Neuronal underpinnings of stuttering

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Sammendrag

Flytende tale er avhengig av solide forbindelser mellom hjerneområder involvert i auditorisk prosessering, motorisk planlegging og utførelse. Taleapparatets evne til uanstrengt å produsere flytende uforstyrret tale er forstyrret hos personer som stammer (PWS). Stamming er en sammensatt forstyrrelse av taleflyt som resulterer i ufrivillige gjentagelser av stavelser, utvidelser, og blokkeringer, spesielt i begynnelsen av ord og setninger. Gitt tiår med forskning på området er det ennå ikke klart hvilke mekanismer som ligger til grunn for stammingen.

Hensikten med dette studiet har vært å utforske det nevrale grunnlaget til stamming ved å se på hjernens nevrokjemi ved å ta i bruk proton-magnetisk resonsansspektroskopi (^{1}H-MRS) teknikk. Vi har sett på om nevrotransmitterene: N-acetyl Asparatate (NAA); glutamat og glutamin (Glx) og myo-inositol kan bidra til forståelsen av de biokjemiske manifestasjonene av stamming. Vi har også samlet inn atferdsdata fra PWS-gruppen og korrelert dette med spektroskopi-dataen. Til slutt kombinerte vi målingene av den nevral aktiviteten av taleproduksjon med ^{1}H-MRS målingene for å se på interaksjon mellom nevral aktivering og underliggende nevrokjemisk funksjon. Inferior frontal gyrus (IFG) var målområdet for undersøkelsen, siden området er viktig for motorisk kontroll av tale.

Nevrotransmitteren myo-inositol viste en hovedgruppeeffekt. Metabolittene i hjernen til personer som stammer var karakterisert av en tydelig reduksjon i nivå av myo-inositol i IFG. Myo-inositol er ansett som en glial markør, og dets konsentrasjon kan muligens fortelle om myelinets tilstand i hjernen. Myelineringsprosessen av nerveceller er en modningsprosess som fasiliterer rask signaloverføring fra hjernen til muskelfibrene involvert i tale. Vi foreslår derfor på bakgrunn av foreliggende litteratur på området og våre resultater at forsinket eller hemmet myelinering av tale-relaterte nevrale nettverk i spedbarnsperioden kan føre til senere utvikling av stamming.

Abstract

Fluent speech production depends on robust connections between brain regions that are crucial for auditory processing, motor planning and execution. The ability of the speech apparatus to produce effortless, continuous and uninterrupted flow of speech is compromised in people who stutter (PWS). Stuttering is a multifactorial speech fluency disorder that results in unintended occurrences of sound syllable repetitions, prolongations, and blocks, particularly on the initial part of words and sentences. Decades of research on the topic have produced an extensive amount of data but the mechanism behind the symptoms associated with stuttering is not clear.

The aim of the present study was to investigate the neuronal basis of stuttering by looking at the brains neurochemistry utilizing the proton magnetic resonance spectroscopy (^{1}H –MRS) technique. In particular, we looked at the neurotransmitters N-acetyl Aspartate (NAA), an aggregate of Glutamate and Glutamine (Glx) and myo-inositol (mI) as potential candidates for understanding the biochemical manifestations of stuttering. We have also collected behavioral data from the PWS group and correlated it with their spectroscopy results. Finally, we combined the measurements of neuronal activity behind speech production, probed with functional magnetic resonance imaging (fMRI), with ^{1}H-MRS measurements in order to achieve information on the interaction between neuronal activation and underlying neurochemical function. The inferior frontal gyrus (IFG) was chosen as a target region for this investigation, given its' involvement in speech motor control.

Neurotransmitter mI showed the main group effect. The cerebral metabolite pattern of PWS is characterized by the pronounced reduction in myo-inositol level in the IFG. Myo-inositol is considered a glial marker and its concentration may reflect the condition of myelin in the brain. The myelination process is referred to as the maturation process of the fibers that facilitates rapid neural innervation of speech muscles underlying speech fluency. Hence, given the existing literature on the topic and our main findings we suggested that delayed or impaired myelination of the speech-related neuronal network in the postnatal period might be responsible for the later development of stuttering.

List of abbreviations

ANOVA	analyses of variance
BGTC	basal ganglia-thalamocortical
BOLD	blood-oxygen level-dependent
CNS	central nervous system
Cr	creatine
CV	consonant-vowel
DAF	delayed auditory feedback
DL	dichotic listening
DTI	diffusion tensor imaging
EEG	electroencephalography
FA	fractional anisotropy
FAF	frequency-altered feedback
FL	forced-left attention instruction
FR	forced-right attention instruction
GABA	gamma (γ)-aminobutyric acid
Glu	glutamate
GLM	general linear model
Gln	glutamine
Glx	glutamate and glutamine
^{1}H-MRS	proton magnetic resonance spectroscopy
Hz	Hertz
ICC	intraclass correlation
IFG	inferior frontal gyrus
mI	myo-inositol
MRI	magnetic resonance imaging
NAA	N-acetyl Aspartate
NF	non-forced attention instruction
PDS	persistent developmental stuttering
PET	positron omission tomography
PRESS	point resolved spectroscopy
PT	planum temporale
PWS	people who stutter

REA	right ear advantage
ROI	region of interest
SLF	superior longitudinal fasciculus
SMA	supplementary motor area
SPM	statistical parametric method
SSI-3	stuttering severity instrument-3
STG	superior temporal gyrus
T	Tesla
TE	echo time
WASSP	Wright and Ayre stuttering self-rating profile

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Introduction

Stuttering and components of stuttering

Stuttering is a multifactorial speech fluency disorder that manifests in frequent sound/syllable/word repetitions, prolongations or silent blockages (Guitar, 2014; Maguire, Yeh, & Ito, 2012). These are the "core behaviors" that notably compromise the fluent flow of speech (Van Riper, 1982). The temporal aspect of speech production, such as rate, rhythm, intonation and stress are also affected (Guitar, 2014). The basic speech behaviors of stuttering differ from "secondary behaviors" which are referred to as learned behavior in response to the initial the core behaviors. According to Guitar (2014) these associated symptoms are the results of attempts to escape (head movements, finger snaps: help to terminate the blocks) or avoid (substitutions: feared words are evaded) core behaviors. Bloodstein and Ratner (2008) suggest that the associated symptoms include overt and physiological concomitants. Overt concomitants of stuttering are responsible for visible tension in the face, eye blinks (squinting), "filled pauses" and altered pitch variations. Physiological concomitants result into cardiovascular phenomenons, prolonged eye fixations or aimless horizontal eye movements, flushing and sweating (Craig-McQuaide, Akram, Zrinzo, & Tripoliti, 2014).

Stuttering has a strong emotional component where communicative pressure may escalate stuttering episodes. People who experience problems with fluency may respond to their problem by feeling anxious, frustrated and often embarrassed. Due to negative self-image and created stereotypes stuttering may have a detrimental effect on the life quality, interpersonal relationship and possible job opportunities for the person who stutters (PWS) (Guitar, 2014).

Epidemiology of stuttering

Stuttering can be developmental or acquired. Both share similar neurochemical and physiological mechanisms, however acquired stuttering does not seem to generate secondary co-existent symptoms, such as facial grimacing, eye blinks and the general excessive articulatory struggle that results in muscle tremors. Acquired stuttering is less common and is often caused by a neurological event for example brain damage, tumor, stroke or use of pharmacological agents. It does not induce anxiety and can occur at all ages (Movsessian, 2005).

Persistent developmental stuttering (PDS) usually begins between ages 2 and 5 years (Guitar, 2014). According to (Yairi, 1997) the mean age of onset is 42 months

(3½ years). The primary disturbance in fluent speech includes: repetitions of a single sound (o-o-open), syllable (un-un-under) or a fragment of the word (a single-syllable word repetition) (on-on-on the table) (Bloodstein, 1995). Parents report repetitions as the most frequent signs of the speech labeled as "stuttering" for about 85% children, only 12% exhibit silent blockages and 11% physical concomitants (Taylor 1937; referred in Yairi & Ambrose, 2013). The actual location of a stuttered dysfluency does not seem to be arbitrary. It primarily occurs at syllable or word initial position and varies in a predictable way (Rosenfield, 2008). Bloodstein and Ratner (2008) found that PWS show consistency, while reading the passage a person with PWS would show the tendency to stutter on the same words/syllables. Researches also discovered that PWS show adaptation effect, they would show reduced percentage of words stuttered with repeated reading of the same passage. Furthermore, PWS could anticipate with surprising accuracy which word they would stutter on.

With respect to prevalence, more than 68 million people worldwide stutter. The prevalence of stuttering in children between 3-10 years old is equal 1.99% and 1.15% for school children between 11-17 years old (Boyle et al., 2011). Bloodstein (1995) estimated the adult prevalence to be less than 1%. Stuttering has a male/female ratio 3:1. Females who stutter are more likely to recover during early childhood, thus increasing the proportion of males with the disorder persistent after puberty (around 4:1) (Bloodstein, 1995; Yairi & Ambrose, 2013). Some children experience spontaneous recovery a few years after onset of symptoms while others continue to stutter throughout life. Spontaneous recovery accounts for about 75% of the individuals who ever stuttered (Maguire et al., 2012). Chang, Erickson, Ambrose, Hasegawa-Johnson, and Ludlow (2008) compared children with persistent stuttering, children who recovered spontaneously and age-matched fluent controls. They published a study on the differences in the integrity of white matter in 21children, from 8 to 12 years old. They found a decrease in white matter integrity in the superior longitudinal fasciculus underlying the sensory-motor cortex in both groups, those who persisted with the stuttering and those who recovered naturally. Their findings suggest that regardless of whether children continue to stutter or recover, they might show an aberrant brain connectivity pattern compared to their fluent peers.

Therapeutic interventions for stuttering cover a broad spectrum of difficulties experienced by PWS. Some are focused on stuttering modification or fluency enhancement where the goal is that PWS develop a speech pattern that is volitional

and consciously controlled, i.e. teaching clients to achieve gentle onsets of their vocal targets and speak at a slower pace. Some address the psychological and/or social concomitants of stuttering where the clients learn to cope with the negative feelings and attitudes towards their own stuttering and learn to monitor their speech fears and avoidances (cognitive behavior therapy) (Guitar, 2014; Chang, 2011). Several modern therapies include procedures for all stuttering behaviors and suggest a multidisciplinary approach as an optimal treatment of stuttering (Packman, 2012; Rosenfield, 2008). Many of the treatment options are not yet proven to be effective, hence there is little consensus over which management approach is the best choice for all (Bloodstein & Ratner, 2008). The overall agreement among speech-language therapists who specialize in fluency is that early intervention is important (Guitar, 2014).

Etiology of stuttering

Despite the high incidence of stuttering (11.2%, Reilly et al., 2013) little is known about its etiology. On the surface, stuttering appears to depend on the motor aspects of speech production, however it has been suggested that multiple factors interact in producing the dysfluencies associated with the disorder (Watkins, Smith, Davis, & Howell, 2008).

In the 20th century stuttering was understood as a psychogenic disorder. In the psychoanalytic approach stuttering was viewed as a product of conflict within the individual. In particular, stuttering reflected a neurotic personality. This approach did not lead to improvement in treatment, since focus only on the emotional aspects of the disorder restricted successful outcome. An argument was that since PWS show lack of self-confidence and tend to isolate themselves from social settings, it reflected the inner conflict. However, such behavior could as well be a consequence of the stuttering itself (Boberg & Webster, 1990). Some of the ideas may be found in more recent theories that suggest a link between the childs' personality and its interaction with the parents. They name arousal and nervousness as modulating factors of stuttering severity (Buchel & Sommer, 2004).

A different theory suggested that the family environment was a factor in shaping stuttering, in particular the personality and the behavior of the parents and their reaction to normal childhood dysfluencies (Johnson, 1955; referred in Guitar, 2014). Behaviorist Oliver Bloodstein (1997) proposed that stuttering starts in the child's attempt to avoid dysfluency (learned behavior). In this approach the child was

assumed to experience frustration from communicative failure, start to tense speech muscles and produce fragmented speech. The situation may be further worsened in an environment fraught with communicative pressure, that is when the performance demands excel the childs' speech and language capabilities (Bloodstein, 1997).

More recent studies have investigated the potential genetic components of stuttering (Maguire et al. 2012). Increased susceptibility to the disorder has been associated with disruption, mutations or deletions of multiple genes and combination of those, for example: the dopaminergic gene DRD2, chromosomes 1, 13, 16 (Cox & Yairi, 2000) and chromosome 12q (Riaz et al., 2005). Based on these findings one could conclude that stuttering is a polygenic disorder since no single gene has been identified.

Some associate stuttering already with the pre-motor level and suggest a strong correlation with linguistic and physiological processes (Craig-McQuaide et al., 2014). Specifically, one of the theoretical approaches suggests that a linguistic processing deficit is responsible for stuttered behavior since syntactically demanding speech enhances stuttering episodes (Perkins, Kent, & Curlee, 1991). Kolk and Postma (1997) suggested a "Covert Repair" hypothesis where the covert repair of phonological encoding accounts for stuttering behavior. In other words, they claimed that speech-planning processes in PWS are compromised and that their phonetic plans contain large number of errors. Fluent speakers are able to successfully repair defective speech plans (phonological encoding). PWS are slower in that process and their attempt to correct linguistic errors before they become expressed in overt speech result into repetitions, prolongations and blocks of stuttering (Kolk & Postma, 1997).

Van Riper (1982) claimed that the inability to produce smooth, uninterrupted speech is a result of disturbed timing of muscle movements within the speech motor control system. In particular, he believed that speech is a sequential motor task that needs exact timing to be able to move forward in the speech sequence. He argued that such insufficient initiation of speech segments could explain the stuttering behavior.

Experiments with delayed auditory feedback (DAF), (Black, 1951; Lee, 1951; referred in Guitar, 2014) where normal speakers start producing stuttering-like behavioral: repetitions, prolongations and blocks of utterances made researchers believe that stutterers' abnormal speech may result from certain disturbances of speech-motor output. One explanation of such a phenomenon is that auditory feedback is abnormally integrated with the speech motor execution regions in people

who stutter (Foundas et al., 2004). Fluency could, on the contrary, be enhanced by change in perception of auditory information. For example, by speaking with masked noise, to a rhythmic cue (such as speaking in cadence with metronome), by speaking with frequency shifts (slightly delayed or changed, the so-called frequency altered feedback (FAF), and/or by alternating auditory feedback of speakers own speech (DAF). The most potent fluency-inducing maneuver in stuttering is singing. Due to the acoustic structure of a song it goes through a different type of processing within the speech-motor control system (Chang & Zhu, 2013; Rosenfield, 2008).

