large parts of conversation and testing. Concerning *anatomic structures in rest*, a mild facial paralysis (left side of the face) and a mild frailty in the left side of the palate were presented; *in function*, only mild deviations and insecurities were shown altogether. Impaired movements were mostly seen in the motor tasks for the tongue. Alternating tongue movements were insecure; the elevation of the tongue was hardly feasible.

Table 20. Overview on the outcome of FDA-2 (Enderby & Palmer, 2012).



Note. Illustration based on the layout of Eigentler and colleagues (Eigentler et al., 2012). FDA-2 result of the single case patient POST. The FDA-items (reflexes, respiration, lip movements, jaw, palate, voice, tongue and intelligibility) describe orofacial anatomical structures and (speech) functions. The items are rated due to different conditions and tasks (for example: the item "reflexes" is partly rated by judging the patient's ability

to cough properly). Furthermore, the arithmetic mean is calculated for each task or condition (sum of all tasks or conditions per item / amount of all tasks or conditions per item). The rating scale reaches from 0 points (highly pathophysiological) to 9 points (normal function).

Neurological Examination

At POST, the neurological examination suggested evidence of cerebellar impairment (finger-to-nose and heel-knee-shin tests ataxic on the left side), but also brain stem involvement (gaze elevation palsy of the left eye, disturbed vestibulo-ocular reflex when the head turned to the left) and afferent ataxia (sensation of position of the left extremities reduced).

Neuropsychological Examination

Regarding the estimated premorbid intellectual capability, the patient showed deficits in the following domains when tested POST: psychomotor- and processing speed, shared attention, storing of new visual material and reactive cognitive flexibility (formal-lexical word fluency). These results illustrate a particularly frontal, functional deficit within the scope of an acquired organic psychosyndrome.

BDI

At POST, we applied the BDI (Beck et al., 1961) to test if there were any hints for a possible depressive mood. The outcome was 28 points, this corresponds to a moderate depression (the BDI score is clinical relevant ≥ 18 points).

WHO-5

Concerning the WHO-5-scale (Wit et al., 2007), the patient presented a raw score of 1, pointing towards a very low quality of life at POST.

Structural MRI

FLAIR and T1

At PRE, the T1 data set showed a clear definable acoustic neuroma, which was covering the left vestibular nerve; no additional abnormality was detected (see Figure 26). POST, the patient presented with an extensive lesion comprising approximately 1/5 of the left cerebellum in both T1 and FLAIR images (set out in Table 21, Figure 26). The largest relative amount of damage is detectable in Crus I in the left cerebellar hemisphere (46.93% damage), followed by Crus II (34.33% damage) and VIIb (33.56% damage; see Table 21). The cerebellar vermis is almost not affected by the lesion. Concerning the cerebellar peduncles, solely the middle peduncle is impaired (14.7% damage).

Table 21. Outcome of lesion analysis.

Localisation	Atlas structure	Number of voxels in Atlas structure	Number of voxels in inter-section (lesion mask x Atlas structure)	Relative proportion of lesion in atlas structure (percentage)
Left cerebellum	Left Cerebellum – whole	124715	27232	21,84
Left cerebellum - Subparts	I-IV	15248	0	0,00
	V	19993	385	1,93
	VI	31574	7909	25,05
	Crus I	39313	18451	46,93
	Crus II	38352	13167	34,33
	VIIb	27215	9132	33,56
	VIIIa	23280	5547	23,83
	VIIIb	17681	1124	6,36
	IX	13872	403	2,91
	X	5136	7	0,14
Cerebellar vermis	Crus I	1767	0	0,00
	Crus II	3440	0	0,00
	VI	9004	0	0,00
	VIIb	3289	1	0,03
	VIIIa	5830	32	0,55
	VIIIb	4466	42	0,94
	IX	4662	17	0,36
	X	2085	0	0,00
Cerebellar peduncles	Inferior cerebellar peduncle	968	0	0,00
	Middle cerebellar peduncle	15644	2299	14,70
	Superior cerebellar peduncle	992	0	0,00

Note. Atlas structures were extracted from FSL (JHU white-matter tractography atlas; (Mori et al., 2005); Probabilistic cerebellar atlas with non-linear registration; (Diedrichsen et al., 2009) and afterwards binarised.

Both maximal coordinates of Dimitrova and colleagues (Dimitrova et al., 2006) showing an 61% - 70% ROI overlap for the left dentate nucleus are localized in the patient's lesion (see Figure 28, Figure 29), pointing to a high probability that the dentate nucleus is also affected by the lesion.

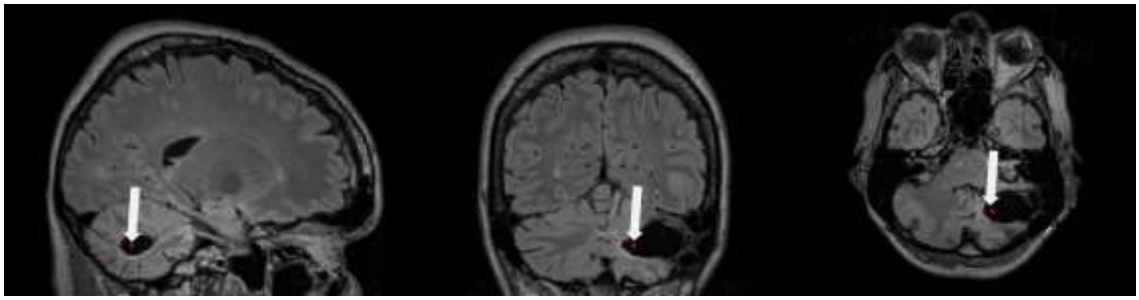


Figure 28. C1 Coordinate of Dentate Nucleus (x -18 y -63 z -40) by Dimitrova et al. (2006) with a sphere of 1 mm in the patient's lesion.

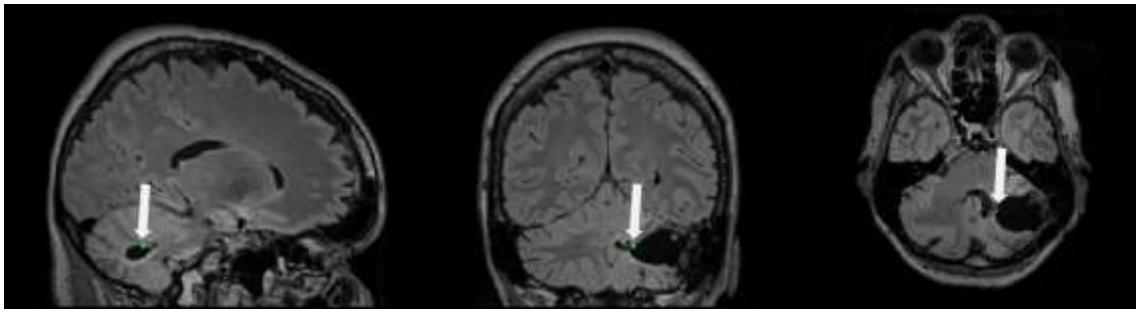


Figure 29. C2 Coordinate of Dentate Nucleus (x 19 y -55 z -36) by Dimitrova et al. (2006) with a sphere of 1 mm in the patient's lesion.

DTI

The TBSS analysis showed a significant fibre decrease in the patient compared to healthy controls (contrast 2: patient < group mean; p <0.05, FWE) from PRE to POST in several brain areas.

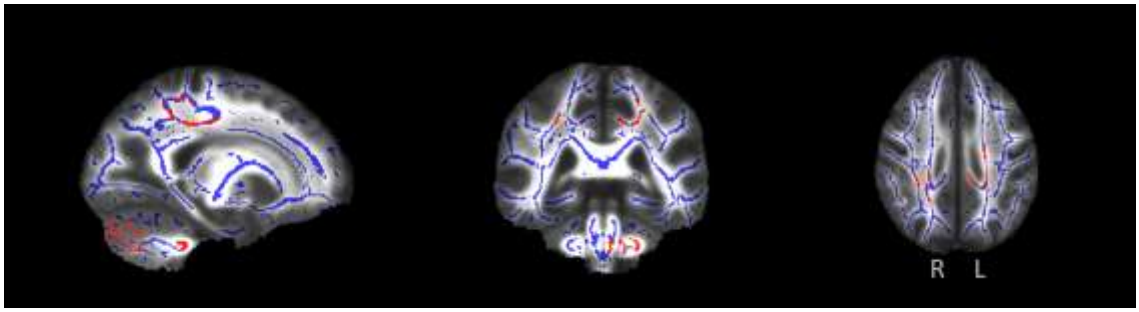


Figure 30. White matter decrease observed in the patient compared to healthy controls - whole brain analysis. Blue (white matter skeleton), red (significant decrease).

On the one hand, areas became significant which arose directly from the lesion itself (e.g. Crus I and Crus II of the left cerebellar hemisphere and the middle cerebellar

peduncle). On the other hand, areas primarily not being located within the lesion showed a significant FA decrease (corpus callosum, right inferior fronto-occipital fasciculus, left anterior thalamic radiation, left cingulum, right posterior corona radiata, see Figure 30, Table 22).

Table 22. Significant cluster of TBSS analysis.