PWS share some diagnostic features with motor-speech disorders such as Tourette's syndrome, dystonia and Parkinson's disease. Common for these disorders is basal ganglia dysfunction, thus a link between stuttering and basal ganglia anomaly has been suggested (Alm, 2005; Ludlow & Loucks, 2003). The basal ganglia are important subcortical gray matter structures located in the forebrain and influence motor behavior, emotions and cognition (Graybiel, 2000). They take part in modulating inhibition and excitation of the frontal lobe cortex (Alm, 2004). Alm (2004) proposed that the basal ganglia-thalamocortical (BGTC) motor circuits through the putamen to the supplementary motor area (SMA) are dysfunctional in PWS. In particular, that the basal ganglia fail to produce accurate timing cues for initiation of the next motor segment in speech. Watkins and colleagues (2008) have also reported on overactivity in the midbrain at the level of the substantia nigra supporting Alms' findings on abnormal function of the basal ganglia.

The left hemisphere is dominant for language in most people (Owens, 2012). A dichotic listening study by Brady and Berson (1975; referred in Rosenfield, 2008) suggested that abnormal cerebral laterality of language in people with PWS stem from abnormal auditory processing of linguistic information. Many of PWS lack the phenomenon right ear advantage (REA), which implies a left hemisphere advantage in language processing. The Orton –Travis theory, one of the most influential theories on the cause of stuttering, postulates that PWS lack normal language laterality (Guitar, 2014). Their electrophysiological (EEG) studies revealed evidence for right hemisphere dominance for language in PWS rather than lack of hemispheric dominance. In 1985, Geschwind and Galaburda hypothesized that delay in left-hemisphere growth during fetal development might be responsible for the difficulties with the fluency experienced by PWS. More recently, Webster (1990) has focused on

SMA and suggested that overactivation in the right hemisphere may disrupt the function of left hemispheric SMA to plan, initiate and sequence speech motor output.

All of the above listed theories on the etiology of stuttering may explain the underlying mechanisms of stuttering to a certain extent, however the real cause of dysfluency in PWS is yet not fully understood. Modern neuroimaging techniques allow researcher to study both anatomical and functional differences between PWS and fluent controls by using magnetic resonance imaging (MRI), functional magnetic resonance imaging (fMRI) and positron emission tomography (PET).

Fluency of speech production and language models

Speech is a process of planning and executing specific motor sequences that requires precise neuromuscular coordination. Fluent speech may be described as an ability of the speech apparatus to produce effortless, continuous and uninterrupted flow of speech (Owens, Metz, & Haas, 2007).

The formula for successful, i.e. fluent, smooth speech production is robust and well-established connections between brain regions that are crucial for auditory processing, motor planning and execution (Owens, 2012; Price, 2010). These functions are most likely to include areas in the frontal cortex of the brain and the temporo-parietal cortex. Subcortical regions are also involved, including the basal ganglia, thalamus and cerebellum. They support the sensorimotor integration of speech production by providing it with internally delivered timing and sequencing cues for speech movement. In addition, they mediate cognition, motor and emotion processing by interacting with the cerebral cortex (Chang, 2011).

The first important contributions to understanding the neuronal basis of language came from the French physician Pierre Paul Broca (1824-1880) and the German physician Carl Wernicke (1848-1905). Their assumptions were based on clinical observations and neuroanatomical examinations. Broca was the first to determine that speech production was associated with the inferior frontal gyrus (IFG) in the left hemisphere. Wernicke described an association between receptive problems with language and damage to the left superior temporal gyrus (STG) (Webb & Adler, 2008). Lesions in the IFG were understood to cause motor aphasia (Brocas' aphasia) and lesions to the STG caused sensory aphasia (Wernickes' aphasia). Dejerine (1895; referred in Friederici, 2011) was the first to suggest that Brocas' and Wernickes' regions were connected by the arcuate fasciculus, a white fiber tract underlying the angular gyrus. According to this speech-language model, the Wernickes' area

formulates the message or ideas that need to be expressed and then transmits those through the arcuate fasciculus to Brocas' area in the frontal lobe, which is responsible for phonetic coding. This coding occurs when phonemes and syllables are translated into a sequence of articulatory plans. Further, the signals are passed to the motor cortex that activates the muscles of speech responsible for initiation and coordination of movement sequences in speech articulators (Owens, 2012; Price, 2010).

A modern variant of such an auditory-motor integration theory is presented in the dual stream model that suggests the connection between the auditory cortex and speech planning and execution areas is achieved through the dorsal stream (Hickok & Poeppel, 2007). One stream connects sensory/phonological networks with conceptual-semantic systems (ventral), and one connects sensory/phonological networks with motor-articulatory systems (dorsal). In particular, the dorsal stream extends from the left posterior temporal lobe via the inferior parietal areas into the left IFG, including premotor areas (Specht, 2013). The dual stream model postulates that the dorsal steam is predominantly left hemisphere lateralized and translates speech signals to articulatory representations for speech production. Researchers suggest that the superior longitudinal fasciculus (SLF) that contains fibers from the arcuate fasciculus is an anatomical homologue of the dorsal stream. The SLF is a major white-matter tract that links brain regions responsible for speech planning (the inferior frontal region), sensory feedback of speech sounds (auditory regions) and speech-motor execution (the motor cortex) by transmitting nerve impulses between them (Chang & Zhu, 2013; M. Sommer, Koch, Paulus, Weiller, & Buchel, 2002; Watkins et al., 2008). The regions underlying the dorsal stream are crucial for intact speech production and rapid information exchange between them is essential for the fluency of the speech production. Thus, if the integrity of the white-matter pathway is compromised, there is a risk of a delay in information flow.

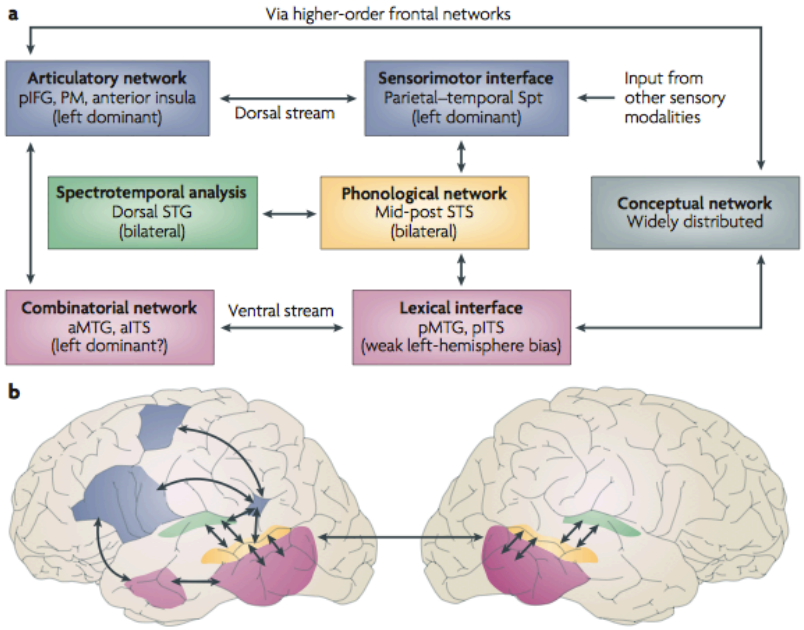

Figure 1: The dual-stream model of the functional anatomy of language. Adopted from "The cortical organization of speech processing", by G. Hickok and D. Poeppel, 2007, *Nature Reviews Neuroscience*, 8(5), s. 395. Copyright 2007 by Nature Publishing Group.

Structural and functional anomalies that might be responsible for the disturbances associated with stuttering

Decades of neuroimaging research has revealed substantial information on the neural processing underpinning spoken language suggesting subtle structural and functional differences in people with PWS in comparison with healthy controls.

Foundas, Bollich, Corey, Hurley, and Heilman (2001) have studied the anatomy of cortical speech-language areas in 16 adults who stutter. The results showed that the right and left planum temporale were significantly larger in PWS and that the leftward asymmetry was reduced in size compared to healthy individuals. Some years later Foundas and colleagues (2004) looked at the correlation between atypical planum temporale (PT) anatomy and changes in the effect of fluency inducing technique - delayed auditory feedback (DAF) on fluency in adults with PWS. They suggested a possibility for anatomically atypical rightward PT to induce stuttering. Moreover, they showed the presence of an extra gyri along the superior bank of the Sylvian fissure (in 14 out of 16 PWS participants). Such an anomaly was never observed in a control. In addition, an atypical structure of the diagonal sulcus

within the frontal operculum was registered. Their findings suggested that anomalies within the perisylvian speech-language areas might put an individual at risk of developing stuttering.

Alm (2005) claims that PWS have a dysfunction of the basal ganglia motor circuits. He proposed that the basal ganglia-thalamocortical (BGTC) motor circuits through the putamen to the SMA are dysfunctional in PWS. The basal ganglia are important subcortical gray matter structures located in the forebrain and influence motor behavior, emotions and cognition (Graybiel, 2000). They take part in modulating inhibition and excitation of the frontal lobe cortex (Alm, 2004). The role of the basal ganglia in stuttering was first uncovered through evidence that acquired stuttering often resulted from lesions in the basal ganglia areas (Ludlow, Rosenberg, Salazar, Grafman, & Smutok, 1987). The main function of the basal ganglia is coordinating the release of the correct motor programs and inhibiting potentially competing motor programs that might interfere with the execution of the specific motor act. According to Alm (2005), the basal ganglia fail to produce accurate timing cues for initiation of the next motor segment in speech. He emphasized the role of the putamen (one of the structures of the basal ganglia that together with the caudate nucleus forms the main input nucleus- the striatum) in speech motor control, whose main function is to regulate movements and influence various types of learning (Neumann et al., 2003). The basal ganglias' function is strongly regulated by dopaminergic activity (Rosenberger, 1980).

Disruption in white matter tract structures has consistently been implicated in the pathophysiology of stuttering. Jäncke, Hanggi, and Steinmetz (2004) found increased white matter volume within the right hemispheric language network in PWS. These areas include superior temporal gyrus (STG), inferior frontal gyrus (IFG), the somatosensory area (including the area underlying the face and mouth representation), and the middle frontal gyrus (MFG). The findings suggested abnormality in white matter pathways that connect speech relevant cortical areas with other areas, possibly indicating aberrant processing strategies in the right hemisphere in PWS. In addition, they reported on leftward asymmetry in white matter volume in fluent controls, while PWS showed symmetric volumes. Their findings are in line with a study conducted by Beal, Gracco, Lafaille, and De Nil (2007) where more white matter volume was found in pathways underlying the right IFG, the right insula and the left middle temporal gyrus. Cykowski, Fox, Ingham, Ingham, and Robin

(2010) also studied adults who stutter and reported low fractional anisotropy (FA) values in white matter underlying the left hemispheric IFG, frontal gyrus and the corpus callosum, suggesting myelination defects. Myelin is a white fatty sheath that covers large peripheral nerves and axons of central nervous system (CNS). Layers of myelin assure rapid transmission of the electrical impulse along the nerve fiber. The cranial nerves that are responsible for neuromotor control of the speech production are myelinated. This facilitates rapid neural innervation of speech muscles underlying speech fluency (Webb & Adler, 2008). Sommer and colleagues (2002) provided evidence for reduced white-matter integrity in the rolandic (or central) operculum of only the left hemisphere, just above the Sylvian fissure. Chang and colleagues (2008) examined the left superior longitudinal fasciculus (SLF) in 22 children. They reported on a subtle decrease in white-matter integrity that compromises the integration of motor plans and sensory feedback during speech production. This was suggested to be responsible for disturbed fluency in stuttering. Watkins and colleagues (2008) argues that reduced integrity of white-matter connections leads to aberrant brain function of the ventral pre-motor cortex in both hemispheres. Connally, Ward, Howell, and Watkins (2014) published a study were they examined 29 PWS who were heterogeneous with respect to age, sex, handedness and stuttering severity. Their results supported previous findings on abnormalities in white matter integrity in language and motor tracts in developmental stuttering.

All these studies have utilized diffusion tensor imaging (DTI) techniques, which use Magnetic Resonance (MR) to study microstructural features of white matter by examining the anisotropy of water molecule diffusion within white matter tracks. A measure describing the degree of anisotropy in the diffusion of water molecules is referred to as fractional anisotropy (FA). FA is thought to reflect myelination in the white matter. There is a claim that myelin plays an important role in determining the observed white matter tract diffusion anisotropy, however a clear association between the direction of anisotropy in the diffusion of water molecules and the myelin integrity remains unclear (Song et al., 2002). According to Sommer and colleagues (2002) “lower FA values can indicate decreased fiber coherence or myelination defects”. Furthermore, low FA values in white matter integrity could be a sign of dysfunctional connectivity between different regions in the brain responsible for auditory processing, movement planning and motor execution (Cykowski et al., 2010; Sommer et al., 2002).

Scientists interested in the brain-activation pattern in individuals who stutter during speech production tasks have reported on diminished activity in the left auditory cortex and hyperfunction in the motor regions of the right hemisphere (Braun et al., 1997; Chang, 2011; Fox et al., 1996). De Nil, Kroll, Lafaille, and Houle (2003) claimed that PWS recruit more neural resources during elementary speech production showing an elevated overall neural activation compared to controls. Even in silent reading tasks they mobilize the primary motor cortex and the cerebellum demonstrating an effort they put on the articulatory aspects (presumably not attributed to actual articulatory movements).

A single word reading study conducted by Salmelin, Schnitzler, Schmitz, and Freund (2000) have shown reverse functional connectivity pattern in adults who stutter compared with fluent individuals. During a fluent speech production right motor cortex has revealed evoked activation. Cortical activation sequence in PWS group showed first activation in left motor cortex followed by signal in left IFG. The results suggest that PWS send up signal to motor area before they accomplish the articulatory coding. Hence, they claim there is a deficiency in the network that includes IFG and motor/premotor areas that effects proficient speech production ability in PWS. Their findings get support in the study by Lu et al. (2010) were they found a weaker connectivity from left IFG to left premotor area in PWS and between right IFG and left motor cortex.

Early Positron Emission Tomography (PET) studies have also demonstrated functional difference in addition to volumetric inequality in brain regions. Brain activity during paragraph reading, stuttering and induced fluency has been investigated by Fox and colleagues (1996). They found hyperactivation of the right hemisphere motor system while reading in PWS. Controls showed largely left lateralized activation in areas of the motor cortex representing the mouth, motor planning areas, auditory regions, visual systems and the cerebellum. Moreover, diminished activation in left frontal-temporal regions in PWS compared to controls has been registered. Sommer and colleagues (2002) reported on signs of disconnection between speech-related cortical areas right below the laryngeal, pharyngeal and tongue representation in the left sensory-motor cortex. Their findings suggested a disturbed temporal pattern of activation in speech-relevant brain areas. The right hemispheric overactivation has been interpreted as a compensatory mechanism that compensates for a left hemispheric deficit and tries to attenuate

stuttering symptoms (similar to aphasia) (Sommer et al., 2002). The fact that right hemispheric overactivation negatively correlates with stuttering severity supports the notion that it is a compensatory activation, not a primary dysfunction (Neumann et al., 2003).

Chang and Zhu (2013) showed a decrease in functional connectivity in BGTC and auditory-motor cortical networks, including the pars opercularis and posterior superior temporal gyrus, primarily in the left hemisphere. The latter may affect speech planning and execution processes essential for fluent speech motor control. In addition, they reported on attenuated connectivity in neural networks responsible for self-initiated timing of speech movement. In general, PWS have decreased connectivity among cortical auditory-motor regions, in particular between the posterior superior temporal gyrus and the insula, the SMA and the IFG focused on the left hemisphere (Chang & Zhu, 2013).

Neurotransmitters and stuttering

The imaging studies presented above suggest that the production of fluent speech requires coordinated communication between the networks of cortical and subcortical brain regions. However, despite the growing body of research the etiology and pathophysiology of the disorder remains poorly defined. The dysbalance on the brains' cortical and subcortical level leads to the idea that there must be an aberrant communication on a micro level, in particular neurotransmitter level.

Neurotransmitters (metabolites) are the brain chemicals that transfer information throughout the brain by sending it between neurons through synapses. Neurotransmitters can be excitatory (stimulate brain function) or inhibitory (to create the balance when excitatory neurotransmitters are overactive). One of the most prominent is *N-acetyl Aspartate (NAA)*. NAA is believed to be a neuronal marker in the brain, with a reduced concentration suggestive of loss or damage to neuronal tissue. *Choline* is a cell membrane marker where increase in choline concentration may indicate cell production or membrane breakdown. *Creatine* is an energy metabolite that supplies energy to all brain cells. Concentrations of creatine are believed to be fairly stable in healthy individuals, although variations can arise in cases of tissue death or cranialcerebral trauma. *Lactate* is a product of anaerobic glycolysis, it accumulates when cells lack oxygen. *Lipids* store energy and constitute a cell membrane. *Myo-inositol* is a glial cell marker and is a breakdown product of

myelin. *Glutamine/glutamate and GABA* are neurotransmitters responsible for fast-action excitatory and inhibitory regulation, respectively (Brandao, 2004).

So far within the stuttering domain the main focus has been on the neurotransmitter *Dopamine.* Dopamine pathways are involved in motor control, movement regulation and emotional responses. Recent findings on neuronal abnormalities suggest elevated dopamine levels in the brains of people who stutter (Civier, Bullock, Max, & Guenther, 2011). The dopamine hypothesis in stuttering was developed after the discovery that drugs that block certain dopamine receptors in the brain can sometimes improve fluency. Since these drugs are dopamine receptor antagonists (e.g. haloperidol) (Healy, 1974), it was proposed that stuttering could result from hyperactivation in the dopaminergic system (Anderson, Hughes, Rothi, Crucian, & Heilman, 1999; Wu et al., 1997). However results are controversial given the work published by Goberman and Blomgren (2003) where the authors contradict the former statements by reporting no significant differences in dysfluency in Parkinson disease in low and high dopamine state. A PET study by Wu and colleagues (1997) provided support for the dopamine access theory of stuttering. They measured the rate of dopamine synthesis in the brain (labeled as FDOPA) and reported that stutterers have three times too high dopamine uptake in both cortical and subcortical areas of the brain. Alm (2004) reported an abnormality in dopamine balance, in particular increased D2 to D1 receptor ratio in the striatum in PWS. He claimed high D2 receptor density in the putamen might be responsible for stuttering behavior.

Research in field of neuroscience suggests that changes in the concentration of neurotransmitters in the human brain may cause a variety of pathological states. The above-mentioned studies on the dopamine hypothesis in stuttering suggests that a well-regulated dopamine release is essential for the proper function of the basal ganglia. Insufficient release of dopamine would lead to general inhibition of movements and impulses that might cause a dysfluency of speech. Given a complex interplay of neurotransmitters, receptors, and general brain plasticity (Hoffmann, 2013) it might be worth investigating the impact of other neurotransmitters in stuttering.

Study metabolites

The metabolites under investigation in this paper are *N-acetyl Aspartate (NAA), Glutamate/Glutamine (Glx) and myo-inositol (ml).* Each metabolite has a

unique chemical structure, which gives rise to a characteristic pattern of peaks in its measured spectrum. The concentration can be derived from the intensity (area) of the peaks. This is possible due to the fact that the area of the peak is proportional to the number of protons contributing to the signal; for an accurate measurement, a correction factor accounting for the relaxation properties of the material is also applied. Since absolute quantification tends to be sensitive to relaxation and partial volume effects (Dramsdahl et al., 2011), it is common to express concentrations relative to an internal reference - most often as ratios over Creatine, Choline or NAA. In this study we use a Creatine reference, since this metabolite is expected to be relatively stable and is commonly used in clinical practice for calculating metabolite ratios (De Graaf, 2007). In particular, we analyzed NAA/Cr, Glx/Cr, and mI/Cr.

N-acetyl Aspartate (NAA) is a highly abundant metabolite in the brain. It is synthesized in the mitochondria and its signal has been used as a marker of neuronal and axonal viability and density. The concentration of NAA decreases in case of neuronal loss as in many white-matter diseases and also in brain tumors. An increase in NAA intensity may indicate increase in the synapse number and axonal recovery (Graaf, 2007).

The second commonly quantified metabolite is *Glx*. Glx is a collective name for the neurotransmitters *glutamate (Glu)* and *glutamine (Gln)*. They have similar chemical structures that lead to very similar spectra, which makes accurate distinction between the two challenging. Accordingly, the combination of Glu and Gln, referred to as Glx, is evaluated as a more reliable measurement. Glutamate is a major excitatory neurotransmitter in the brain and therefore important for neuronal communication through long-range cerebral connections. Approximately 80 % of all neurons use glutamate (Agarwal & Renshaw, 2012). It also serves as the precursor for the main inhibitory neurotransmitter gamma (γ)-aminobutyric acid (GABA) and is involved in the reduction of neuronal excitability throughout the CNS. Glutamate also plays an important role in the migration, differentiation and death of cells (Bracken et al., 2011; Brandao, 2004; van der Graaf, 2010). Moreover it is a critical molecule responsible for synapse formation and dendrite pruning (Agarwal & Renshaw, 2012). Glutamine is synthesized from Glu and its signal has been used as an astrocyte marker. However, the main function of Gln is in ammonia (bi-product of the breakdown of protein) detoxification. *Myo-inositol (mI)* has been proposed as a glial disease marker. The concentration of ml increases with glial-cell size and with glial

proliferation (van der Graaf, 2010). In addition it is believed to represent the product of myelin degradation (Brandao, 2004).

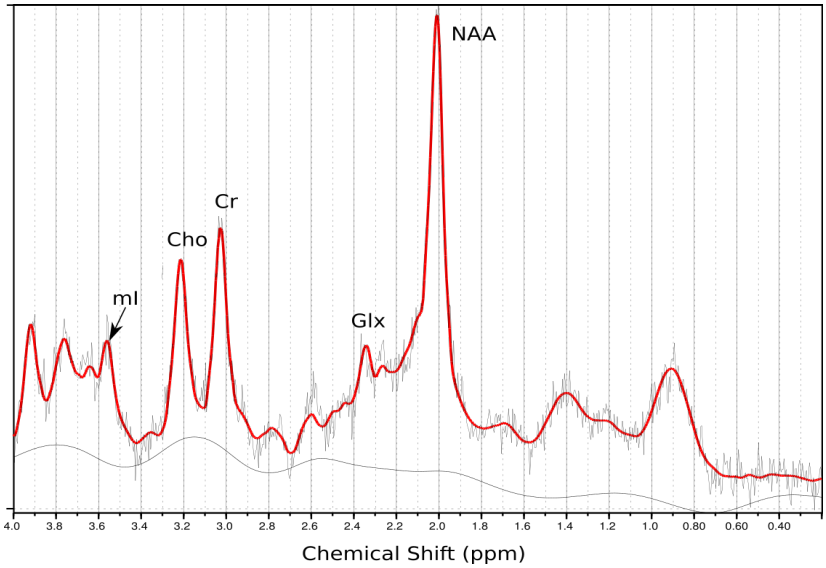

Figure 2. A representative ^{1}H-MRS spectrum from the inferior frontal gyrus (IFG). We can see characteristic peaks from which NAA, Glx and mI (marked with the arrow) are measured.

Variation in the concentration levels of the above mentioned metabolites provides chemical information on the region of interest, which may relate to observed structural, functional or behavioral changes. In the current study the brain region considered was the inferior frontal gyrus (IFG).

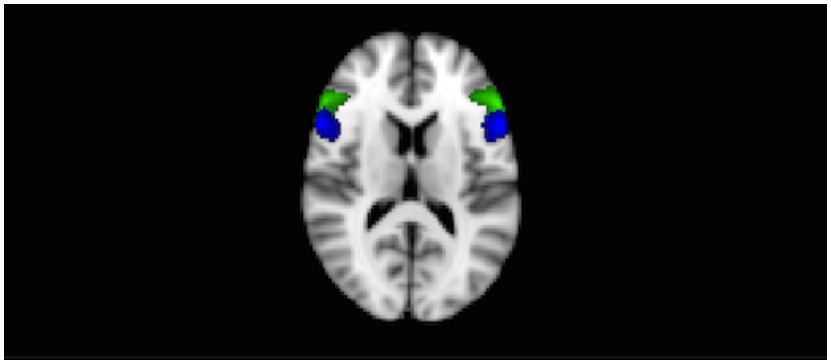

Figure 3. Highlighted is the area of the IFG, the pars opercularis (blue) and the pars triangularis (green). Axial view

Region of interest – the inferior frontal gyrus (IFG)

Stuttering is a neurodevelopmental disorder associated with left inferior frontal structural anomalies. Traditionally, the (pre)frontal lobe is understood as having execution functions and as being responsible for control, organization and synthesis of sensory and motor information, functions essential for successful speech production (Owens, 2012). For example, Greenlee and colleagues (2004) conducted a brain stimulation study and provided evidence that the IFG has a functional corticocortical connection with the orofacial representational area of the primary motor cortex. The authors conducted an electrophysiological experiment by stimulating the brain areas with electrical impulses. Their findings yielded that the IFG and the orofacial motor cortex connect in a reciprocal way. Electrical stimulation of the precentral motor cortex has shown to cause disruptions of speech fluency, loss of voluntary control of phonation (laryngeal representational area) and vocal fold adduction (Greenlee et al., 2004).

The IFG is a part of the neuronal network that underlies the region that accounts for speech motor control (Beal, Gracco, Brettschneider, Kroll, & De Nil, 2013). Functional neuroimaging studies claim that the IFG is a region within the neuronal network that supports both word production (phonology) and word comprehension (semantics) (Binder et al., 1997). There are three macroanatomical regions that compose the gyrus, the pars opercularis, the pars triangularis and the pars orbitalis. The pars opercularis lies in the anterior area of the IGF and cytoarchitectonically corresponds to Brodmann area 44. The pars opercularis is associated with sequencing linguistic and nonlinguistic events as well articulatory events (Papoutsi et al., 2009). The pars triangularis lies behind the pars opercularis and is associated with Brodmann area 45, which together with Brodmann area 44 comprises the well-known Brocas' speech area. Finally, the pars orbitalis is an orbital part of the IFG and is associated with Brodmann area 47. It is located anterior and inferior to the pars triangularis and appears to be active during vocalization processes (Schulz, Varga, Jeffires, Ludlow, & Braun, 2005). Since all the constituents of the IFG contribute to language-related tasks the integrity of all the regions is essential for normal speech and language function (Greenlee et al, 2004).

Functions specific to the left IFG, in particular Brocas' area, include motor-based speech production and articulatory coding. Moreover it coordinates sensory and semantic information that is received from the posterior parts of the neural language

network (Binder et al., 1997). In particular, Brodmann regions 44 and 45 have been proven to support the computation of syntactic movements (Friederici, 2011).

Structural MRI shows that the IFG develops abnormally in children who stutter. Chang and colleagues (2008) published findings were children who stuttered had less gray matter volume in the bilateral IFG compared to fluent controls. Adults who stutter have also been shown to differ in gray matter volume in the left IFG compared to controls where the study group showed more gray matter than the fluent controls (Lu et al., 2010). Watkins and colleagues (2008) reported on significantly lower fractional anisotropy (FA) of white matter in the PWS group compared to control group in the pars orbitalis in the right IFG, left and right posterior IFG. Left posterior IFG on the contrary have shown to have higher FA in the PWS group in opposition to controls. Other studies also report on abnormalities in white matter pathways in PWS lying underneath IFG area (Beal et al., 2013; Beal et al., 2007; Cykowski et al., 2010; Jäncke et al., 2004).

Research questions and hypothesis

Overall aim: Investigate the neuronal underpinnings of stuttering and its variability/severity depending on individual level/concentration of a certain neurotransmitter.

Question 1: Are there differences in neurotransmitter concentrations (NAA, Glx and mI) in people who stutter compared to controls?

Question 2: Are there correlations between individual levels of concentration of certain neurotransmitter and severity of stuttering (correlation between ^{1}H-MRS and behavioral measurements)?

Question 3: Are there correlations between fMRI and ^{1}H-MRS data? Is there a direct relationship between the studied neurotransmitters and the blood-oxygen level-dependent (BOLD) response within the measured regions? Does the concentration of the neurotransmitters in the IFG (NAA, Glx and ml) correlate with the IFG (pars opercularis, pars triangularis and pars orbitalis) BOLD response?

Hypothesis:

- Hypothesis related to the first research question:

  H1: There are differences in neurotransmitter concentrations between people who stutter and fluent controls, i.e. the concentration of NAA is expected to be comparable between groups and hemispheres; we expectd a higher concentration of Glx in the right hemisphere in the PWS group (reflecting

right hemisphere overactivation in PWS); the level of mI is expected to be lower in both hemispheres in the study group compared to the controls (reflecting decreased white matter integrity in PWS).

H0: There are no differences in the levels of neurotransmitters between people who stutter and fluent controls.

- Hypothesis related to the second research question:

H1: There is a correlation between ^{1}H-MRS data and behavioral measurements i.e. negative correlation for mI level for both sides; positive correlation for right hemisphere Glx; no correlation with NAA

H0: There is no correlation between ^{1}H-MRS data and behavioral measurements.

- Hypothesis related to the third research question:

H1: Levels of neurotransmitters from the IFG correlate with the IFG BOLD response, i.e. we expected increased Glx corresponding with greater neuronal activity in the right hemisphere in people who stutter compared to controls.

H0: Levels of neurotransmitters from the IFG do not influence the BOLD response from the IFG.

Methods

The present study is considered a quantitative cross-sectional correlational design study where we focused on brain chemistry and stuttering. We studied the phenomenon through the numerical representation of observations, using neuroimaging technique and behavioral measurements with a cross-sectional research design focused on studying existing differences between people and phenomena at a specific point in time. Such a research design does not entail the manipulation of variables, hence the inferences based on findings rely on existing differences rather than change following intervention (Cozby, 2005; Howitt & Cramer, 2005).

We will be using *Magnetic Resonance Imaging (MRI)* and *Proton magnetic resonance spectroscopy (^{1}H –MRS)* technique to examine concentrations of *N-acetyl Aspartate (NAA)*, *Glutamate/Glutamine (Glx)* and *myo-inositol (mI)* in both individuals who stutter and healthy controls.