Cluster	Voxels	MAX	MAX X (mm)	MAX Y (mm)	MAX Z (mm)	JHU WM Tract	ICBM WM Labels	Cerebellar FNIRT
9	1900	0,955	-20	-71	-39			2% Left Crus I 44% Left Crus II
8	988	0,955	-6	-20	25		Body of Corpus Callosum	
7	512	0,955	25	-48	31	13% Inferior fronto-occipital Fasciculus R		
6	486	0,955	-10	-34	-41	3% Anterior thalamic radiation L 11% Corticospinal tract L	Middle cerebellar peduncle	
5	278	0,955	-6	-29	23	3% Anterior thalamic radiation L	Body of Corpus Callosum	
4	134	0,955	-10	-50	-49			5% Left VIIIb 67% Left IX
3	109	0,955	-19	-62	-29			42% Left VI
2	93	0,955	-13	-53	53	3% Cingulum (cingulate gyrus) L		
1	15	0,955	20	-30	38		Posterior corona radiata R	

Note. Significant decrease in patient's FA values from pre to post. Outcome of an randomise analysis with FSL; conducted with TFCE, FWE, $p<0.05$. MAX (significance level, p = 1-MAX); MAX X – MAX Z (Coordinates in mm); JHU WM Tract (JHU white-matter tractography atlas (Mori et al., 2005)); ICBM WM Labels (ICBM-DTI-81 white-matter labels atlas (Mori et al., 2005)); Cerebellar FNIRT (Probabilistic cerebellar atlas with non-linear registration (Diedrichsen et al., 2009)). Including clusters >10 voxel.

Functional MRI

The patient's brain activation changed from PRE to POST. The most pronounced activation difference was evident in the speaking condition – at POST compared to PRE; there was an increase of activation in parietal and temporal areas; but less activation in frontal areas (see Figure 31). In comparison, during humming, the difference map revealed smaller activation differences between both points of time. An overlap of the differences from PRE to POST between humming and speaking condition was mainly shown in motor regions (see Figure 31).

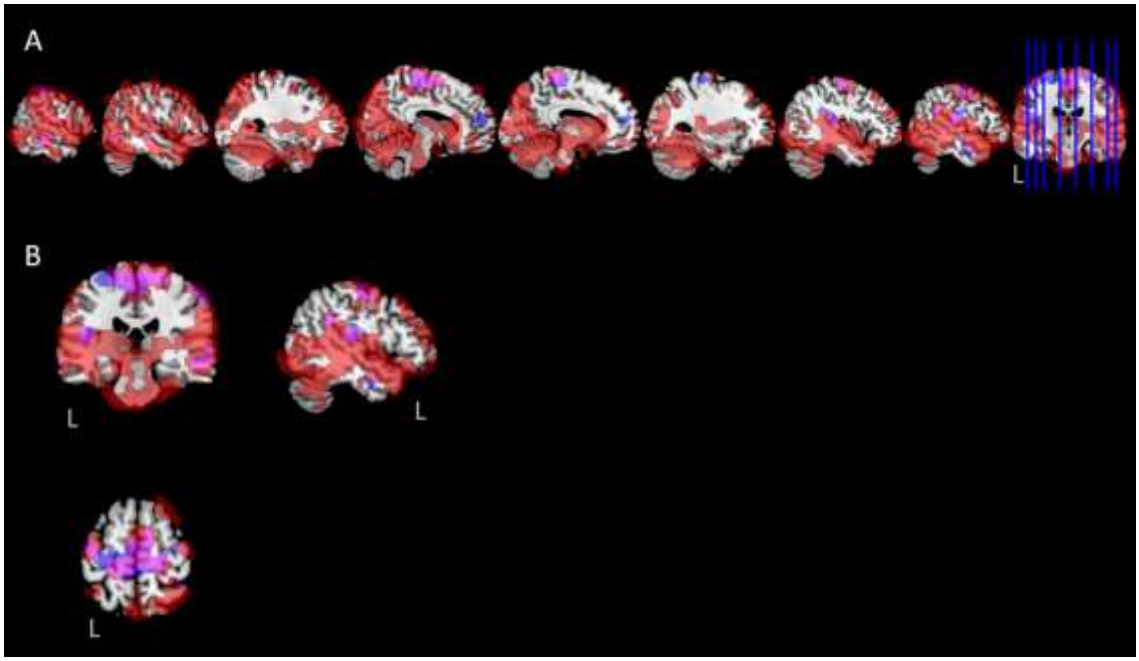


Figure 31. Difference maps POST- PRE, Single Case Patient. (A) Multislice presentation in sagital view. (B) Sagittal, coronal and axial view. Covert speaking (red areas), covert humming (blue areas), overlap (purple).

As shown in Figure 32, the group of stuttering controls showed less difference in activation from PRE to POST in both conditions. Especially in the covert speaking condition, the controls showed less activation differences from PRE TO POST than the single case patient. A slight increase of activation was detected in small portions of the frontal lobe. An overlap of the difference maps of both conditions became again obvious in the primary motor area (M1), see Figure 32.

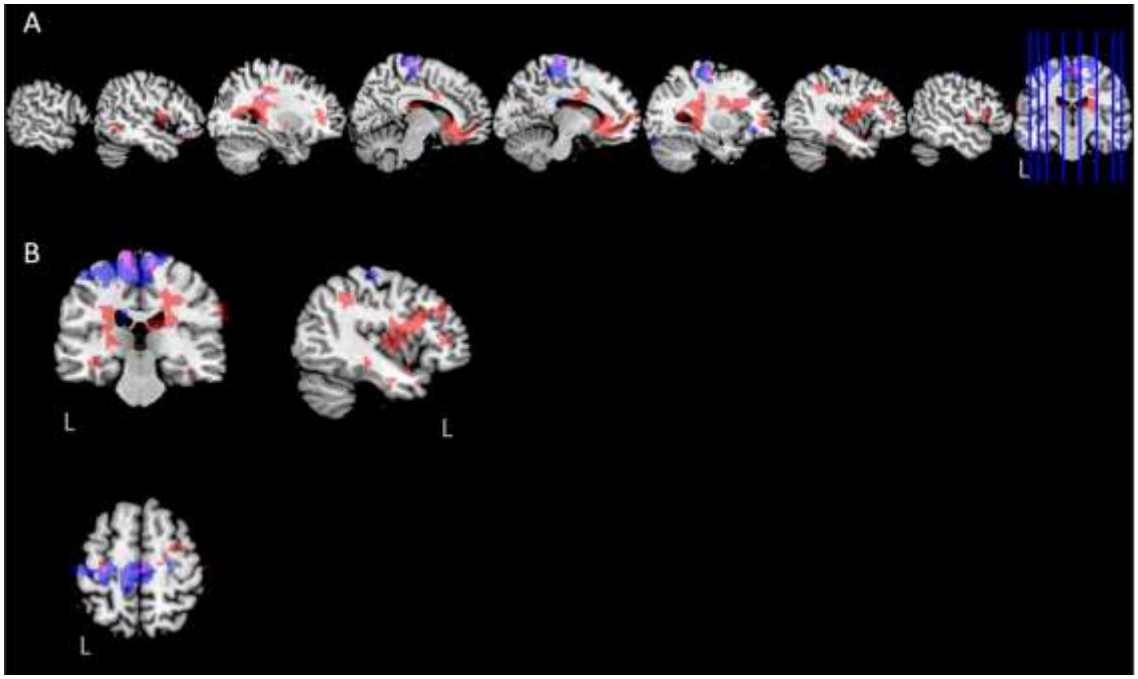


Figure 32. Difference maps POST- PRE, stuttering control group. (A) Multislice presentation in sagital view. (B) Sagittal, coronal and axial view. Covert speaking (red areas), covert humming (blue areas), overlap (purple).

To evaluate specific activation changes in our ROI BA 44, we compared the beta values of the patient's PRE and POST measurement with the beta values of healthy and stuttering controls. In the covert speaking condition, an increase of activation is present in the single case patient from PRE to POST (see Figure 33 and Figure 34). In left and right BA 44, this increase is even larger than the variance of the beta values within the stuttering control group (e.g. SC post, Figure 33 and Figure 34). In the right hemisphere, the POST beta value in BA 44 during covert speaking is sharply higher in the single case patient compared to healthy as well as stuttering controls PRE and POST.

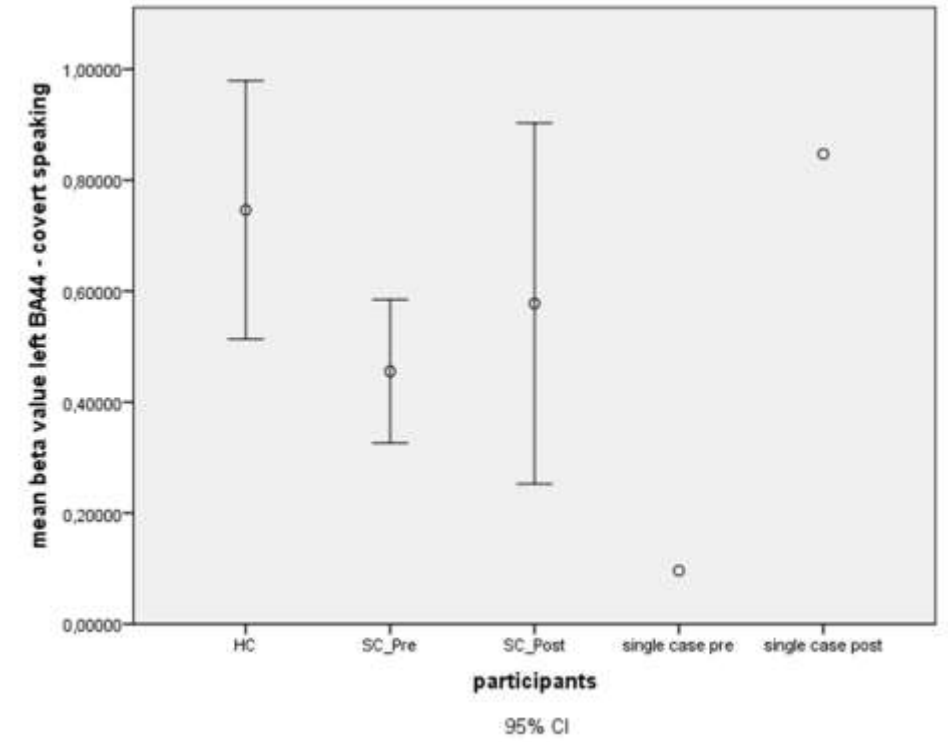


Figure 33. Mean beta value for left BA 44 - covert speaking. HC (healthy controls), SC_Pre (stuttering controls PRE), SC_Post (stuttering controls POST), CI (confidence interval).

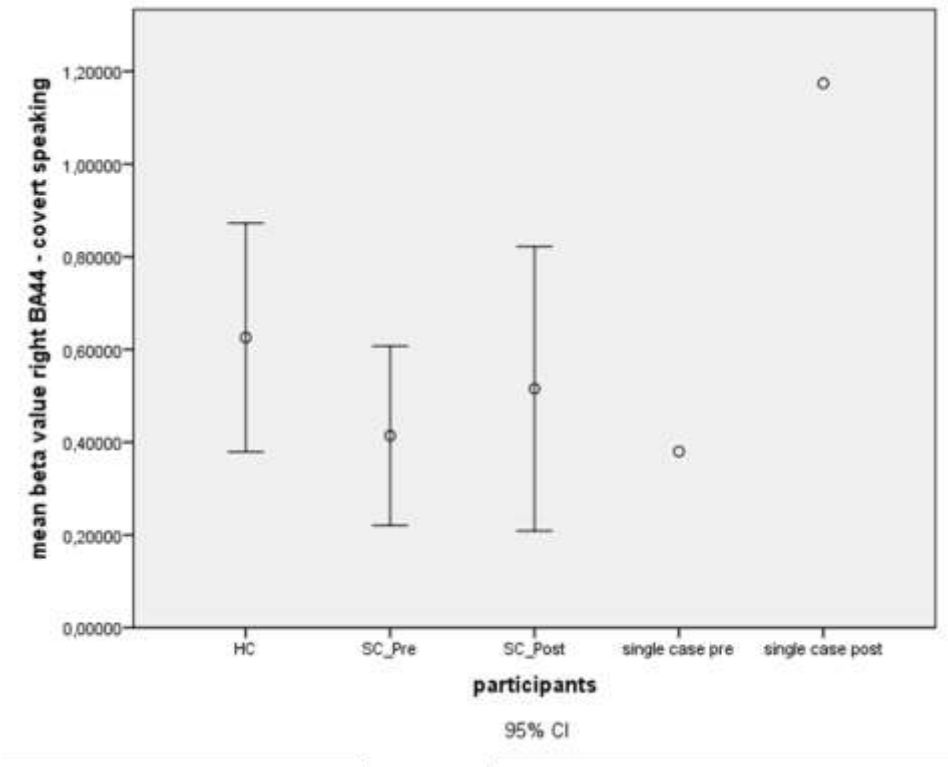


Figure 34. Mean beta value for right BA 44 - covert speaking. HC (healthy controls), SC_Pre (stuttering controls PRE), SC_Post (stuttering controls POST), CI (confidence interval).

Contrary to the speaking condition, a decrease of activation becomes evident from PRE to POST in the covert humming condition in left and right BA 44 (Figure 35 and Figure 36). The single case patient's decrease of activation in left BA 44 is larger than the beta values' variance within the group of stuttering control participants (e.g. SC pre; Figure 35). The activation during covert humming in left BA 44 is much lower in the single case patient POST than all mean beta values PRE and POST from healthy and stuttering controls.

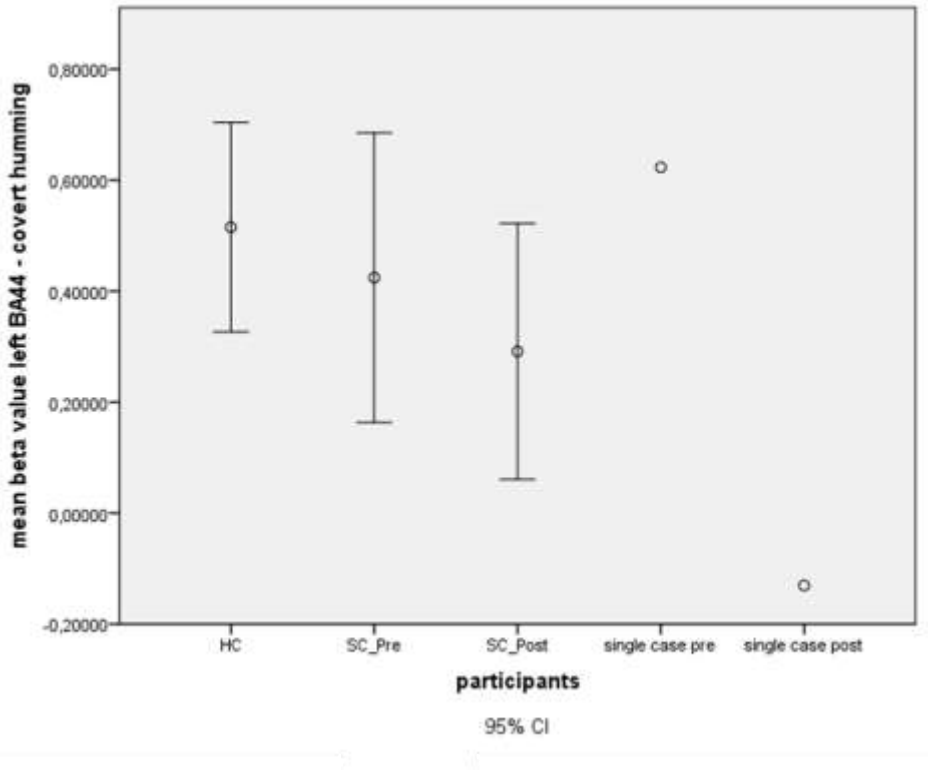


Figure 35. Mean beta value for left BA 44 - covert humming. HC (healthy controls), SC_Pre (stuttering controls PRE), SC_Post (stuttering controls POST), CI (confidence interval).

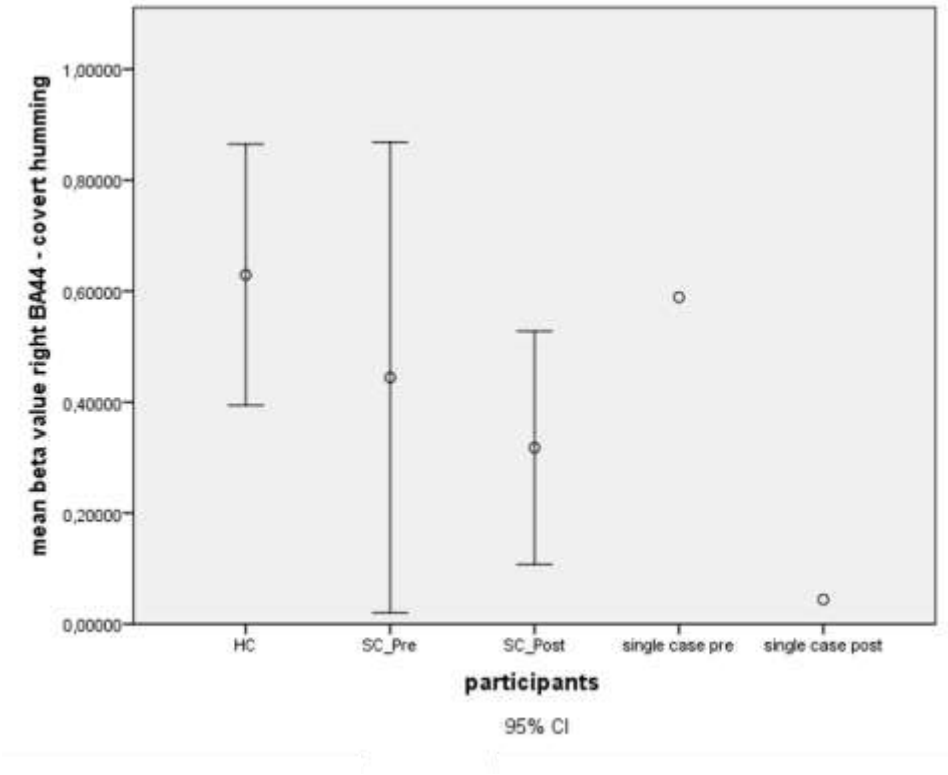


Figure 36. Mean beta value for right BA 44 – covert humming. HC (healthy controls), SC_Pre (stuttering controls PRE), SC_Post (stuttering controls POST), CI (confidence interval).

4.4 Discussion

The study presented here is one of the first investigations to explore the neurophysiological backgrounds of a cessation of persistent developmental stuttering in a patient with a left-cerebellar haemorrhage. Following, we discuss the research questions determined earlier.

4.4.1.1 Damage of anatomical structures and their possible impact on the cessation of stuttering

To summarize the outcome of the applied lesion analysis, approximately 1/5 of the left cerebellum was impaired by the lesion according to the structural images POST. While the cerebellar vermis was hardly affected by the haemorrhage, the cerebellar left lobules Crus I, Crus II and VIIb in the posterior part of the cerebellum showed the largest relative

damages caused by the lesion. But also in lobule VI that belongs to the anterior part of the cerebellum, 25% of the tissue was impaired by the lesion.