MRI and fMRI

Magnetic Resonance Imaging (MRI) uses the properties of nuclear magnetic resonance to generate high spatial resolution anatomical images of the brain. The MRI technique has been introduced in 1970s and has had a dynamic development. Since

the early 1990s *functional MRI (fMRI)* as an extension of MRI has provided a non-invasive measure of brain activity by accessing hemodynamic changes in response to increased neuronal activity (Westbrook, Roth, & Talbot, 1998). In other words, it measures increase or decrease in blood oxygenation while a subject performs an experimental task. The fMRI method, which takes advantage of different magnetic properties of oxygenated and deoxygenated blood (MR signal intensity changes between stimulus and rest) referred to as the blood-oxygen level-dependent (BOLD) contrast. The BOLD technique is based on the assumption that the increase in blood oxygenation reflects increases in neuronal activity (Ogawa & Lee, 1990). Increased neuronal activity leads to increased demand on oxygen; in order to meet this demand, blood flow to the region increases. The increased supply of oxygenated hemoglobin actually exceeds the neuronal demands, hence a surplus of oxygenated hemoglobin is observed. Oxygenated blood is diamagnetic (has only weak magnetic properties) while deoxygenated blood is paramagnetic which means it has both unpaired electrons and a significant magnetic field, introducing local inhomogeneities and alterations in the MR signal (Huettel, Song, & McCharthy, 2009). Hence, the BOLD fMRI technique indirectly measures neuronal activity by measuring changes in the magnetic field as a result of changes in blood oxygenation. The BOLD response for each experimental condition was obtained by contrasting the task response and the baseline condition.

^{1}H –MRS

Proton magnetic resonance spectroscopy (^{1}H –MRS) is another technique that provides clues and suggests the role of neurotransmitters in the case of diseases (stuttering) in vivo. It is a non-invasive technique that is based on the magnetic properties and energies of nuclei and it is able to quantify biochemical compounds (metabolites) in the brain (Soares & Law, 2009). MR spectroscopy can measure a number of nuclei including fluorine, carbon, sodium and hydrogen nuclei (^{1}H). The latter is the most abundant in biological tissue and the most widely used in MR imaging. The strength of the magnetic field determines a frequency on which ^{1}H-nuclei resonate, in accordance with the Lamor equation:

$$\omega = -\gamma B$$

In different chemical environments the ^{1}H-nuclei will have somewhat different effective fields because of chemical shielding from surrounding electrons, leading to slight shifts in the observed resonant frequency of ^{1}H in that material.

^{1}H-MR spectra of the brain can be assessed by using point resolved spatially localized pulse sequence (PRESS) and can particularly be obtained from a single well-defined volume in a specific region of the brain (single-voxel spectroscopy). The metabolites get demonstrated in the plot of the series of peaks (a water-suppressed spectrum) where signal intensity versus chemical shifts are depicted and show the relative contribution of different elements within a tissue sample. The spectrum characteristics vary from substance to substance as a result of local shielding and spin coupling within the molecular structure, allowing particular metabolites to be identified and measured from the spectra (De Graaf, 2007).

Participants. Inclusion and exclusion criteria

Sixteen persons with PWS and twenty-seven controls were included in the study. The PWS were between 17 and 41 years of age (M=26.8; SD=1.7). The PWS were recruited through the "Norwegian organization for people who stutter" (NIFS) and "Centre for Adult Education" in Bergen, Norway. All the participants from the experimental group have been previously diagnosed with persistent developmental stuttering (PDS). The sex ratio is 13 males and three females in the PWS group and 12 male and 15 female participants in the control group. The controls were recruited from the student population in Bergen using e-mail. They were also between 20 and 36 years of age, but with a somewhat lower age mean (M=24.5; SD=0.7).

Participants were all native Norwegian speakers. People who stutter were excluded from the sample if they had co-existent deficits, namely speech- and/or language disorders, reading- and/or writing disabilities, dyslexia or Attention Deficit Hyperactivity Disorder (AD/HD). For safety concerns with regard to MR procedure see *Ethical issues.*

Hearing test

Due to the verbal/oral nature of the speech perception and speech production paradigm, the hearing threshold was assessed with the Hughson-Westlake audiometric test (Oscilla USB-330, Inmedico, Lystrup, Denmark). The test was conducted on a separate computer and covered hearing thresholds in frequencies of 250 Hz, 500 Hz, 1000 Hz, 2000 Hz, and 3000 Hz. Participants with an averaged inter-aural acuity difference of more than 10 dB were excluded.

Handedness test

The subjects handedness was checked by means of the Edinburgh Handedness Questionnaire (Oldfield, 1971). In the control group 23 subjects were strictly right

handed, three showed a preference for the right hand, and one showed a left hand preference. In the PWS group 13 subjects were strictly right handed, one strictly left handed, and two showed a left hand preference.

Data collection procedure

The current paper will be using already existing data on auditory perception, speech lateralization and stuttering. Four master students carried out the data collection under the supervision of Dr. Karsten Specht from autumn 2008 to summer 2012.

The MRI experiment was conducted at Haukeland University Hospital, Radiological department. The general MR procedure relevant for the present study consisted of the following sequences that were run on 3 Tesla GE Signa HDx MR-scanner: T1-weighted structural imaging, fMRI while performing modified DL task (described below), T2-weighted sequence and ^{1}H-MRS sequences in the left and right IFG.

^{1}H-MRS. In-vivo ^{1}H-spectra was measured from two voxels placed bilaterally in the IFG, using a single-voxel point resolved spectroscopy (PRESS) sequence (echo time TE=35ms; repetition time TR=1500ms, 128 averages, voxel size 20 x 20 x 20 mm).

^{1}H-MRS spectra was analyzed using LCModel software (Version 6.2-1A; Provencher, 1993), allowing for quantification of the metabolite levels. The levels of NAA, Glx and mI, are quoted as ratios over creatine. The fact that the concentration of the total creatine is relatively stable makes it a common choice of metabolite for internal referencing (De Graaf, 2007). The data were checked for suitable quality (line width and signal-to-noise ratio) and reliability of fit (% SD of estimate).

Dichotic listening task. The current study used fMRI- DL paradigm presented as a pseudo- randomized block design composed of two experimental conditions and one control condition. Dichotic listening is an experimental task that consists of two consonant-vowel syllables (i.e. /ba/ and /pa/) that are presented simultaneously to the listener, one to the right ear and the other syllable to the left ear (Hugdahl & Andersson, 1986; Hugdahl et al., 2009). The test is used as a behavioral measure of hemispheric lateralization of speech perception (Hugdahl, 2011). Traditionally, there are three instruction conditions in the DL task: the non-instructed or non-forced (baseline condition), the forced-right (FR) is when the participant is instructed to

focus the attention on the right ear stimulus and the forced-left (FL) when the participant is instructed to focus the attention to the left ear stimulus.

We used a modified version of such paradigm, which was designed to measure both speech perception and speech production, and included only the non-forced (NF) DL condition. In the experimental conditions the syllables were presented with different instructions. The first condition required subjects to attend to the stimuli allowing measurement of activity associated with speech perception. In the second condition the participants were additionally required to produce the syllable they thought they heard best, allowing the measurement of activity associated with speech production. Control condition consists of a resting task block where the participants were relaxing. The blocks were alternated and therefore it is possible to determine which voxels were activated as a function of differences between the blocks. Each block with one of three conditions was composed of 10 stimulations and the order of the tasks were counterbalanced. The presentation time was 400-500 ms with sound intensity of 87 dB. The paradigm was administered by E-prime software (2.0 Psychology Software Tools, Inc) and the participants listened to the stimuli through MR compatible headphones.

The fMRI experimental design. The fMRI was performed using an echo-planar imaging (EPI) sequence which was oriented using the structural image. A sparse sampling protocol was used with a TR = 5.5 seconds and TA of 1.5 seconds, leaving silent gaps of 4.0 seconds between the scans. This is when the auditory stimuli were resented and the participants could give an oral response without the interference of scanner noise and movement artifacts as a result of articulation.

Image pre-processing and statistical analysis were performed using the Statistical Parametric Mapping (SPM 8) analysis software package (Wellcome Department of Cognitive Neurology, London, UK). EPI images were first realigned, movement corrected, normalized, re-sampled and smoothed. Next, our data were subjected to a general linear model (GLM) that indicated the separate start of stimulation for each condition. Based on a stimulus onset time of both experimental conditions (perception/production), we obtained single contrasts specified for each condition and participant separately. The SPM contrast for ROI analysis was “NF condition minus rest condition” where we decided to concentrate on speech production activity.

In order to explore regional effects, the heamodynamic response of activation was studied from the same regions of interest as the ^{1}H-MRS measurement, i.e. the IFG. The BOLD signal from the regions of interest: the pars opercularis, pars triangularis and the pars orbitalis has been extracted in order to compare fluctuation in neuronal activity and the BOLD signal change that occurs due to the DL stimulus presentation with the metabolites detected by the ^{1}H-MRS technique.

Behavioral data. The behavioral part of the data collection was performed in an audiolab in a sound-attenuated room on the 9th floor at the Institute for biological and medical psychology, University of Bergen. This part of the data collection included only the individuals who stutter. Administration of the *Stuttering Severity Instrument-3 (SSI-3)* (G. Riley, 1972) was video recorded and was used for re-analysis. In particular, a videotaped sample of 257 syllables on a reading task (Norwegian text "Bestefar og sønnesønn") and approximately 500 syllables from the conversational speech were transcribed. Part-word repetitions, monosyllabic whole-word repetitions, sound prolongations and blockages of sound were identified and marked in the transcript. Rephrasing, repetition of phrases or whole words, and pausing without tension were not counted as stuttering (J. Riley, Riley, & Maguire, 2004). The percentage of syllables stuttered (%SS) gave us an overview over the frequency of stuttering. In addition, the duration of stutters, i.e. the average length of the three longest stuttering events were assessed and physical concomitants were evaluated.

Objective measures on severity of stuttering were further supplemented with the subjective measure from *The Wright and Ayre Stuttering Self-Rating Profile (WASSP)*- Danish version *(Wright & Ayre, 2000)*. In the self-rating WASSP questionnaire participants are asked to rate 24 items using a 7-point Likert scale to describe self-perceived stuttering severity (1-none, 7-very severe). The items are grouped into five sections: stuttering behaviors (8 items), thoughts about stuttering (3 items), feelings about stuttering (5 items), avoidance due to stuttering (4 items), and disadvantage due to stuttering (4 items). The participants from the stuttering group completed the WASSP questionnaire after SSI-3 testing took place.

Statistical analysis and results

The statistical analysis was done using the Statistical Package for the social sciences (SPSS), version 21.0 (IBM Corp., New York, USA). We used $p < .05$ as a significance level for all statistical analysis.

Individual levels of NAA, Glx and mI were determined in a resting state, using proton magnetic resonance spectroscopy (^{1}H-MRS) from two voxels placed bilaterally in the IFG. The concentrations of these metabolites were compared between the PWS and fluent controls. The correlation coefficient was calculated to investigate the relationship between stuttering severity as measured through the SSI-3 and WASSP (Wright & Ayre, 2000) with the concentration of each of the study metabolites. Finally, we aimed to investigate the complex interplay between neurotransmitters in the modulation of neuronal activation, where high or low concentration of the study metabolites could be responsible for the local effect during task performance. Hence, we tried to supplement the above findings with the additional correlation analysis of fMRI (ROI)/^{1}H-MRS-data to investigate the relationship between the neurotransmitter mI and the BOLD response from the regions of interest (pars opercularis, pars triangularis and pars orbitalis).

Spectroscopy data. Group comparisons were made between the PWS and fluent controls as categorical variables and between levels of NAA/Cr, Glx/Cr and mI/Cr as dependent variables from both left and right hemispheres. Three 2 x 2 repeated measures analyses of variance (ANOVA) within the framework of the GLM statistical model were performed. The general linear model (GLM) repeated measures is a statistical technique that provides the analysis of variance when the same measurement is performed several times on each of the subjects. It can be used to test the null hypothesis about the main effect between and within subjects' factors. Moreover, with GLM repeated measures one can investigate covariate effects and interaction between covariates and between subject factors (Cohen, Cohen, West, & Aiken, 2003).

The results from the study demonstrate that PWS have different concentration levels of metabolites compared to fluent controls. The cerebral metabolite pattern of PWS is characterized by the pronounced reduction in myo-inositol level in the IFG. Concentration of NAA and Glx in the IFG are also somewhat reduced but they follow a similar pattern with the fluent controls and the concentration peaks are not significantly different from the controls.

Correlation analysis. The results from the ^{1}H-MRS study were correlated with the participants' results on the SSI-3 and WASSP to investigate possible connections/dependences. There were no significant correlations between the subjects SSI-scores and each of the study metabolites. However, the correlation between the

^{1}H-MRS data and the results from the WASSP did reach a conventional level of statistical significance on one of the measurements, *stuttering behaviors*. The subtest consists of items on frequency and range of stuttering behaviors, in addition to the persons' defense and coping reactions to stuttering.

Combined fMRI/^{1}H-MRS analysis were further set up as a factorial design within the framework of the GLM statistical model. First, a 2 (groups) x 2 (sides) x 3 (ROI) repeated measures analysis of variance (ANOVA) was conducted to check whether there was a difference in the regions of interest between the groups based on hemispheric side. In the next 2 x 3 ANOVA calculations we treated groups as a between-subject factor and the regions of interest as a within-subject factors. We performed this analysis separately for the right and left hemisphere. In addition, we added the neurotransmitter myo-inositol that showed a fundamental difference between the PWS and controls as a covariate to check whether the BOLD signal from the region of interest could possibly be influenced by the mI concentration level (ANCOVA). The findings did not show any correlation between local ml concentration in either the left or right hemispheres and the strength of the BOLD response within the measured regions.

Probability adjustment. In order to avoid false positive interpretation of the results, i.e rejecting the null-hypothesis when it is in fact is true (Type-I error) one usually applies various correction procedures such as the Bonferroni correction. Especially, when the same dataset has been subjected to multiple statistical testing. However, Bonferroni correction may be considered too conservative, and increases the risk of a Type-II error, whereby the null-hypothesis is accepted when it is false (Polit & Beck, 2012). In our study, given the main effect of group on mI and strongly significant p-value on the correlation analysis the probability adjustment was not considered necessary.

Discussion

The aim of this study was to investigate the neuronal underpinnings of stuttering and its variability/severity depending on individual level/concentration of a certain neurotransmitter.

The main findings

Our results suggested a role of the neurotransmitter myo-inositol in stuttering. Myo-inositol is considered a glial marker. The type of glial cells called oligodendrocytes are responsible for the production of myelin. Myelin is a white fatty

sheath that covers large peripheral nerves and axons of CNS. Layers of myelin increase the speed at which impulses propagate along the nerve fibers. The cranial nerves that are responsible for neuromotor control of the speech production are myelinated. This facilitates rapid neural innervation of speech muscles underlying speech fluency (Webb & Adler, 2008). The process of myelination continues during the first postnatal year and is completed in the second year of life. Some of the white matter tracts continue to myelinate past the first decade of life. During early childhood, the volume increase in myelinated white matter in the speech-related areas parallels the time of language acquisition and development, however it happens later than in the sensorimotor region (Brauer, 2009). Hence, the myelination process is also referred to as the maturation process of the fibers.