There is anatomical evidence for a structural dichotomy in the cerebellum (Grimaldi & Manto, 2012; Mariën et al., 2014; Neef et al., 2017; Stoodley & Schmahmann, 2010): the anterior lobe of the cerebellum, in most papers described as an entity of lobes I-V and lobule VI, is mainly processing sensorimotor functions, while the posterior lobe (VI, VII and VIIb, Crus I and II) coordinates higher level processes, cognition and emotion.

Because the main lesion of the patient is situated in the posterior lobe, the acquired organic psychosyndrome of the patient could be a sequelae of the impairment in lobules VIIb, Crus I and II. Especially the reported impairment of the patient's cognitive flexibility concerning lexical word fluency as well as attention and working memory is conclusive. It is in line with literature describing the function of the cerebellar posterior lobe in neurocognitive and affective processing (Stoodley & Schmahmann, 2009) as well as in working memory (Durisko & Fiez, 2010). The high BDI score as well as the low WHO-5 score, pointing to a low quality of life in the patient, might reflect the cognitive and emotional sequelae of the cerebellar lesion, too.

The damage caused by the haemorrhage in the anterior part of the cerebellum (lobule VI) is probably responsible for the increase of speech fluency: neuroimaging studies reported that articulation was localized in medial parts of lobule VI bilaterally (Carreiras, Mechelli, Estévez, & Price, 2007), linked to sensorimotor areas of the cerebral cortex. A damage to this important area might affect speech fluency and reduce stuttering symptoms. Disregarded the cessation of stuttering, the lesion of lobule VI was surely responsible for the atactic dysarthria the patient developed (Schoch, Dimitrova, Gizewski, & Timmann, 2006).

Further we could show that the dentate nucleus was affected by the lesion. The dentate nucleus, processing the greatest amount of efferent signals from the cerebellum, is activated in speech articulation (Thürling et al., 2011). Lesions in the dentate nuclei are related to an overshoot of the target as well as a decomposition of multi-joint movements (Bastian, Martin, Keating, & Thach, 1996; Thach, Goodkin, & Keating, 1992). It is discussable if an impaired dentate nucleus is able to facilitate fluency in a person who stutters, but the fact that it can trigger hypermetria or disassemble complex movements might be meaningful in the possible aetiology of PDS presumed by Etchell and colleagues (Etchell, Johnson, & Sowman, 2014). They assume that a neurophysiological deficit in stuttering consists of a deficit in brain timing networks and that a dysfunction in the internal timing might cause stuttering symptoms. In our single case patient, the cerebellar haemorrhage could have confounded the internal timing and sequencing of movements which probably has already been dysfunctional before due to the PDS. This confound could afterwards have led to an in-time motor speech articulation i.e. a cessation of stuttering.

Concerning the cerebellar peduncles, only the middle cerebellar peduncle was hit by structural damage. This specific peduncle is receiving the bulk of the afferent input directly from the cortical motor neurons. The information is then processed to the posterior cerebellar lobe and afterwards sent back to the contralateral motor cortex via dentate nucleus and then to the superior cerebellar peduncle (Connally et al., 2014). An impairment of the middle cerebellar peduncle could therefore be responsible for a loss of information concerning planned movements and in succession for a misguided control of planned motor movements. On the one hand, this might explain how the atactic dysarthria arose. On the other hand, in the light of the internal timing hypothesis of Etchell and colleagues (Etchell et al., 2014; Etchell et al., 2017), this can again be seen as a facilitating factor for the internal timing of the patient: less afferent information is

arriving the cerebellum due to the lesion, there is no infobesity of afferent input and the internal time which might have been out of balance before can now function properly.

4.4.1.2 Changes in speech-related brain activation and differences in activations between patient and control groups

The patient's activations showed a modality-related effect when comparing the difference between POST and PRE activation: in the condition of covert humming, only small activation differences between both points of time were visible, while in covert speaking, the patient exhibited an increase of activation in parietal and temporal areas, but less activation in frontal areas POST (see Figure 31). This increase is valid for both hemispheres. There are two imaginable reasons for this hyperactivation. On the one hand, it could reflect a pathophysiological reaction to the damage of cerebellar cells – a physiological imbalance (diachisis) was established and the overactivation is a symptom of this. On the other hand, the hyperactivation could be seen as a compensational, facilitating reaction. Because regular circuities are not functioning anymore due to the brain damage, other, more extensive areas are activated to process motor control and motor planning properly.

Both explanations are conceivable and are discussed controversively in studies of language and motor recovery after stroke (Rehme, Eickhoff, Rottschy, Fink, & Grefkes, 2012; Rosen et al., 2000; Ward, Brown, Thompson, & Frackowiak, 2003). The fact that we see a modality-related effect of improved speech fluency in our patient confirms more the facilitatory function of the hyperactivation – speech fluency increased, the stuttering disappeared and a bilateral hyperactivation during covert speaking POST might be the neurophysiological grounding for this.

Interestingly, studies reported laterality differences between song and speech processing in the cerebellum. Lobule VI in the left cerebellar hemisphere and the right cortex process prosody and melody, while the right cerebellar lobule VI and the left

cortex process segmental information of speech (online sequencing of gestures into larger utterances; (Ackermann, 2008; Callan et al., 2006; Callan, Kawato, Parsons, & Turner, 2007). In our patient, the lesion affected 25% of the left cerebellar lobe VI. This means that the right cerebellum, which was processing motor speech aspects, was still intact. The lesioned left cerebellum, operating prosody and melody functions, was causing a hyperactivation during covert speaking but not covert humming. This hyperactivation of the collaborational efferents between left cerebellar hemisphere and right (sensorimotor) cortex might have activated additional melodic and prosodic processing cues that were responsible for the enhancement of fluency. It is a well described phenomenon that external timing cues like speaking in unison with a metronome, speaking with a slow voice onset or singing are enhancing speech fluency and inhibiting the core symptoms of stuttering (Guitar & McCauley, 2010). Therefore, fluency shaping stuttering therapy approaches around the world are using these techniques to improve speech fluency in stuttering patients (Euler et al., 2009; Neumann et al., 2017). Correspondingly, the patient demonstrated a monotonous prosody which was presumably caused by her atactic dysarthria. This specific symptom might also have a fluency-inducing function and probably contributed to the cecasstion of stuttering.

Regarding the difference images of stuttering controls, we could descriptively show that they revealed less difference in activation from PRE to POST in both conditions compared to the difference image of the single case patient (Figure 32). For the covert speaking condition, the stuttering controls exhibited a smaller change of activation from PRE to POST than the single case patient. This again is highlighting the supporting function of the hyperactivation in the single case patient: the cerebellar lesion seems to trigger the bilateral hyperactivation, probably leading to a cessation of stuttering in the single case patient.

We compared specific activation changes between the single case patient, stuttering controls and healthy controls not only in the whole brain, but also in the ROI BA 44

(Broca's Area). For this comparison, we used beta values (parameter estimates for brain activity). Again, this analysis yielded a modality related effect seen before in the global difference maps of the single case patient: the patient showed an increase of beta values in BA 44 from PRE to POST in the covert speaking condition (Figure 33, Figure 34), which was evident in the left as well as in the right hemisphere. In the covert humming condition, the patient exhibited a decrease of beta values from PRE to POST (Figure 35; Figure 36).

4.4.1.3 Longitudinal changes of white matter integrity in the patient and comparison to changes in the healthy control group

Certainly, the haemorrhage itself was causing a decrease of white matter in the patient, involving diverse cerebellar brain parts (e.g. different cerebellar lobules, middle cerebellar peduncle, see Table 22). This white matter decrease is the primary biomechanical mechanism following the haemorrhage: because of the hematoma, fibres stretch and disrupt in the end. In the centre of the hematoma, a recovery of whiter matter fibres is not possible, though peri-hematomical preservation of fibres can partly occur reliant on bleeding speed and size of the hematoma (Tao, Hu, Li, & You, 2017).

Interestingly, we also found evidence for secondary white matter impairment: a decrease of white matter integrity could be shown in the corpus callosum, in the right inferior fronto-occipital fasciculus, the left anterior thalamic radiation, the left cingulum and the right posterior corona radiata (see Figure 30, Table 22). It seems to be common that patients after a haemorrhage or a stroke show this fibre decrease in motor processing white matter fibre bundles. Li and colleagues (Li, Wu, Liang, & Huang, 2015) discovered that thirteen patients with a subcortical unilateral stroke revealed significantly decreased FA in the corpus callosum and the bilateral corticospinal tracts compared to a control group. A correlation existed between the patients' motor deficit score and FA in the corpus callosum. The smaller the FA values in the corpus callosum was, the more severe was the motor impairment. In addition, Li and colleagues used fibre tracking and observed

significant changes in inter-hemispheric fibre connections between left and right motor cortex. In detail, these changes were observed in the corpus callosum, left anterior thalamic radiation and interior fronto-occipital fasciculus, bilateral corticospinal tract (CST), anterior/superior corona radiate, cingulum and superior longitudinal fasciculus (Li et al., 2015). The authors speculated that these white matter impairments are a sign for inter-hemispheric network disturbances and can be used to predict motor and neurological disorders in stroke patients. In our single case patient, exactly the same brain regions as in the study of Li and colleagues (2015) exhibited a fibre decrease in the second measurement, with exception of the superior longitudinal fasciculus. It seems as if a haemorrhage in the left cerebellar hemisphere leads to a comparable FA decrease known from a unilateral stroke and also shows similar neurophysiological network disturbances. Nevertheless, in the current case report these declines of white matter integrity might not only be a sign for inter-hemispheric network disturbances, but were also facilitating for the speech fluency of the patient.

4.4.1.4 Hypotheses about neurophysiological mechanisms being responsible for the cessation of stuttering after cerebellar haemorrhage

The cerebello-thalamo-cortical pathway seems to be a central point for integrating all previously described remarks in an encompassing neurophysiological theory for the cessation of stuttering in our single case patient. Again, this circuitry is operating the control of motor planning and execution by receiving afferent information through the motor cortex via pons and the middle cerebellar peduncle, then processing information in the anterior lobe of the cerebellum and finally sending efferent feedback back to the motor cortex via dentate nucleus and the superior cerebellar peduncle.

The anterior cerebellar lobe (in particular lobule VI) as well as the dentate nucleus were impaired in our patient. These parts of the cerebellum are involved in articulation processes and sensorimotor functioning of the vocal tract (Carreiras et al., 2007; Mariën et al., 2014; Thürling et al., 2011). Lesions in these important areas for motor speech

execution are responsible for the pathogenesis of atactic dysarthria and they could presumably be causative for the cessation of stuttering: In an intact cerebellum, the dentato-thalamo-cortical pathway is facilitatory per se, but a cerebellar inhibition is active - Purkinje cells of the cerebellar cortex are inhibiting the dentate nucleus and cause a disfacilitation of the motor cortex (Grimaldi et al., 2014). When this cerebellar inhibition is active, the dentate nucleus inhibits efferents that are sent to the thalamus. Grimaldi and colleagues note that in patients with lesions in the middle cerebellar peduncle, this cerebellar inhibition is still functioning, while in patients with lesions in the dentate nucleus, the cerebellar inhibition is reduced (Grimaldi et al., 2014). Evidence for the motor sequels of a reduced cerebellar inhibition is given by studies claiming that lesions in the dentate nucleus lead to hypermetria, a target overshoot in movements (Grimaldi & Manto, 2012), i.e. an “overactivation” of movement execution.

Our patient shows both impairments in the middle cerebellar peduncle and in the dentate nucleus. Therefore we assume that the cerebellar inhibition is reduced. The reduced inhibition turned into a disinhibition and became evident in the massive overactivation the single case patient showed during covert speaking in temporal and parietal areas as well as in BA 44 POST. This overactivation could have been further facilitated by the observed decline of white matter fibres responsible for interhemispheric motor communication. Less white matter integrity and myelination measured POST in our case patient might have supported the heavily fired neural potentials, in other words the hyperactivation during covert speaking.

But how can this reduced cerebellar inhibition be beneficial for speech fluency? One hypothesis refers to the lateralisation of the cerebellum. The left cerebellar hemisphere (in specific: lobule VI) is collaborating with the right cortex and is specialised for processing prosody and melody (Ackermann, 2008; Callan et al., 2006; Callan et al., 2007; Callan & Manto, 2013). A cerebellar disinhibition which is leading to an overactivation of the right motor cortex might therefore facilitate unique melodic and

prosodic cues that are beneficial for an enhancement of speech fluency. These assumptions match the hypothesis of Etchell and colleagues (Etchell et al., 2014): they suppose that an internal timing deficit causes stuttering. This deficit might be erased in our patient via the released prosodic support which is caused by the cerebellar disinhibition – prosodic and melodic elements consist of rhythm and timing information.

In addition to the previously explained theory, we hypothesise that the cerebellar disinhibition in our patient POST was probably leading to an overactivation in the thalamus. As a consequence, this thalamic overactivation might have obliterated the stuttering symptoms. Several studies report an association between DBS in the thalamo-cortical pathway and an improvement of stuttering: Bhatnagar and Andy were able to show that acquired neurological stuttering was improved by unipolar self-stimulation of the centromedian nucleus of the thalamus (via DBS; Bhatnagar & Andy, 1989; Craig-McQuaide et al., 2014). Also Maguire et al. (Maguire et al., 2012) reported a decrease of developmental stuttering after DBS of the ventral intermediate nucleus in the thalamus. Furthermore, Thiriez and colleagues (2013) presented a case where the use of bilateral subthalamical nucleus DBS diminished the patient's developmental stuttering symptoms. To conclude, the cerebellar disinhibition in our patient POST was probably leading to an overactivation in the thalamus which might have triggered comparable effects to the DBS in the study of Bhatnagar and Andy (1989), Maguire and colleagues (2012) as well as Thiriez and co-authors (2013): it facilitated the speech fluency of our patient.

4.4.2 Conclusion

The precise role of the cerebellar hemispheres and nuclei in speech fluency remains under debate (Budde et al., 2014; Howell et al., 2012; Lu et al., 2012; Sitek et al., 2016; Yang et al., 2016) but the earlier described case report on acquired stuttering in adulthood after right cerebellar lesions (Tani & Sakai, 2010) as well as our presented

case study highlight the involvement of the cerebello-thalamo-cortical circuitry in speech fluency.

Can cerebellar lesions make dysfluent people fluent? The principle of operation in the cerebello-thalamo-cortical pathway and its discontinuity of inhibition caused by cerebellar lesions seem to be a plausible explanation to this question.

The presented study is limited due to its single case character. Other aspects that we were not able to control for might have also contributed to the cessation of stuttering, e.g. the rehabilitation therapies and processes the patient underwent after the haemorrhage. Nevertheless, this study adds beneficial support for the role of the cerebellum as a neural correlate of stuttering and underlines the importance of reporting as well as supporting interesting single cases in science.

In the future, noninvasive brain stimulation could be used to up- or down-regulate cerebellar hemispheres selectively, thereby probing the mechanisms of interference in the cerebello-thalamo-cortical pathway and their influence on speech fluency.

5 General Discussion

In this thesis I evaluated the long-term effect of an intense German stuttering therapy on white matter plasticity and brain activity changes in PWS. Furthermore, I presented a rare single case study of an adult who lost stuttering after a cerebellar haemorrhage. The outcome of these three studies is discussed in the following subsections.

Long-term white matter plasticity changes in PWS induced by stuttering therapy (chapter 2):

One aspect of this study was to replicate former findings of a decline of white matter integrity in PWS compared to healthy controls. We were able to confirm this reduced white matter integrity in right hemispheric brain regions including parts of the inferior longitudinal fasciculus close to the callosal body, cingulum, inferior-fronto-occipital fasciculus and the corticospinal tract. Eleven months after the baseline scan, the effects of therapy in the intervention group were manifold. On the behavioural level, a significant reduction of stuttering severity as well as of the impact of stuttering on life quality were found and associated with undertaking the stuttering therapy. Concerning white matter plasticity changes, a significant increase of FA in the left superior longitudinal fasciculus (Neef et al., 2015) was observed. Contrary to the intervention group, a significant decrease of white matter integrity was found in both control groups. This white matter decrease might underlie aging effects which came to light in the longitudinal study design. These novel findings highlight the lasting impact of stuttering therapy on white matter brain structure.

Long-term brain activity changes in PWS induced by stuttering therapy (chapter 3):

Compared to healthy and stuttering control participants, stuttering patients presented with a hypoactivation in prominent motor processing (e.g. left and right rolandic operculum) as well as in cognition and emotion processing (e.g. left amygdala) regions

at pre-test. At post-test, a therapy-induced increase of brain activation in these regions was observed. The therapy effect was also measurable on the behavioural level. Only stuttering patients who took part in the intervention developed a significant decrease of stuttering severity as well as of the OASES and BDI score. These findings point to an improved coping with adverse feelings and attitudes connected with stuttering in the patient group. Furthermore, a positive correlation between brain activity and stuttering severity, which was calculated over all participant groups and measurements, was affirmed. This implies the higher the stuttering severity score has been, the higher was the functional activity in specific motor regions. Brain regions showing these significant correlation results were the left and right rolandic operculum, the right area 44 and the left precentral gyrus, but also non-motor regions like right middle temporal gyrus and subiculum.

Cessation of stuttering after left cerebellar haemorrhage – a case report (chapter 4):

After experiencing a perioperative haemorrhage some months after the initial MRI scan, the 52 years old patient presented with a large cerebellar lesion, including approximately 1/5 of the left cerebellum. The largest damages were located in Crus I, Crus II and VIIb in the posterior part of the cerebellum as well as in lobe VI in the anterior cerebellum. Affected structures were also the middle cerebellar peduncle and the dentate nucleus. A TBSS analysis revealed a primary white matter decrease caused by the haemorrhage in the previously described parts of the cerebellum. Additionally, a secondary white matter impairment was detected in the corpus callosum, right inferior fronto-occipital fasciculus, left anterior thalamic radiation, left cingulum and right posterior corona radiata. These decreases of white matter integrity can be interpreted as neurophysiological network disturbances and seem to emerge prevalently after stroke or traumatic brain injuries (Li et al., 2015). In the whole-brain fMRI analysis, the patient showed a modality-related difference in brain activity from pre to post-test. In the covert

speaking condition, a prominent increase of activation in parietal and temporal areas became obvious, which was not present in the covert humming condition. The ROI analysis in the left and right BA 44 revealed a hyperactivation of the single case patient during covert speaking POST compared to stuttering and fluent control participants. This hyperactivation was again modality-related and not found in the covert-humming condition. The results were interpreted in reference to the cerebello-thalamo-cortical pathway. The cerebellar disinhibition caused by the lesion might have led to an overactivity in thalamus and motor cortex which might have facilitated the cessation of stuttering.