The work by Cykowski and colleagues (2010) suggests that factors responsible for the clinical symptoms of stuttering involves a deficit in the myelogenesis in the white fibers of the areas responsible for spoken language. In particular, they showed a robust left-hemispheric FA reduction in the third division of the superior longitudinal fasciculus (SLF III) extending rostromedially into the left anterior corona radiata and left forceps minor. They referred to those regions as late-myelinating associative and commissural fibers (Cykowski et al., 2010). The SLF is a major white-matter tract that links brain regions responsible for speech planning (the inferior frontal region), sensory feedback of speech sounds (auditory regions) and speech-motor execution (the motor cortex) by transmitting nerve impulses between them (Chang & Zhu, 2013; M. Sommer et al., 2002; Watkins et al., 2008). A number of studies reported on reduced FA in left-sided white-matter tracks in both children and adults that stutter (Chang et al., 2008; Cykowski et al., 2010; M. Sommer et al., 2002; Watkins et al., 2008). Reduced FA in the SLF could therefore imply that PWS might have a dysfunctional connectivity in the language dorsal stream (see the Dual stream model, Hickock and Poeppel, 2007).

Combining the hypothesis suggested by Cykowski and colleagues (2010), existing literature on the white matter deficiency, and our findings on diminished concentration of mI in the study group we may hypothesize that insufficient formation of the myelin sheath during brain development may cause disruption in functional connectivity between the areas responsible for fluent speech production. In other words, delayed or impaired myelination of the speech-related neuronal network in the postnatal period might be responsible for the later development of stuttering.

Ethical issues

The present study has received approval from the Regional Committee for Medical Research Ethics in Western Norway (REK-Vest), number 212.08 and informed consent was obtained from each subject before the experiment (see attachment 2, 3). Respect for the patients' autonomy is an important concept in health-research and ethics which promotes that every human being has the right to decide whether they wish to participate in the study or not (Cozby, 2005; Dunn, Nowrangi, Palmer, Jeste, & Saks, 2006). The informed consent form is concerned with the protection of the experimental group from physical and mental violations of integrity (De nasjonale forskningsetiske komiteene, 2006). All participants have been treated in accordance with the Helsinki Declaration (World Medical Association, 2008). The Helsinki Declaration is a set of ethical principles in medical research involving human subjects. It postulates that the health of the patients/participants is the first consideration of the physician who is obligated to act only in the patient/participants interest.

The informed consent sheet contained information about the purpose of the project, participants right to withdraw from the project at any time, and that the data was processed anonymously. With regard to the MR procedure, the participants were thoroughly checked for any condition that may pose a threat for their health and safety. Because of the strong magnetic field people who had metal in their bodies, such as surgical clips, heart pacemaker, metal splinter or those that underwent neurosurgical procedures were not allowed at the scanner. Pregnant women as well as those who suffer from claustrophobia were not recommended to participate in the MR study. In addition, participants completed an MR safety checklist presented by radiographers as a complementary safety measure. The radiographers monitored participants throughout the entire MR session, held contact via microphone, made sure that safety protocols are followed. The MR scanner is very noisy and participants had to lie still in a rather narrow tube for more then an hour. This can lead to uneasiness and discomfort. In case a participant felt uncomfortable during scanning procedure and wanted to abort the experiment, they could do so any time with an alert button. The participants were also informed that the doctor routinely scrutinized MR images and if there was found anything that might represent illness, for instance a brain tumor, they would be contacted for further medical investigation. This otherwise unnoticed discovery might lead to an earlier intervention and a better outcome. At the

end of the experiment participants were thanked for their cooperation and compensated for their expenses. Controls received 200 NOK and PWS received 300 NOK, which was justified by the fact that they underwent behavioral testing in addition to MR scanning. The compensation was considered equitable (appropriate for the time and effort subjects devote to participation) and did not violate the principles of true volunteering (De nasjonale forskningsetiske komiteene, 2006).

Internal and external validity

The main concern with this dataset is the sample size. Polit and Beck (2012) argue that there must be at least 20 participants in a sample to get a stable and statistically significant outcome that cannot be explained by random chance. This means that our possible findings will need further evidence in greater data sets. Our study can however give direction to further studies. It may also indicate a tendency (show a trend) if not statistical power.

Because the data sample has an unequal number of male and female participants in the PWS group (13 male and 3 female), sex differences within the stuttering group should be interpreted with caution. This unequal number reflects the fact that more males than females stutter (3:1). One could claim that this dataset supports the notion of a higher rate of spontaneous recovery in females during early development. Such a discrepancy in sex ratios negatively impacts the internal validity. It may however, strengthen the external validity by making the sample more representative for gender distribution. In order to make statements about the whole population of PWS we would have needed a higher number of participants. The control samples size of 27 participants was also much larger than the PWS sample of 16 participants, probably causing a mismatch of the variance between the PWS and control groups. This makes the results from the PWS group more susceptible to individual differences.

I. E. Sommer, Aleman, Bouma, and Kahn (2004) performed a meta-study to access language-related brain activation. A total of fourteen fMRI studies were included and the result revealed no significant differences in language lateralization between men and women. Gender is a mediating factor in hemispheric asymmetry with men showing stronger lateralization than women when tested by means of DL (Hirnstein, Westerhausen, Korsnes, & Hugdahl, 2013). The fact that we included four left-handed subjects in the sample of participants means that the data has not been kept homogenous with respect to handedness. In 90% of people who are right-handed

the language is processed in the left hemisphere of the brain. Left-handers, on the contrary show a difference in brain asymmetry, often showing rightward lateralization of language areas (Hirnstein, Hugdahl, & Hausmann, 2014; Westerhausen et al., 2006). Because left-handers show a different pattern of language lateralization, it might compromise internal validity. Inclusion of the left-handed participants could strengthen the external validity, however our sample size remains too small to make inferences about the general population of PWS, with regard to handedness (e.g., Knecht et al., 2000)

The ^{1}H-MRS literature on hemispheric asymmetry is rather contradictory. For example Sijens et al. (1999) found in their cohort study a chemical asymmetry in the frontal lobe in the general population, while C. M. Braun and colleagues (2002) did not observe similar findings. With regard to gender, they found a higher concentration of NAA/H_2O in the frontal lobe of women compared to men. Furthermore, with respect to right hand preference, C. M. Braun and colleagues (2002) reported an interaction with the hemisphere, which they explained as right-handers had significantly higher concentration of NAA/H_2O in the left hemisphere. Bernard et al. (1996) studied an interaction between hemispheric asymmetry, gender and hand preference (using NNA ratio over choline) and found a strong interaction in the frontal lobe. When Braun and colleagues tried to replicate the findings they failed to achieve similar effects (Braun et al., 2002). They named a choice of internal reference metabolite as a confounding factor that complicated the interpretation of the effects. Given the above examples we can assume that MR spectroscopy is able to reveal some chemical brain asymmetries, separate men from women and distinguish dextrals from sinistrals. However, it remains unclear to which extent those differences influence the biological and physiological state of the brain.

SSI-3. The SSI-3 is the most commonly used measure of overt stuttering behaviors and is a useful tool for tracking changes in severity during and after intervention (Guitar, 2014; Howell, Soukup-Ascencao, Davis, & Rusbridge, 2011). The SSI-3 measures three parameters, stuttering frequency (percentage of stuttered syllables), duration of the three longest selected stutters and observed physical concomitants (e.g. distracting sounds, facial grimaces, etc.). The total score is obtained by adding together the scores for the three dimensions above. Rileys' (1994) assessments of intra-, and inter-judge reliability of the SSI-3 were recognized as satisfactory. Mean agreements for intra-judge reliability ranged from 75% to 100%,

while the mean agreement for inter-judge reliability ranged from 94.6% to 96.8% for %SS, from 58.1% to 87.2% for duration, from 59.8% to 97.5% for physical concomitants, and from 71% to 100% for overall scores (G. Riley, 1994). The construct validity (whether the test measures what it claims) was also considered and proved to be acceptable. Riley reported on significant correlations between the total SSI score and each of its components (frequency, duration and physical concomitants). In 1995, Lewis replicated statistical evaluations of the SSI-3. She reported significantly lower agreement levels and concluded that the test does not meet a criterion for reliable or valid measure (Lewis, 1995). McCauley (1996) criticized the sample of children and adults on which SSI-3 was normed and questioned the reliability and validity issues as those that were not convincing.

When a human judgment is involved, the reliability of the measurement is compromised. In order to check whether the evaluator of a stuttering behavior was consistent, the evaluator analyzed recorded SSI-3 test situations for each participant twice with a three-week interval. The time interval was held in order to start on the second observations free from memories of the earlier judgment (Guitar, 2014). This approach is called for intra-rater reliability. Reliability of the measurements on the first and second attempt was assessed using “point-to-point agreement”. Three weeks later the assessment procedure was repeated using a fresh copy of the transcript. An intraclass correlation (ICC) analysis using IBM SPSS Statistic 21.0 (IBM Corp., New York, USA) was performed. The correlation coefficient showed 98% reliability between test and re-test situations. According to Cordes and Ingham (1994) 80% agreement is a lower limit for the sample to be considered reliable.

A challenging part was the assessment of frequency. Some, in order to avoid stuttering used interjections or non-lexical utterances as “uh”, “um” “well”, etc. before the target word. Guitar (2014) suggests counting a target word as stuttered when one is certain that the client has just applied a successful avoidance behavior. However, the habit of filling up the pauses in spontaneously uttered speech is not so uncommon for a fluent speaker. Certainty about what was the feature of normal dysfluency and what was avoidance for anticipated stuttering proved to be difficult.

Variability is a hallmark of stuttering (Bloodstein, 1995). Stuttering has been shown to vary from one test situation to another and the data collected on a certain day may not be representative of a client’s true performance (Cordes & Ingham, 1994; Yaruss, 1997). There is an ongoing discussion in the field of psychology about

the issue of laboratory versus field experiments. The main concern is whether the laboratory or audiolab in our case is a suitable setting to detect the real-life behavior of the client. This is referred to as ecological validity and concerns the occurrence of an effect in real-life (Hammond, 1998). An artificial atmosphere in the lab, i.e. pressure of the video recoding during the SSI-3 assessment and face-to-face interview with an unknown person may have induced behavior that is not representative of a real-life situation. Especially, given the fact that nervousness is considered a modulating factor of stuttering. Hence, while the controlled test environment strengthens the internal validity, the external validity gets compromised. It would be ideal to gather two samples of the assessment, one recorded in the clinic and one recorded in the clients' typical environment.

WASSP. In addition, the PWS filled out the self-report questionnaire, WASSP (Wright & Ayre, 2000). In 2006 the WASSP was translated and revalidated for use in Denmark. Reliability and validity testing was successfully replicated (Fibiger & Fabaech Knudsen, 2006). The correlation between the subjects' SSI-3 scores and the WASSP results did not show any significant overlap or interrelation. A close look at the data reveals an example where one of the participants rated himself as a severe stutterer on the WASSP questionnaire (stuttering behaviors (average score 5,88), thoughts about stuttering (average score 6,30), feelings about stuttering (average score 7,00), avoidance due to stuttering (average score 5,75), and disadvantage due to stuttering (average score 6,00)) while his score on the SSI-3 placed him in the mild group (23).

A main concern with self-reports is credibility. Self-perception might be shaped by self-consciousness, true self-image, self-deception and memory (Paulhus & Vazire, 2008). The advantage of self-report is that the people who stutter are the best-qualified witnesses to their own stuttering. They have access to the thoughts, feelings and sensations connected to their dysfluency that is inaccessible for the therapist. People usually invest more time and effort when reporting on their own disability profile, which ensures greater validity. However, even if the participants are doing their best on reporting insightful and factual information, their replies are often inaccurate.

Individual response bias has been widely discussed connected to the way people fill out rating scales in self-reports. The findings show that people have an individual tendency to respond in a certain way and be consistent across the

questionnaire. For example, the so-called extreme responders, fill out the lower or higher scores while others stick to the midpoint and rarely use the outer points on the rating scales. Some tend to exaggerate or fake their answers, in order to give socially desirable responses, while others unconsciously are defensive and show denial. There are earlier studies on correlations between extreme responding and personality traits. Some of them report on more pronounced tendencies to extreme reporting in high-anxiety participants (Berg and Collier, 1953; Norman 1969; referred in Austin, Deary, Gibson, McGregor & Dent, 1998). It has also been suggested that PWS have higher levels of social anxiety then compared to controls (Iverach & Rapee, 2014). As a result, such behavior may reflect something different (response bias) then what the test has been designed to measure (interferes with the validity of the response).

Despite the pros and cons of the above-discussed assessment tools, combining standardized and self-rating measures of stuttering behavior can provide a speech – language therapist with a multidimensional view of the disorder.

Neuroimaging pros and cons. One of the possible limitations of the single voxel ^{1}H-MRS as a method is that the spectroscopy voxel covers a relatively large volume, and measures the total quantity of metabolite within that region, possibly incorporating tissue from other structures in the vicinity of the structure of interest, and without distinguishing between intracellular and extracellular metabolite (Ross & Sachdev, 2004).

In localized ^{1}H-MRS metabolites levels are often expressed as ratios, the so-called relative quantification rather than as absolute concentrations. Cr is considered the most stable brain metabolite (no change reported with age or variety of disease) and it makes it a common choice of metabolite in clinical practice for calculating metabolite peak ratios. For example the ratios are less sensitive to relaxation properties of the signal and partial volume effects compared to absolute metabolite concentrations (De Graaf, 2007; Dramsdahl et al., 2011). However, the referencing should be done with caution since there have been detected deviant concentrations of Cr in certain pathological states as for example tumors or frontal lobe epilepsy (R. De Graaf, 2007). Hence, there is a chance that changes in the mI/Cr ratio may be a result of changes in the concentration of Cr. However, since we only see an effect for mI and not other metabolite ratios, it is unlikely that changes in Cr are driving this effect.

The neurotransmitters glutamate (Glu) and glutamine (Gln) have similar chemical structures that lead to very similar spectra. Hence, at 3T field strength, their

^{1}H-MRS absorption peaks are partially overlapping. Using the PRESS sequence with TE=35 ms the separation between Glu and Gln becomes unreliable. The sum of these two metabolites may still be calculated with high accuracy, however, optimization of the sequence to improve contrast would be advantageous for future studies.