In the following subchapters, I discuss these findings in a more comprehensive context.

5.1 Implications for the aetiology of stuttering

In this subchapter, I will discuss certain brain regions out of the three presented papers that have the potential to play a role in the complex aetiology of stuttering. Some of these structures or areas have been discussed rarely in relation to stuttering and its aetiology in former research studies. Therefore, it is worth to elucidate their possible connection to stuttering.

The first relevant finding of this thesis is the barely considered role of the **cerebellum and the cerebello-thalamo-cortical pathway** for the onset of stuttering. The single case study underlines that not only cerebral cortices and subcortical structures are involved in the pathomechanisms of dysfluent speech, but also the cerebellum and its connections via the thalamus to the cortex might influence how fluent a person speaks. This is not surprising, because the cerebellum and the cerebello-thalamo-cortical pathway operate the control of motor planning and execution (Trepel, 2004) as well as the control of the vocal tract and clarity of speech (Mariën et al., 2014). Possibly, they constitute a constituent in the pathomechanisms of stuttering – this interesting hypothesis should be put into the focus of future research. Former studies already found

results which confirm this hypothesis: Lu and colleagues (2012) showed an abnormal increase of RSFC in the cerebellum and Yang et al. (2016) were able to detect lower RSFC between left and right cerebellar regions as well as between left cerebellum and right BA 4/6 (M1/premotor cortex and SMA) in PWS compared to fluent control. This functional dysconnectivity between the cerebellum and motor processing cerebral regions in PWS provides further insights into the role of the cerebellum as a neural correlate of stuttering.

The second interesting finding of this thesis is **the bilateral hypoactivation at pre-test and its trend to normalisation at post-test in brain areas processing emotion and cognitive functions** in the group of stuttering patients**.** Left-hemispheric (mostly frontal) brain regions processing motor and motor speech production have shown an increased activity after stuttering therapies in several studies (De Nil, Luc F. et al., 2003; Neumann et al., 2018). The novel outcome that also regions like the left amygdala and the right supramarginal gyrus show this increase of activation opens up an interesting new perspective. In studies which compared brain activation changes after an intense stuttering therapy, only Neumann and colleagues (2018) found an increased activation in the bilateral amygdala at post-measurement. Since the same kind of stuttering therapy was applied (Kasseler stuttering therapy) and both studies showed this increased activation in the amygdala after therapy, one might speculate that this effect is induced by therapy. It could originate from specific acquired therapy techniques or the successful revision of negative emotions and attitudes related to stuttering. In addition, Toyomura et al. (2018) investigated speech disfluency-dependent amygdala activity in PWS. They demonstrated that amygdala activation during conversations is involved in stuttering. This is why I conclude that a hypoactivated left amygdala at pre-test and an increase of amygdala activation after stuttering therapy might arise from the importance of the amygdala in the aetiology of stuttering. Future studies will provide further insights how the amygdala is influencing speech fluency and/or psychosociological symptoms of

stuttering. I assume that the amygdala is meaningful in the stuttering brain because it regulates emotions and fears. PWS often show social and linguistic avoidance behaviour, have a history of social withdrawal and characterise their stuttering symptoms as fearful events during communication. Therefore, to it seems plausible to me that the amygdala was hypoactivated in PWS at pre-test and its activation increased later on.

Another indication for the importance of emotion regulating brain regions for the aetiology of stuttering is the influence of emotion processing on motor control. Previous studies were able to demonstrate that emotional context can modulate neural activation in areas which are involved in movement control: transcranial magnetic stimulation (TMS) studies showed that the SMA provided greater motor cortex excitability in reference to emotionally positive and negative connoted images compared to non-emotional neutral images (Hajcak et al., 2007; Oliveri et al., 2003). An increase of corticospinal excitability has been also observed as a response to adverse emotional settings (Coelho, Lipp, Marinovic, Wallis, & Riek, 2010). In the single case paper of this thesis I pointed out that the cerebellum which plays a major part in motor control is also involved in emotional processing (Mariën et al., 2014; Stoodley & Schmahmann, 2010). A study from Mazzola and colleagues (2013) even reported effects of different emotional contexts on the cerebello-thalamo-cortical pathway activation during action observation. Left SMA and right cerebellar anterior lobe activity increased while observing an action in a negative emotional context. The authors concluded that the emotional context is able to modulate the cerebello-thalamo-cortical pathway (Mazzola et al., 2013). As described earlier, the single case patient showed a cessation of stuttering probably due to a disinhibition of her cerebello-thalamo-cortical pathway. The idea that emotional context or specific feelings and attitudes have a direct impact on this specific pathway and possibly other motor processing circuits is again stressing the importance to consider non-motor brain regions and networks as possible neural correlates for stuttering. This line of reasoning agrees with the findings of networks studies of Chang

et al. (2017), Xuan et al. (2012) and O'Neill et al. (2017). These researchers demonstrated that not only motor networks, but also networks related to attention and default mode show deviations in PWS.

The third relevant finding of this thesis is our confirmation **of right-hemispheric reductions of white matter integrity in PWS as well as the new finding of an increase of white matter integrity after intense stuttering therapy**. Former studies presented in the review of Neef and colleagues (2015) reported a reduction of FA in mostly left hemispheric brain regions as the left rolandic operculum or left perisylvian regions in PWS (e.g. Cykowski et al., 2010). In the last five years, several studies confirmed the reduction of white matter integrity in right-hemispheric brain regions of PWS (Cai et al., 2014; Chang et al., 2015; Cieslak et al., 2015; Kronfeld-Duenias et al., 2016; Misaghi et al., 2018; Neef et al., 2018). Despite of the advanced diffusion imaging techniques, the locations where a reduction of white matter integrity in PWS was demonstrated differ from each other. For sure, the larger growing body of diffusion imaging studies literature strengthens the knowledge and evidence that PWS manifest a weak white matter integrity as a neural correlate of stuttering. But from a scientific point of view, it would be interesting to investigate how the different locations (left or right hemisphere, specific brain structure) in these diffusion MRI studies are substantiated. Are the distinct regions of reduced white matter integrity caused by the heterogenous population of stuttering participants (different stuttering severities, symptoms, coping strategies, age groups)? Or are the diverging results evoked by small study groups, different MRI techniques and statistical analysis approaches? Future studies with larger groups of PWS including a greater variability of potential confounding factors are needed to answer this still open question. The region that showed a reduction of FA in our group of PWS which is most frequently replicated by other researchers (Cai et al., 2014; Chang et al., 2015; Neef et al., 2018; Sitek et al., 2016) is the right superior longitudinal fasciculus. This fibre bundle connects posterior and inferior frontal lobe with perisylvian

speech areas in the parietal lobe and processes vocalisation control in humans (García et al., 2014). From my point of view, reduced white matter integrity in this structure has therefore the potential of being a neural hallmark of stuttering and should be taken into consideration for further research approaches. Furthermore, we found an increase of white matter integrity in the left superior longitudinal fasciculus after stuttering patients took part in the intense stuttering therapy. Why the increase of FA might occur in this specific structure will be explained in chapter 5.2.

To complete this subchapter, the implications of our research for the aetiology of stuttering are discussed. Recently, the Packman & Attanasio "3-factors causal model of moments of stuttering" (Packman, 2012; Packman & Attanasio, 2010) was used to debate interference in the stuttering research community. This model describes three factors which are responsible for the aetiology of stuttering (see Figure 37).

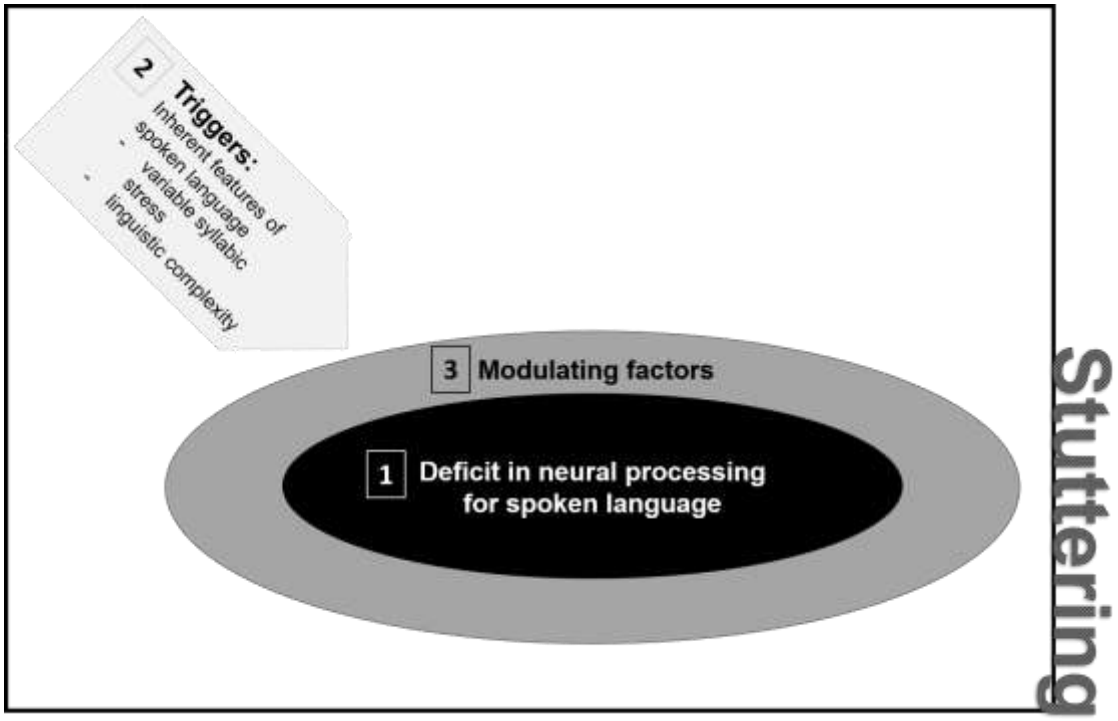


Figure 37. The Packman and Attanasio 3-factor causal model of moments of stuttering (based on figure 1 of the paper of Packman, 2012, p. 227).

The first factor is the impaired neural processing of speech production. This factor leads to unstable and dysfluent speech and is a necessity for the occurrence of stuttering. The

second factor postulated by Packman and Attanasio are two triggers that disturb the production of speech and therefore cause individual stuttering symptoms. These triggers are the variable syllabic stress and linguistic complexity. Both language features result in a rise of motoric demands on the speech production system. The third factor concerns modulating factors that lower the threshold for the occurrence of stuttering symptoms, they are interindividually different. Packman and Attanasio (2012) consider physiological arousal (reaction to stressful situations and stimuli) as the major modulating factor, but also emotions like fear or a special demand of cognitive resources could represent modulating factors. If a stuttering person is not able to perform multiple tasks at the same time, the factor “multiple tasking” could lower the threshold for the occurrence of stuttering symptoms, for example. Packman claims: “Modulating factors, then, can be seen as the major contributor to the variability of stuttering within individuals, across communicative context” (Packman, 2012, p. 228). In the model, all three factors interact. Stuttering symptoms occur if the impaired neurophysiological processing of a stuttering patient (necessary condition) is temporally connected with the presence of linguistic triggers and/or modulating factors (sufficient conditions).

The results of this thesis underline the hypothesis behind the 3-factor-model (Packman, 2012; Packman & Attanasio, 2010). We were able to replicate former findings of a decreased white matter integrity in PWS. These decreases were detected in different locations in the right hemisphere, e.g. in the superior longitudinal fasciculus (as described previously). This decline of white matter integrity in speech relevant fibre bundles of the brain represents the first factor of the 3-factor-model of Packman and Attanasio. In our group of PWS, the myelin sheaths might be insufficiently developed, which initially can be caused by genetic aberrations (for a review, see Kraft & Yairi, 2012). As a result, the neural transmissions may not work properly and this might represent the necessary neural deficit for the occurrence of stuttering symptoms (see also Packman, 2012). In our studies, the emotion processing brain regions like the left

amygdala and the right supramarginal gyrus were hypoactivated in comparison to stuttering and fluent controls before therapy, and they showed an increase of activation after therapy. These findings might be interpreted in reference to factor 3. They could be regarded as possible modulating factors of the Packman and Attanasio model. The hypoactivation at the pre-test could be seen as a sign for an insufficient coping with fear, emotional arousal and psychological strain caused by stuttering symptoms. The 'dysfunctional coping' then represents a modulating factor which might lower the threshold for the occurrence of stuttering symptoms. At the post-measurement, we detected a tendency for the normalisation of amygdala activity. This could provide some explanation why we measured a therapy effect on the behavioural level. By learning a new pattern of speech as well as functional coping strategies for negative emotions and attitudes towards stuttering, the modulating factor functional coping may have raised the threshold at which stuttering symptoms occur and the patients' stuttering severity therefore decreased significantly after therapy. To conclude, this thesis has been able to partly substantiate multifactorial mechanisms of the aetiology of stuttering represented by the 3-factors-model of Packman and Attanasio (Packman, 2012; Packman & Attanasio, 2010).

5.2 Implications for the treatment of stuttering

Several findings described in this thesis have important implications for the (neurophysiological) comprehension of the effects of stuttering therapy. That is why the advancement of stuttering therapy approaches and procedures should take these findings into account. The implications are discussed in the following subchapters.

Neurophysiological effects of an intense fluency-shaping stuttering therapy on the brain

To the best of our knowledge, we found the first evidence for structural brain changes evoked by an intense stuttering intervention. A significant increase of FA in a specific

ROI within the left superior longitudinal fasciculus (third portion) became evident at the post-measurement. In more detail, the ROI was located in the inferior parietal lobe and in the vicinity of the angular gyrus and the posterior supramarginal gyrus. We chose this ROI for our analysis because this specific region is one of the three clusters where PWS obtained a significant decrease of white matter integrity in the meta-analysis of Neef and colleagues (2015). In their analysis, Neef et al. used deterministic DTI tractography to illustrate that this cluster obtains connections with the ventral premotor cortex, postcentral gyrus and the pars opercularis of BA 44 (Neef et al., 2015).

Are there any assumptions why exactly this region exhibited an increase of FA 11 months after the intense Kasseler stuttering therapy (Euler et al., 2009)? A possible explanation for the white matter plasticity in this specific region is the operating principle of the Kasseler stuttering therapy. During the initial intense therapy phase, patients learn a new, global speech pattern including a soft voice onset and a prolonged, slower manner of speech production. An intensive training of this new speech pattern which is effective in the alleviation of stuttering symptoms might most likely address brain areas which process motor planning and motor production. Therefore, the finding of an increase of white matter integrity in a region which is connected via white matter fibres to prominent motor processing regions is not surprising. The premotor cortex, BA 44 (Broca's area) and the postcentral gyrus are functionally involved in the organisation of the sensorimotor cortex for speech articulation (Bouchard, Mesgarani, Johnson, & Chang, 2013). A modification of the global motor speech pattern is the chief constituent of the Kasseler stuttering therapy. Therefore, it seems plausible that we found a therapy-induced increase of white matter plasticity in this specific brain area. In addition, Broca's area is associated with the processing of rhythm, music and working memory of pitch (Koelsch & Siebel, 2005; Platel et al., 1997). It is conceivable that the intense, bio-feedback supported training of a gentle voice onset, a prolonged speech manner and a

slowed velocity of speech is facilitated by the additional recruitment of Broca's area and its fibre connection to the cluster in the left superior longitudinal fasciculus.

One unanticipated finding was the therapy-induced increased functional brain activity in previously hypoactivated brain regions responsible for emotional regulations, such as the left amygdala (Javanbakht et al., 2015; Toyomura et al., 2018) and right supramarginal gyrus (Silani et al., 2013). As discussed previously, this changed activation pattern could represent a therapy-evoked improvement of coping strategies with negative emotions and attitudes related to stuttering. Further research should be undertaken to emotion-regulating brain regions in stuttering modification therapies additionally to fluency shaping approaches. As opposed to speech restructuring therapy approaches, stuttering modification therapies focus on the desensitisation of the patient prior to the training of speech techniques (Natke et al., 2010; van Riper, 1973; Zückner, 2014; Zückner, 2017). The desensitisation phase includes the patient's confrontation with his own negative emotions evoked by the stuttering symptoms and the confrontation with aversive listener reactions. Cognitive psychological approaches like behavioural therapy (e.g. cognitive reorganisation) can be applied in the therapy process to reframe these negative connoted feelings and attitudes (Zückner, 2014). PWS' emotion processing brain networks and structures might show even larger therapy-induced activation changes after an intense stuttering modification therapy, since the regulation of emotions is the major interest in this therapy approach.

To conclude this subchapter with a revisited reference to the 3-factors-model of moments of stuttering by Packman and Attanasio (Packman, 2012), our research provides the first evidence that the deficit in neural processing (factor 1, necessary for the occurrence of stuttering symptoms) can be modulated through stuttering therapy. This possibility has been under debate for several years (Packman, 2012). Future studies that may replicate or expand our study results of increased white matter integrity

as a result of an intense stuttering therapy are necessary to further determine and refine this neurophysiological effect.

Derived conclusions and suggestions for stuttering treatments

The research presented in this thesis confirms the importance of integrating the treatment of inner, psychosociological symptoms of stuttering into stuttering therapy programmes. We assume that the therapy-induced increase of brain activation in emotion-processing brain regions like amygdala and supramarginal gyrus is an effect of the training of functional coping strategies. If PWS learn to successfully reduce or ease their stuttering symptoms and learn to handle negative emotions, this might have an additional facilitating effect on motor control and production (see chapter 5.1). If we further consider the successful handling of negative connoted inner stuttering symptoms as a modulating factor in the 3-factor-model of Packman and Attanasio (Packman & Attanasio, 2010), the threshold for the occurrence of stuttering symptoms might be elevated so that the patient's speech becomes increasingly fluent. These described synergy effects should be taken into account by speech-language pathologists. Not only the training of new speech patterns (global speech restructuring approaches) or locally applied speech techniques (stuttering modification approaches) should be a stable component of the intervention, but also the desensitisation of stuttering patients (e.g. against negative listener reactions or against their own stuttering symptoms and consequently developed negatively connoted feelings).

Moreover, we could use the knowledge of these studies to further evaluate and implement additional therapy methods for stuttering. Chesters and colleagues (2018) provided the first evidence that transcranial direct current stimulation (tDCS) applied in addition to a metronome-timed and choral speech training had a facilitating effect on speech fluency. PWS who received 20 min of stimulation in combination with a daily fluency-enhancing training showed a significant improvement of speech fluency

compared to the control group of PWS receiving sham stimulation. The authors conclude that tDCS presents a new perspective as an adjunct for stuttering therapies. In this context, this thesis offers ideas for possible target areas for non-invase brain stimulation.