As discussed earlier the BOLD technique for fMRI is based on the assumption that neuronal activity and haemodynamic response are interrelated (Heeger & Ress, 2002). fMRI is characterized as showing a relatively poor temporal resolution – due to the relatively slow hemodynamic response, and the performance of the scanning sequence. In order to measure more direct consequence of neuronal activity it is important to model the heamodynamic response in relation to the various stimulus events in the study design, i.e. optimize a behavioral task. The optimal experimental design maximizes signal-to-noise and statistical power, hence a possibility of finding a reliable answers to the formulated research question. We used a block-design, when stimulus and contrasting periods are alternated in blocks, which offers the simplest way of comparing an experimental condition to a control condition, using a subtraction paradigm (Donaldson & Buckner, 2001). The main advantage of block designs is that they provide a considerable statistical power and intuitive analysis. The main critique is that randomization is limited within a block, hence the learning effect could become a confounding factor. In order to increase the spatial specificity of the BOLD signal the scanner with a high magnetic field was used (i.e. 3T GE Signa MR scanner). In order to increase a temporal resolution we used the echo-planar imaging (EPI) that allows fast measurement of the signal. This type of scanning technique is necessary if one wants to capture dynamics of the heamodynamic response.

Research contribution and conclusion

When conducting literature research on the topic using search engines such as Web of Science, Pubmed, the Cochrane Library and Google scholar we did not come across any studies using ^{1}H-MRS technique in order to examine changes in the neurotransmitter system in stuttering. Hence, a scientific advantage of the current study is that we tried to elucidate the neuronal basis of stuttering by looking at the brains neurochemistry utilizing the ^{1}H-MRS technique. The results we obtained may show some indications for further research in the field of stuttering. In particular, we looked at the neurotransmitters as important potential candidates for understanding the biochemical manifestations of stuttering. We suggested a relationship between biochemistry and stuttering and addressed the role of the neurotransmitter myo-

inositol as a possible marker of deficient myelogenesis in persistent stuttering. Given that the myelin sheath facilitates rapid neural innervation of the speech muscles underlying speech fluency, an improper formation of the myelin sheath during brain development might cause disruption in functional connectivity between the areas responsible for fluent speech production. However, our findings and claims await further research in bigger and more matched datasets.

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Myo-inositol as a marker of deficient myelogenesis in stuttering: a proton magnetic resonance spectroscopy (^{1}H-MRS) study

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Sammendrag

Til tross for den høye forekomsten av stamming er lite kjent om dens etiologi. Forskning innenfor nevrovitenskap har gitt kunnskap om struturelle og funksjonelle forskjeller i kortikal og sub-kortikale hjerneområder hos personer som stammer sammenliknet med personer med normal taleflyt. Mekanismene bak stammesymptomene er allikevel uklare. Vi har undersøkt forholdet mellom biokjemi og stamming, og presenterer ulike nevrotransmitteres rolle i stammingen. Vi tok i bruk protonmagnetisk-resonansspektroskopi (^{1}H-MRS) for å undersøke om nivået av N-acetyl Aspartate (NAA), glutamat og glutamin (Glx), og myo-inositol (mI) er forskjellig for personer som stammer og kontroller med flytende tale. Vi tok i tillegg i bruk atferdsdata for å se om det var korrelasjoner mellom nivå av nevrotransmittere og alvorlighetsgrad av stamming. Til slutt kombinerte vi fMRI og ^{1}H-MRS for å se på forholdet mellom myo-inositol og nevrale responser i oppgavesituasjon og i hvilesituasjon. Våre funn bekrefter det nære forholdet mellom biokjemi og stamming. Vi foreslår at myo-inositol kan fungere som en markør for manglende myelogenese. Resultatene trenger imidlertid bekreftelse i forsøk med større og bedre matchede datasett.

Abstract

Despite the high incidence of stuttering little is known about its etiology. Decades of neuroimaging research revealed structural and functional differences in cortical and subcortical brain areas in people who stutter (PWS) compared to fluent controls, however the mechanism behind the symptoms associated with stuttering is not clear. We examined the relationship between biochemistry and stuttering and suggested the role of the neurotransmitters in the cause of the disorder. We used proton magnetic resonance spectroscopy (^{1}H –MRS) to investigate whether the levels of concentration of N-acetyl Aspartate (NAA), an aggregate of Glutamate and Glutamine (Glx) and myo-inositol (mI) are different between PWS and fluent controls. In addition, we used behavioral data to check whether there is a correlation between inter-individual variations in levels of certain neurotransmitter and the severity of stuttering. Finally, we combined functional magnetic-resonance imaging (fMRI) and ^{1}H –MRS to investigate the relationship between neurotransmitter myo-inositol and neuronal responses during tasks and resting state. Our findings confirmed the close relationship between biochemistry and stuttering and suggested the role of the neurotransmitter myo-inositol as a marker of deficient myelogenesis. However, the results await further research in bigger and more matched datasets.

Keywords: speech dysfluency, people who stutter (PWS), proton magnetic resonance spectroscopy (^{1}H –MRS), functional magnetic resonance imaging (fMRI), blood-oxygen level-dependent (BOLD), the inferior frontal gyrus (IFG), myo-inositol (mI), white matter integrity, myelin, myelogenesis.

Introduction

Stuttering is a multifactorial speech disorder that manifests in involuntary part-word repetitions, sound prolongations, and/or silent blocks (Guitar, 2014). Other coexistent symptoms may include visible tension in the face, head movements, eye blinks or tremor of muscles involved in speech. A person who stutters (PWS) may avoid words or situations that escalate the stuttering episodes. Stuttering appears to have a genetic component, however according to twin and family studies the genetic factors that are thought to be involved must interact with environmental factors for stuttering to appear (Guitar, 2014; Maguire, Yeh, & Ito, 2012). Stuttering has a male/female ratio 3:1. Females who stutter are more likely to recover during early childhood, thus increasing the proportion of males with the disorder persistent after puberty (around 4:1). Despite the high incidence of stuttering (11.2%) little is known about its etiology (Reilly et al., 2013). On the surface, stuttering appears to depend on motor aspects of speech production. It has, however, been suggested that multiple factors interact in producing the dysfluencies associated with the disorder (Smith, Goffman, Sasisekaran, & Weber-Fox, 2012; Watkins, Smith, Davis, & Howell, 2008). For example, syntactically demanding speech or communicative pressure may increase stuttering frequency while changes in perception, such as delayed auditory feedback (DAF) or frequency-altered feedback (FAF) as well as choral speech/reading or singing may decrease stuttering episodes. The often dramatic improvement in fluency after the above-named fluency-inducing conditions compromise the general speech-motor instability approach and suggest specific causal mechanisms leading to the speech problems.

Language processing, both receptive and expressive, is left hemisphere dominant in most adults (Owens, 2012). The formula for successful, i.e. fluent speech production is robust and well-established connections between subcortical-, cortical-, and cerebellar-motor output systems and auditory cortical fields (Scott, 2012). Theoretically, if the connection between the brain regions crucial for auditory processing, motor planning and execution is compromised, a dysfluent or stuttered speech may be a possible outcome.

As early as the 1870's Carl Wernicke proposed a speech-language network where the auditory cortex is connected to speech planning and execution areas (Hickok & Poeppel, 2007). A modern variant of such an auditory-motor integration theory is presented in the dual stream model that suggests the connection between the

auditory cortex and speech planning and execution areas is achieved through the dorsal stream (Hickok and Poeppel, 2007). One stream connects sensory/phonological networks with conceptual-semantic systems (ventral), and one connects sensory/phonological networks with motor-articulatory systems (dorsal). In particular, the dorsal stream extends from the left posterior temporal lobe via the inferior parietal areas into the left IFG, including premotor areas (Specht, 2013). The dual stream model postulates that the dorsal steam is predominantly left hemisphere lateralized and translates speech signals to articulatory representations for speech production. Researchers suggest that the superior longitudinal fasciculus (SLF) that contains fibers from the arcuate fasciculus is an anatomical homologue of the dorsal stream. The SLF is a major white-matter tract that links brain regions responsible for speech planning (the inferior frontal region), sensory feedback of speech sounds (auditory regions) and speech-motor execution (the motor cortex) by transmitting nerve impulses between them (Chang & Zhu, 2013; Sommer, Koch, Paulus, Weiller, & Buchel, 2002; Watkins et al., 2008). The regions underlying the dorsal stream are crucial for intact speech production and rapid information exchange between them is essential for the fluency of the speech production. Thus, if the integrity of the white-matter pathway is compromised, there is a risk of a delay in information flow.

Decades of neuroimaging research revealed structural and functional differences in cortical and subcortical brain areas in PWS compared to fluent controls that are involved in the process of stuttering.

Foundas and colleagues (2001) have studied the anatomy of cortical speech-language areas in 16 adults who stutter. The results showed that the right and left planum temporale (PT) were significantly larger in PWS and that the leftward asymmetry was reduced in size compared to healthy individuals. Some years later Foundas and colleagues (2004) looked at the correlation between atypical PT anatomy and changes in the effect of fluency inducing technique - delayed auditory feedback (DAF) on fluency in adults with PWS. They suggested a possibility for anatomically atypical rightward PT to induce stuttering. Moreover, they showed the presence of an extra gyri along the superior bank of the Sylvian fissure (in 14 out of 16 PWS participants). Such an anomaly was never observed in a control. In addition, an atypical structure of the diagonal sulcus within the frontal operculum was registered. Their findings suggested that anomalies within the perisylvian speech-language areas might put an individual at risk of developing stuttering.

Alm (2005) claimed that PWS have a dysfunction of the basal ganglia motor circuits. He proposed that the basal ganglia-thalamocortical (BGTC) motor circuits through the putamen to the supplementary motor area (SMA) are dysfunctional in PWS. The basal ganglia are important subcortical gray matter structures located in the forebrain and influence motor behavior, emotions and cognition (Graybiel, 2000). They take part in modulating inhibition and excitation of the frontal lobe cortex (Alm, 2004). The main function of the basal ganglia is to coordinate the release of the correct motor programs and inhibiting potentially competing motor programs that might interfere with the execution of the specific motor act. According to Alm (2005), the basal ganglia fail to produce accurate timing cues for initiation of the next motor segment in speech. He emphasized the role of the putamen (one of the structures of the basal ganglia that together with the caudate nucleus form the main input nucleus - the striatum) in speech motor control, whose main function is to regulate movements and influence various type of learning (Neumann et al., 2003). The basal ganglias' function is strongly regulated by dopaminergic activity (Rosenberger, 1980).

Disruption in white matter tract structures has consistently been implicated in the pathophysiology of stuttering. Jäncke, Hanggi, and Steinmetz (2004) found increased white matter volume within the right hemispheric language network in PWS. These areas include superior temporal gyrus (STG), inferior frontal gyrus (IFG), the somatosensory area (including the area underlying the face and mouth representation), and the middle frontal gyrus (MFG). The findings suggested abnormality in white matter pathways that connect speech relevant cortical areas with other areas, possibly indicating aberrant processing strategies in the right hemisphere in PWS. In addition, they reported on leftward asymmetry in white matter volume in fluent controls, while PWS showed symmetric volumes. Their findings are in line with a study conducted by Beal and colleagues (2007) where more white matter volume was found in pathways underlying the right IFG, the right insula and the left middle temporal gyrus. Cykowski and colleagues (2010) also studied adults who stutter and reported low fractional anisotropy (FA) values in white matter underlying the left hemispheric IFG, frontal gyrus and the corpus callosum, suggesting myelination defects. Myelin is a white fatty sheath that covers large peripheral nerves and axons of CNS. Layers of myelin assure rapid transmission of the electrical impulse along the nerve fiber. The cranial nerves that are responsible for neuromotor

control over the speech production are myelinated. This facilitates rapid neural innervation of speech muscles underlying speech fluency (Webb & Adler, 2008).

Connally and colleagues (2014) published a study were they examined 29 PWS who were heterogeneous with respect to age, sex, handedness and stuttering severity. Their results supported previous findings on abnormalities in white matter integrity in language and motor tracts in developmental stuttering. Sommer and colleagues (2002) provided evidence for reduced white-matter integrity in the rolandic (or central) operculum of only the left hemisphere, just above the Sylvian fissure. Chang and colleagues (2008) examined the left superior longitudinal fasciculus (SLF) in 22 children. They reported on a subtle decrease in white-matter integrity that compromises the integration of motor plans and sensory feedback during speech production. This was suggested to be responsible for disturbed fluency in stuttering. Watkins and colleagues (2008) argued that reduced integrity of white-matter connections leads to aberrant brain function of the ventral pre-motor cortex in both hemispheres.

All these studies have utilized diffusion tensor imaging (DTI) techniques, which use Magnetic Resonance (MR) to study microstructural features of white matter by examining the anisotropy of water molecule diffusion within white matter tracks. A measure describing the degree of anisotropy in the diffusion of water molecules is referred to as fractional anisotropy (FA). FA is thought to reflect myelination in the white matter. There is a claim that myelin plays an important role in determining the observed white matter tract diffusion anisotropy, however a clear association between the direction of anisotropy in the diffusion of water molecules and the myelin integrity remains unclear (Song et al., 2002). According to Sommer and colleagues (2002) “lower FA values can indicate decreased fiber coherence or myelination defects”. Furthermore, low FA values in white matter integrity could be a sign of dysfunctional connectivity between different regions in the brain responsible for auditory processing, movement planning and motor execution (Cykowski et al., 2010; Sommer et al., 2002).

Scientists interested in the brain-activation pattern in individuals who stutter during speech production tasks have reported on diminished activity in the left auditory cortex and hyperfunction in the motor regions of the right hemisphere (Braun et al., 1997; Chang, 2011; Fox et al., 1996). De Nil, Kroll, Lafaille, and Houle (2003) claimed that PWS recruit more neural resources during elementary speech production

showing an elevated overall neural activation compared to controls. Even in silent reading tasks they mobilize the primary motor cortex and the cerebellum demonstrating an effort they put on the articulatory aspects (presumably not attributed to actual articulatory movements).

Early Positron Emission Tomography (PET) studies have also demonstrated functional differences in addition to volumetric inequality in brain regions. Brain activity during paragraph reading, stuttering and induced fluency has been investigated by Fox and colleagues (1996). They found hyperactivation of the right hemisphere motor system while reading in PWS. Controls showed largely left lateralized activation in areas of the motor cortex representing the mouth, motor planning areas, auditory regions, visual systems and the cerebellum. Moreover diminished activation in left frontal-temporal regions in PWS compared to controls has been registered. Sommer and colleagues (2002) reported on signs of disconnection between speech-related cortical areas right below the laryngeal, pharyngeal and tongue representation in the left sensory-motor cortex. Their findings suggested a disturbed temporal pattern of activation in speech-relevant brain areas. The right hemispheric overactivation has been interpreted as a compensatory mechanism that compensates for a left hemispheric deficit and tries to attenuate stuttering symptoms (similar to aphasia) (Sommer et al., 2002). The fact that right hemispheric overactivation negatively correlates with stuttering severity supports the notion that it is a compensatory activation, not a primary dysfunction (Neumann et al., 2003).

Chang and Zhu (2013) showed a decrease in functional connectivity in BGTC and auditory-motor cortical networks, including the pars opercularis and posterior superior temporal gyrus, primarily in the left hemisphere. The latter may affect speech planning and execution processes essential for fluent speech motor control. In addition, they reported on attenuated connectivity in neural networks responsible for self-initiated timing of speech movement. In general PWS have decreased connectivity among cortical auditory-motor regions, in particular between the posterior superior temporal gyrus and the insula, the SMA and the IFG focused on the left hemisphere (Chang & Zhu, 2013).