The presented single case study elucidates the importance of the cerebellum and the cerebello-thalamo-cerebral pathway for speech fluency. A future study could analyse the effects of a conventional stuttering therapy combined with complementary noninvasive brain stimulation. Various locations would hereby qualify as possible target regions for stimulation. First, the selection of the cerebellar hemisphere should be of significance for stimulation effects because of the reported laterality differences between song and speech processing in the cerebellum (Callan et al., 2006; Callan et al., 2007). While the left cerebellar hemisphere and the right motorcortex are specialised for processing prosodic and melodic properties, the right cerebellar hemisphere in combination with the left motor cortex is more specialised for speech (Callan et al., 2007). This could imply that stimulating the left cerebellar hemisphere probably supports the effect of a fluency shaping therapy and its trained prolonged and slowed pattern of speech. A stimulation of the right hemisphere might facilitate the production of speech in a more general way and could be helpful in the maintenance phase of an already progressed therapy. The maintenance phase usually aims to stabilise improvements with the handling of inner stuttering symptoms as well as the implementation of trained speech patterns or speech techniques. A complementary stimulation of the right cerebellar hemisphere might boost this stabilisation and support a successful transfer of therapy achievements. Lobule VI with its responsibility for the processing of articulation (Carreiras et al., 2007) is a promising region where cerebellar non-invasive stimulation could be evaluated. Likewise, the stimulation of the cerebellar vermis could be the subject of a future research study, because this structure exhibited a normalisation of RSFC in PWS after conducting a stuttering intervention (Lu et al., 2012). Nevertheless, it is questionable if

the establishment of a montage with small electrodes is possible to exactly target these regions.

Chesters et al. (2018) already showed that a stimulation of the left inferior frontal cortex enhanced the effects of their stuttering intervention. They placed the cathode over the right supra-orbital ridge and the anodal electrode on BA 44 “with the electrode extending posteriorly to cover ventral portions of premotor and primary motor cortex, where the representation of the articulators is located” (Chesters et al., 2018, p. 1163). Interestingly, the ventral premotor cortex as well as BA 44 which Chesters et al. used as target regions are two of three regions connected to cluster 1 (left superior longitudinal fasciculus) in the meta-analysis of Neef and colleagues (2015). In this cluster, we found an increase of white matter integrity after our patient group finished the intense Kasseler stuttering therapy. This overlap between our result and the study parameter as well as findings of Chesters et al. (2018) indicates that a complementary stimulation in these brain areas might be beneficial for PWS taking part in the Kasseler stuttering therapy. A future study should investigate if tDCS in these regions as well as in the postcentral gyrus (the third region connected to cluster 1) can further boost the therapy-induced increase of white matter integrity we observed in our study. Beyond that, this stimulation and its montage should be used together with other therapy approaches, e.g. an intense stuttering modification therapy. This might add new insights to the question if achievements in these forms of therapies will also benefit from supplementary stimulation. Chesters and colleagues (2018) merely evaluated the short-term effects of complementary stimulation (the assessment of speech fluency took place 1 and 6 weeks after their 5-day intervention). Therefore, another resulting research question is to determine if stimulation effects are still traceable after a long-term follow up > 6 months for different forms of stuttering treatments. Furthermore, it is questionable if a specific group of PWS, e.g. stuttering patients with severe and long core symptoms and distinct

motor accompanying symtpoms, are more likely to benefit from this complementary stimulation.

5.3 Conclusion

This thesis includes three studies about the longitudinal effects of an intense stuttering therapy or a brain lesion on brain structure and function in PWS. Our research provides first evidence of white-matter plasticity induced by an intense stuttering therapy. It confirms that it is important to consider long-term longitudinal evaluations of brain activity changes in PWS, since we detected therapy-evoked activation increases not only in motor, but also in emotion-regulating brain regions. And it elucidates the necessity of considering the cerebellum and its neural pathways as relevant constituents for the occurrence of stuttering. Apparently, future research is essential to confirm and complement our new findings of white matter integrity changes in PWS. It is also necessary to further synthesise, weight, classify and align the growing knowledge about the complex aetiology of stuttering.

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7 List of Abbreviations

BA	*Brodmann area*
BDI	*Beck Depression Inventory*
BOLD	blood oxygenation level dependent
CNS	*central nervous system*
CST	*corticospinal tract*
CWS	*children who stutter*
DBS	*deep brain stimulation*
DTI	*diffusion tensor imaging*
EPI	*echo-planar imaging*
FA	f*ractional anisotropy*
FDA-2	*Frenchay Dysarthrie Assessment - 2*
FLAIR	*fluid-attenuated inversion recovery*
fMRI	*functional magnetic resonance imaging*
FMRIB	*Oxford centre for functional MRI of the brain*
FOV	*field of view*
FSL	*The FMRIB Software Library*
FWE	*family wise error*
FWHM	*full width at half maximum*
HC	*healthy controls*
IFG	*inferior frontal gyrus*

KALPHA	*Krippendorff's Alpha*
M1	*primary motor cortex*
MRI	*magnetic resonance imaging*
OASES	*Overall Assessment of the Speaker's Experience of Stuttering*
PDS	*persistent developmental stuttering*
PET	*positron emission tomography*
POST	*post-test*
PRE	*pre-test*
PWS	*persons who stutter*
ROI	r*egion of interest*
RSFC	*resting state functional connectivity*
SC	*stuttering controls*
SMA	*supplementary motor area*
SP	*stuttering patients*
SSI-4	*Stuttering Severity Index 4*
STAI	*The State-Trait Anxiety Inventory*
TBSS	*tract-based spatial statistics*
tDCS	*transcranial direct current stimulation*
TE	*echo time*
TFCE	*threshold-free cluster enhancement*
TI	*inversion time*
TMS	transcranial magnetic stimulation

TR	*repetition time*
WHO	*World Health Organization*
WHO-5	*The WHO-5 Well-Being Index*

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9 Acknowledgements

It would have been impossible to finalise this thesis without the support of several people.

First, my sincere gratitude goes to Martin Sommer for being the supervisor of my thesis. Without him, the implementation of our research projects and this thesis would not have been possible. Thank you for your ongoing advice, encouragement, ideas and fruitful discussions. I also appreciated our clinical collaboration in the stuttering ambulance a lot.

Second, I thank Annekathrin Schacht and Tanya Behne for co-supervising my projects and for all the good ideas and suggestions which fostered my progress. In this coherence, I would also like to thank Peter Dechent, Hansjörg Scherberger and Arezoo Pooresmaeili for being members of my examination committee. I am very grateful for your time and participation! In addition, I wish to express my thankfulness to Chris Schlögl and Rebecca Jürgens – you completed an amazing job in organising and supporting my PhD time in BeCog!

Furthermore, my deep gratitude goes to Peter Dechent, Roberto Goya-Maldonado and Sarah Wolter for their great support and help with the imaging analysis. I am so happy that you shared your knowledge as well as time with me and broadened my horizon.

I also thank Ilona Pfahlert and Britta Perl for their constant support and commitment to realise all the MRI measurements which were necessary for our research projects. Even at the weekends, you made it possible to scan our well-travelled patients and always offered them and me a very pleasant and welcoming environment. I have never taken this for granted – thank you!

Moreover, I want to express my gratitude for the collaboration with my Speech-Language Pathologist fellow Bettina Helten – your focused and accurate support in the speech fluency analysis saved me from drowning in tons of video recordings and made it possible to finish all SSI analyses in time. I really liked to work in a team with you, it was a pleasure!

I also want to express my gratitude to Professor Paulus and all the members of the Klinik für Klinische Neurophysiologie. Thank you for the support and the facilities which further aided our research projects. In specific, I thank Michael Bartl who partly digitalised the huge amount of filled in behavioural questionnaires.

Beyond this, my appreciation goes to Alexander Wolff von Gudenberg and his Kasseler Stottertherapie team – without our collaboration, it would have been impossible to include enough stuttering patients into our studies. Special thanks are addressed to

Verena Schneider, Birgit Schröck and Anna Merkel who provided me with essential information about therapy courses and the organisatory processes in Bad Emstal – thank you for inviting me to the KST information day each month. Moreover, I would like to thank Andreas Starke and Robert Richter for announcing our study in the VIERMALFÜNF stuttering therapy groups.

Next, I want to express my cordial thanks to all the patients and participants who took part in our studies. I really appreciate that you donated your time, your patience with the long measurement in the scanner and your interest for stuttering and science to our projects! I got to know a huge amount of congenial people who sometimes shared their pleasant as well as sad experiences and stories with me. This really opened up new perspectives – thank you all.

My further gratitude goes to all my embosomed colleagues and friends – it was enlightening to learn from you and enjoyable to spend leisure time with you: Zsolt Turi, Ivan Alekseichuk, Giorgi Batsikadze, Yuichiro Shirota, Gabriel Amador de Lara, Aguida Foerster, Leonie Schmalfuß, Sarah Wolter, Albert Lehr, Elina Zmeykina, Alexandra Korzeczek and Christina Stier – it has been a blast! I also like to thank Silvia Gosewinkel, Albert Heinle and all my other friends near and far for encouraging me in my ups and downs and therefore helping me to get this thesis finished. A huge “thank you” also goes out to Anke Kohmäscher for discussing the foundations of stuttering with me.

Last, but not least, I thank my family which is always the biggest support to me. Thanks to my mother and father as well as Margit and Stephan for comforting me and always having a sympathic ear for me. And thanks to Martin who always listens to my joys and doubts and is there for me unconditionally. I really appreciate that you showed me that it is worth to fight against all the obstacles in one’s way. And I am deeply thankful for all our discussions about stuttering and the brain – you are probably one of the few corrosion engineers worldwide who is also an expert in stuttering by now.

10 Curriculum Vitae

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