Despite steadily increasing knowledge on the cause of stuttering, little is known regarding the underlying biochemistry. So far, within the stuttering domain the main focus has been on the role of the neurotransmitter dopamine. Dopamine access

theory of stuttering suggests that there is an excessive dopamine activity associated with stuttering. Dopamine is a neuromodulatory neurotransmitter responsible for executive function, motor control and temporal sequencing. Neurobiological features of dopamine enables it to regulate working memory, cognitive flexibility, reasoning and language and emotional processing (Civier, Bullock, Max, & Guenther, 2011; Hoffmann, 2013). In a study by Wu and colleagues (1997) using PET they measured the rate of dopamine synthesis in the brain, labeled as FDOPA. PWS were reported to have three times higher dopamine uptake in both cortical and subcortical areas of the brain. Given the role of the basal ganglia in stuttering (to provide an appropriate pattern of inhibition and activation of various frontal cortex areas) and the relation between dopamine and basal ganglia discussed earlier one may conclude that well-regulated dopaminergic system is essential for the proper functioning of the basal ganglia circuits, hence for normal brain activation and speech fluency.

The chemical architecture of the brain is highly heterogeneous. Different neurotransmitters assist different higher cortical and subcortical function. The fast acting excitatory and inhibitory neurotransmitters are modulated by a number of long-range slower acting neurotransmitters. Given a complex interplay between the neurotransmitters, receptors and brain plasticity, it would be interesting to investigate the impact of other neurotransmitters on regional cerebral metabolism in stuttering. In the current study we use *proton magnetic resonance spectroscopy (^1H –MRS)* to investigate whether the levels of concentration of *N-acetyl Aspartate (NAA)*- believed to be a neuronal marker in the brain; *an aggregate of Glutamate and Glutamine (Glx)*- excitatory neurotransmitters responsible for fast-action information transmission, *and myo-inositol (mI)*- a glial cell marker and a breakdown product of myelin are different between PWS and fluent controls. In addition, we use behavioral data to check whether there is a correlation between inter-individual variations in levels of certain neurotransmitter and the severity of stuttering as measured through the "*Stuttering severity instrument*" *the third edition (SSI-3)* and *Wright and Ayre Stuttering Self-Rating Profile (WASSP)* (Wright & Ayre, 2000). Finally, we used the combination of functional magnetic-resonance imaging (fMRI) and ^1H –MRS to investigate the relationship between neurotransmitter myo-inositol and neuronal response during tasks and resting state.

The inferior frontal gyrus was chosen as a target region for this investigation.

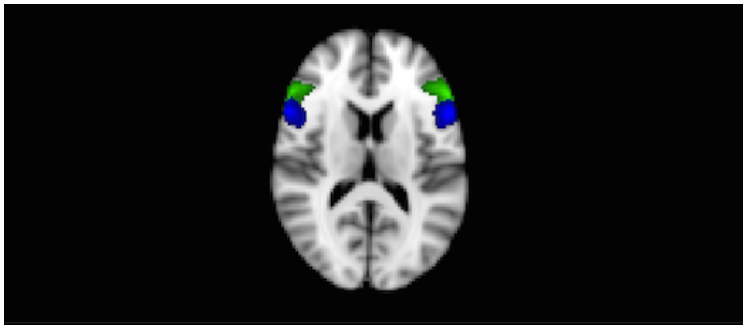

Figure 1: Highlighted is the area of the IFG, the pars opercularis (blue) and the pars triangularis (green). Axial view

Neuroanatomical studies suggest that IFG is composed of three separate subregions with different functional significance, the pars opercularis, the pars triangularis and the pars orbitalis. The pars opercularis and the pars triangularis comprise the classic Broca's speech area, while the pars orbitalis appears to be active during vocalization processes (Schulz, Varga, Jeffires, Ludlow, & Braun, 2005). Cytoarchitectonically, these three areas are associated with Brodmann areas 44, 45 and 47, respectively. Functions specific to the left IFG, in particular Broca's area, include motor-based speech production and articulatory coding, it also coordinates sensory and semantic information received from the posterior parts of the neural language network, and therefore plays a language executive role (Binder et al., 1997). In particular, Brodmann regions 44 and 45 have been proven to support the computation of syntactic movements (Friederici, 2011). Structural MRI shows that the IFG develops abnormally in children who stutter. Chang and colleagues (2008) published findings were children who stutter had less gray matter volume in the bilateral IFG compared to fluent controls. Adults who stutter have also been shown to have more gray matter volume in left the IFG compared to controls (Lu et al., 2010). Watkins and colleagues (2008) reported on significantly lower fractional anisotropy (FA) of white matter in the PWS group compared to control group in the pars orbitalis in the right IFG, left and right posterior IFG. Left posterior IFG on the contrary have shown to have higher FA in the PWS group in opposition to controls. Other studies also report on abnormalities in white matter pathways in PWS lying underneath IFG area (Beal, Gracco, Brettschneider, Kroll, & De Nil, 2013; Beal et al., 2007; Cykowski et al., 2010; Jäncke et al., 2004).

Individual levels of NAA, Glx and mI were determined in a resting state, using ^{1}H-MRS from two voxels located bilaterally in the IFG. Correlation coefficient was calculated to investigate the relationship between stuttering severity as measured through the SSI-3 and WASSP with the concentration of each of the study metabolites. Finally, this study used a variant of DL paradigm (described later) presented as pseudo- randomized block design composed by two experimental conditions and one control condition. We wanted to look at the complex interplay between neurotransmitters in the modulation of neuronal activation, where high or low concentrations of study metabolite could be responsible for the local effect during task performance.

We hypothesized that:

1. There are differences in the concentration of neurotransmitters between people who stutter and fluent controls, i.e. the concentration of NAA is expected to be comparable between groups and hemispheres; we expected higher concentration of Glx in the right hemisphere in the PWS group (reflecting right hemisphere overactivation in PWS); the level of mI is expected to be lower in the both hemispheres in the study group compared to controls (reflecting decreased white matter integrity in PWS).

2. We expected a negative correlation between SSI-3 and WASSP measurements and mI level, positive for Glx in the right hemisphere and no correlation with NAA.

3. Finally, we expected levels of neurotransmitters from the IFG to correlate with the IFG BOLD response, i.e. we expected more neuronal activity in the right hemisphere in PWS compared to controls.

Materials and methods

Subjects

Sixteen persons with PWS and twenty-seven controls were included in the study. The PWS were between 17 and 41 years of age (M=26.8; SD=1.7). The PWS were recruited through the ”Norwegian organization for people who stutterer” (NIFS) and “Centre for Adult Education “ in Bergen, Norway. All the participants from the experimental group have been previously diagnosed with persistent developmental stuttering (PDS).

The sex ratio is 13 males and three females in the PWS group and 12 male and 15 female participants in the control group. The controls were recruited from the

student population in Bergen using e-mail. They were also between 20 and 36 years of age, but with a somewhat lower age mean (M=24.5; SD=0.7).

Handedness was checked by means of the Edinburgh Handedness Questionnaire (Oldfield, 1971). In the control group 23 were strictly right handed, three showed a preference for the right hand, and one showed a left hand preference. In the PWS group 13 were strictly right handed, one strictly left handed, and two showed a left hand preference. Due to the verbal nature of the speech perception and speech production paradigm, the hearing threshold was assessed with the Hughson-Westlake audiometric test (Oscilla USB-330, Inmedico, Lystrup, Denmark). Participants with an averaged inter-aural acuity difference of more than 10 dB were excluded. They were all native Norwegian speakers. People who stutter were excluded from the sample if they had co-existent deficits, namely speech- and/or language disorders, reading- and/or writing disabilities, dyslexia or Attention Deficit Hyperactivity Disorder (AD/HD). With regard to MR procedure, the participants were thoroughly checked for any condition that could pose a threat for their health and safety by our screening questions in the informed consent sheet. Because of the strong magnetic field people who have metal in their bodies, such as braces or implants or people who have had brain surgery were not allowed in the scanner. Pregnant women or those who suffer from claustrophobia were not recommended to participate in the MR study. In addition, participants completed an MR safety checklist presented by radiographers as a complementary safety measure. At the end of the experiment participants were thanked for their cooperation and compensated for their expenses.

The current paper will be using already existing data on auditory perception, speech lateralization and stuttering. The data collection was carried out by four master students under the supervision of Dr. Karsten Specht from autumn 2008 to summer 2012. The study received approval from the Regional Committee for Medical Research Ethics in Western Norway (REK-Vest), number 212.08 and informed consent was obtained from each subject before the experiment (see attachment 2 & 3).

Stuttering severity assessment (SSI-3 and WASSP)

The behavioral part of the data collection was performed in an audiolab in a sound-attenuated room on the 9th floor at the Institute for biological and medical psychology, University of Bergen. This part of the data collection included only the individuals who stutter. Stuttering severity was examined by SSI-3. In addition PWS filled out a supplementary self-reporting survey WASSP, Danish version (Fibiger &

Fabaech Knudsen, 2006).

SSI-3 is a standardized and norm-referenced stuttering severity evaluation instrument that provides a multidimensional view of stuttering (Guitar, 2014). In particular, it allows assessment of four areas of speech behavior: frequency of stuttered syllables, stuttering duration, physical concomitants and naturalness of individuals' speech. The total score is obtained by adding together the scores for the three dimensions above. Administration of SSI-3 was video recorded on JVC HD Everio GZ-HD6 camera (Victor Company, Japan) and was used for re-transcription and re-calculations. As a result, all the participants from the study group ranged in their severity from very mild (6) to severe (34), (M=19.13 (mild), SD=8.15) (see Table 1).

The same person has performed the transcription and analysis of the recorded data twice in order to determine measurement reliability of SSI score ratings. We used an intraclass correlation (ICC) coefficient, IBM SPSS Statistic 21.0 (IBM Corp., New York, USA) to establish the consistency and reliability of the repeated measures and whether the person who did test/re-test used the measurement techniques correctly. The ICC for the overall SSI-3 measurements was 0.98, which indicated excellent agreement between test and re-test situations (the intra-rater reproducibility).

To avoid the bias of only one measurement obtained on stuttering severity, WASSP questionnaire has also been used. In the self-rating WASSP questionnaire participants are asked to rate 24 items using a 7-point Likert type scale to describe self-perceived severity (1-none, 7-very severe). The items are grouped into five sections: stuttering behaviors (8 items), thoughts about stuttering (3 items), feelings about stuttering (5 items), avoidance due to stuttering (4 items), and disadvantage due to stuttering (4 items). The participants varied in their self-rating between 1.5% to 6.33 % (Table 1). The correlation between the PWS' results on SSI-3 and WASSP did not show any significant overlap or interrelation.

MR imaging

The MRI experiment was carried out at Haukeland University Hospital, Radiological department, according to a fixed protocol lasting approximately one hour per subject. Imaging data were acquired on a 3T GE Signa MR scanner using an eight-channel head coil. All participants underwent a scout sequence and structural imaging before fMRI and ^{1}H-MRS. The structural imaging was performed with a T1-weighted spoiled gradient echo (SPGR) pulse sequence was used for positioning the

spectroscopy voxel; an axial T2-weighted image was also obtained. Two functional echo-planar imaging (EPI) acquisitions were performed (including the DL sequence described later) before ^{1}H-MRS. ^{1}H-spectra were measured from two voxels located bilaterally in the IFG, using a single-voxel point resolved spectroscopy (PRESS) sequence (echo time TE=35ms; repetition time TR=1500ms, 128 averages, voxel size 20 x 20 x 20 mm).

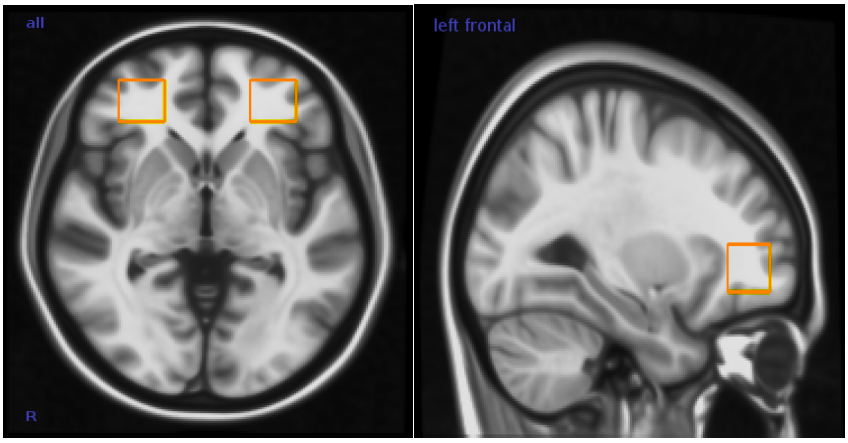

Figure 2. An individual placement of two voxels in the left and right hemispheres of the IFG. Axial and Sagittal views.

fMRI was performed using an echo-planar imaging (EPI) sequence which was oriented using the structural image. EPI volumes covered the cerebrum and the most of the cerebellum and contained 24 axial slices. In total, 164 whole brain images were acquired with a 64 x 64 image matrix and a voxel size of 3.44 mm x 3.44 mm x 5.5 mm. A sparse sampling protocol was used with a TR = 5.5 seconds and TA of 1.5 seconds, leaving silent gaps of 4.0 seconds between scans. This is when the auditory stimuli were presented and the participants could give an oral response without the interference of scanner noise and movement artifacts as a result of articulation (see attachment 5).

DL paradigm. An fMRI pseudo- randomized block design integrates a variation of DL-paradigm, and is designed to measure both speech perception and speech production. The paradigm is composed of two experimental conditions and one control condition and is based on the dichotic presentation of consonant-vowel syllables i.e. /ba/, /da/, /ga/, /pa/, /ta/, /ka/. Stop consonant-vowel syllables were spoken by a Norwegian male voice in constant intonation and intensity, on a total of 36 different dichotic stimulus pairs. In the experimental conditions the syllables were

presented with different instructions. The first condition required subjects to listen to the stimuli allowing measurement of activity associated with speech perception. In the second condition they were additionally required to produce the syllable they thought they heard best, allowing the measurement of activity associated with speech production. We employed only non-forced (NF) DL condition. Control condition compriseed a resting task block where participants were relaxing. Blocks alternated and made it possible to determine which voxels activated as a function of differences between the blocks. Each block with one of three conditions was composed of 10 stimulations and the order of the tasks was counterbalanced. The presentation time was 400-500 ms with sound intensity 87 dB. The paradigm was administered by E-prime software (2.0 Psychology Software Tools, Inc) and the participants listened to the stimuli through MR compatible headphones.

Image pre-processing and statistical analysis

1H-MRS data. ^{1}H-MRS spectra were analyzed using LCModel software (Version 6.2-1A; Provencher, 1993), allowing for quantification of metabolite levels. Levels of NAA, Glx and mI, are quoted as ratios over creatine (Cr). The fact that the concentration of total creatine is relatively stable makes it a common choice of metabolite for internal referencing (de Graaf, 2007). Data were checked for suitable quality (line width and signal-to-noise ratio) and reliability of fit (%SD of estimate).

Three 2 x 2 repeated measures analyses of variance (ANOVA) were conducted using SPSS 21.0 (IBM Corp., New York, USA) software, with the between-subject factor group (PWS and controls), and the within-subject factor - concentration of certain neurotransmitter (NAA/Cr, Glx/Cr and mI/Cr) in the left and right hemispheres, respectively. We used $p < .05$ as a significance level. Results from ^{1}H-MRS study were further correlated with stuttering severity indexes (SSI-3 and WASSP) to look for possible connections/dependences.

fMRI data. After MRI data was acquired, it was analyzed using the Statistical Parametric Mapping (SPM8) tool. During the pre-processing steps the EPI images went through the correction techniques for movement artifacts (realignment and unwarp). The latter will maximize the functional signal-to-noise ratio (minimize signal variability), hence improve the validity of the assumptions for the statistical testing. The images were then normalized to standard stereotactic space using the Montreal Neurological Institute (MNI)-template and resampled to a voxel size of 3

mm. In addition, spatial smoothing performed by means of an 8 mm Gaussian filter was applied to accommodate remaining anatomical inter-subject variations.

Next, our data was subjected to a general linear model (GLM) that indicated the separate start of stimulation for each condition. Based on a stimulus onset time of both experimental conditions (perception/production), we obtained single contrasts specified for each condition and participant separately. The SPM contrast for ROI analysis was "NF condition minus rest condition" where we decided to concentrate on speech production activity.

In order to explore regional effects, the hemodynamic response of activation was studied from the same regions of interest as the ^{1}H-MRS measurement, i.e. the IFG. The BOLD signal from the regions of interest: the pars opercularis, the pars triangularis and the pars orbitalis has been extracted in order to compare fluctuation in neuronal activity and BOLD signal change that occurs due to the DL stimulus presentation with the metabolites detected by ^{1}H-MRS technique.

A 2 (groups) x 2 (sides) x 3 (ROI) repeated measures analysis of variance (ANOVA) was conducted to check whether there is a difference in the regions of interest between the groups based on hemispheric side. In the next 2 x 3 ANOVA calculations we treated groups as between-subject factors and the regions of interest as within-subject factors. We performed this analysis separately for the right and left hemisphere. In addition, we added neurotransmitter myo-inositol that showed a fundamental difference between the PWS and controls ($F(1,32) = 5.09$, $p < .031$) as a covariate to check whether the BOLD signal from the region of interest (pars opercularis, pars triangularis or/and pars orbitalis) could possibly be influenced by the mI concentration level (ANCOVA).

Results

Spectroscopy data

N-acetyl Aspartate (NAA)/Cr: the main effect of group revealed an F ratio of $F(1.37) = 2.00$, $p > .166$. The results show that there is no significant difference between the groups as a function of NAA concentration. Side effect was though shown to be significant $F(1.37) = 23.48$, $p < .001$. There were not detected any side X group interaction.

Glutamate/Glutamine (Glx)/Cr: the main effect of group revealed an F ratio of $F(1.29) = 0.15$, $p > .704$. The results show that there is no significant difference between the groups as a function of Glx concentration. Again, the side effect reached

a conventional level of statistical significance F (1.29) = 19.19, p < .001. There was not detected any side X group interaction.

Myo-inositol (mI)/Cr: the main effect of group revealed an F ratio of F (1.32) = 5.09, p < .031. The results show that there is a statistically significant difference between the groups as a function of mI concentration.

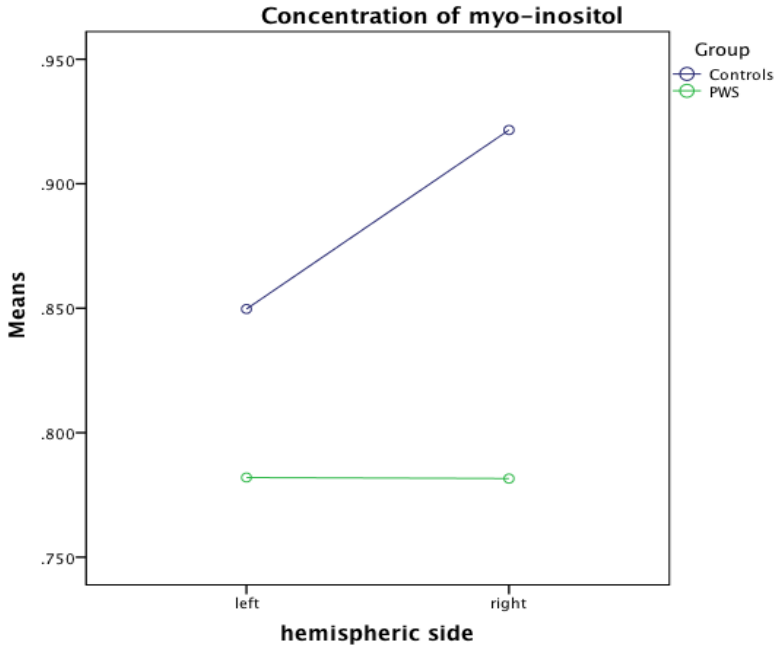

Figure 3. Main effect of mI on the difference between PWS and controls.

The within subject test indicates that there is no significant side effect, in other words the groups are not different on ml concentration depending on the hemispheric side, however the result from the descriptive statistics show more variance in the left hemisphere (M = 0.79, SD = 0.23) in the PWS group then in the right hemisphere (M = 0.79, SD = 0.12). The side X group interaction effect was non-significant.

To check for possible gender effect, we conducted an ANOVA (2 x 2) that yielded a non-significant F ratio of F(1.32) = 1.06, p > .310.

Correlation between ^{1}H-MRS, SSI-3 and WASSP

There was no significant correlation between SSI scores and each of the metabolites:

		mI/Cr R	mI/Cr L	NAA/Cr R	NAA/Cr L	Glx/Cr R	Glx/Cr L
SSI	Pearson Correlation	.545	.098	-.060	-.254	.455	-.118
	Sig. (2-tailed)	.054	.740	.830	.343	.102	.688

Table 2. Results of the correlation analysis between ^{1}H-MRS and SSI-3.

The correlation between concentration of mI/Cr in the right hemisphere and SSI-3 showed a trend to significance (r= .545, p= .054), however it did not reach a conventional level of statistical significance.

The correlation between ^{1}H-MRS data with self-rating WASSP questionnaire did reach a conventional level of statistical significance on one of the measurements. The measurement was on *stuttering behaviors*, which consists of items on frequency and range of stuttering behaviors, as well as a persons' defense and coping reactions to stuttering. The results show a statistically significant dependence, where 56% of the variability in the WASSP was accounted for by the concentration of mI in the left hemisphere – mI/Cr left (r= .748, p= .002).

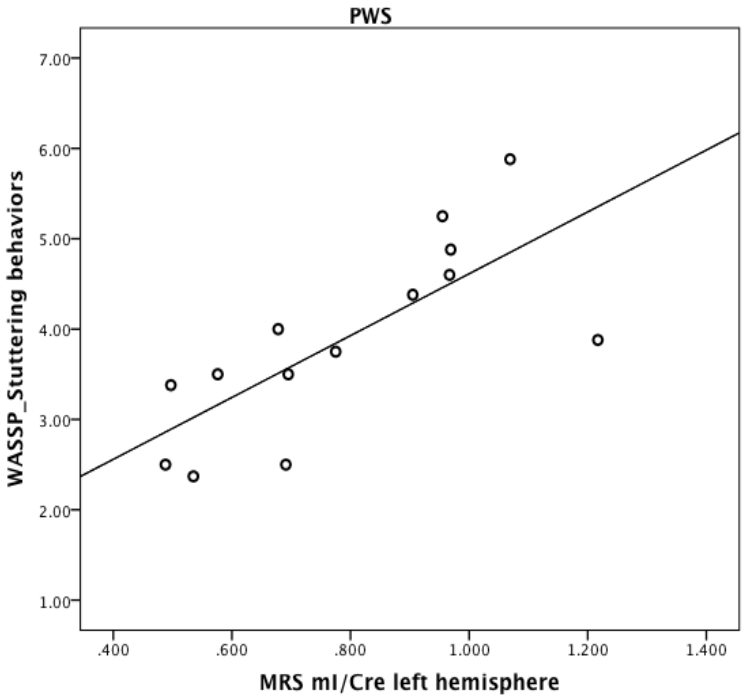

Figure 4. Correlation between WASSP (stuttering behaviors) and level of mI/Cr in the left hemisphere

fMRI (ROI) and ^{1}H-MRS correlation analysis

In the 2 x 2 x 3 ANOVA the test of between-subjects effect showed statistically insignificant result, i.e. the main effect of the group yielded an F ratio of F (1.39) = 0.06, p > .813.

In the 2 x 3 ANCOVA with mI as a covariate none of the hemispheric sides showed any significant results with regard to group: right F(1.34) = 0.52, p > .476; left F(1.33) = 0.10, p > .749 or myo-inositol concentration: right F(1.34) = 0.23, p > .623; left F(1.33) = 0.86, p > .362.

Given the main effect of group differences between PWS and controls on mI and strongly significant p-value on the correlation analysis the probability adjustment was not considered necessary.

Discussion

The aim of the present study was to investigate the role of neurotransmitters in stuttering. Group comparisons were made between the PWS and fluent controls as categorical variable and between levels of NAA/Cr, Glx/Cr and mI/Cr as dependent variables from both left and right hemispheres. The results from the study demonstrate that PWS have different concentration levels of metabolites compared to fluent controls. The cerebral metabolite pattern of PWS is characterized by the pronounced reduction in the myo-inositol level in the IFG. Concentration of NAA and Glx in the IFG are also somewhat reduced but they follow a similar pattern with the fluent controls and the concentration peaks are not significantly different from the control group.

The level of concentration of myo-inositol between the groups showed a significant group effect regardless of hemispheric side. However, the variance of mI in the PWS group in the left hemisphere was larger compared to fluent controls. The findings suggest that there is a general effect across hemispheres, but perhaps more variability in the left hemisphere. This claim is further supported with evidence from fMRI and PET studies that the language dominant hemisphere is compromised in PWS. In particular, they have reported on diminished brain activation pattern in PWS left motor and auditory areas in contrast to enhanced right hemisphere involvement (Braun et al., 1997; Fox et al., 1996). The right-sided overactivity is often explained as a compensatory reaction to the left-sided deficit (Chang et al., 2008; Cykowski et al., 2010; Sommer et al., 2002). Sommer and colleagues (2002) reported on impaired

neuronal communication in the left hemisphere between areas responsible for language preparation and execution, possibly due to atrophy in white matter fibre tracts. In particular, they reported on attenuated white matter coherence in the left SLF below the laryngeal and tongue representation in the sensorimotor cortex.

The fact the myo-inositol is considered a glial marker and that a type of glial cells called oligodendrocytes are responsible for the production of myelin may suggest a role of the neurotransmitter in stuttering. Myo-inositol has also been presented as breakdown product of myelin. In other words, enhanced concentration of myo-inositol may be understood as a partial loss of glial cells, hence the activation of cells-death signaling pathways. In that case the increased level of mI would have been in line with insufficiently myelinated axons possibly in the presence of ongoing demyelinating processes (Brandao, 2004). Although, this is not necessarily the case in PWS.

Our findings, on diminished concentration of mI in the study group may find support in a hypothesis suggested by Cykowski and colleagues (2010). They claim that the factor responsible for the clinical symptoms of stuttering involves a deficit in the myelogenesis in the white fibers of the areas responsible for spoken language. In particular, they showed a robust left-hemispheric FA reduction in the third division of the superior longitudinal fasciculus (SLF III) extending rostromedially into the left anterior corona radiata and left forceps minor. They referred to those regions as late-myelinating associative and commissural fibers (Cykowski et al., 2010).

The SLF is a major white-matter tract that links brain regions responsible for speech planning (the inferior frontal region), sensory feedback of speech sounds (auditory regions) and speech-motor execution (the motor cortex) by transmitting nerve impulses between them (Chang & Zhu, 2013; Sommer et al., 2002; Watkins et al., 2008). According to the Dual stream model suggested by Hickok and Poeppel (2007) SLF connects sensory/phonological networks with motor-articulatory systems, hence translates speech signals to articulatory representations for speech production. Researchers suggest that the superior longitudinal fasciculus (SLF) is an anatomical homologue of the dorsal stream. Reduced FA in the SLF could therefore imply that PWS might have a dysfunctional connectivity in the language dorsal stream. A number of studies reported on reduced FA in left-sided white-matter tracks in both children and adults (Chang et al., 2008; Cykowski et al., 2010; Sommer et al., 2002; Watkins et al., 2008).

When studying adults who have stuttered from childhood it remains unclear whether differences existed before the onset of stuttering or if they are a result of the lifetime experiences of the individual who stutters. The fact that the myelination process is also referred to as the maturation process of the fibers, suggests that PWS might have problems with developing the proper myelin sheath that will accelerate the conduction of impulses along the axon, hence facilitate the functional connectivity between the speech production areas.

With regard to development, the process of myelination continues during the first postnatal year and is completed in the second year. Some of the white matter tracts continue to myelinate past the first decade of life. During early childhood, the volume increase in myelinated white matter in the speech-related areas parallels the time of language acquisition and development, however it happens later than in the sensorimotor region (Brauer, 2009). The fact that persistent developmental stuttering usually begins between 2 and 5 years of age (Guitar, 2014) could suggest a link between the defective or delayed myelination in postnatal period and dysfluency of speech production. By the time the development of the myelin sheath finally completes, one may speculate that most of the children will experience a spontaneous recovery. Spontaneous recovery accounts for about 75% of individuals who ever stuttered (Maguire et al., 2012). Some children experience spontaneous recovery a few years after onset of symptoms while for some myelogenesis remains incomplete and they may risk continuing to stutter throughout life.

Combining the hypothesis suggested by Cykowski and colleagues (2010) existing literature on the topic and our findings we assume that the delayed or impaired myelination of the speech-related neuronal network in the postnatal period might be responsible for the later development of stuttering. However, there is a claim that myelination modulates neuronal development according to environmental experience, hence whether the myelination is a cause or a concomitant of stuttering behavior remains unclear.

The correlation analysis revealed that in the adults who stutter, the degree of self-rated communication impairment was positively correlated with the metabolite peak. This indicates that self-placement into the more severe stuttering group was positively correlated with the concentration of myo-inositol in the left hemisphere IFG. This result could reflect confounding factors, such as onset of stuttering, start-up of therapy, or extent of earlier therapeutic intervention. Therapy delivered in early

childhood during a dynamic growth in the speech-related regions gives a greater chance of full recovery without relapse. The neuronal ability of the brain to organize or even reorganize itself according to what it experiences is referred to as plasticity (Webb & Adler, 2008). Plasticity stands for normal processes such as development, learning, aging, as well as response to therapy (Raymer et al., 2008). As a result of the neuroplastic processes that underlie therapy-induced recovery during early childhood, the brain may regain the same function and structure that resembles normally fluent children or it may adapt by developing successfully compensatory connections between language-related areas (Chang, 2011; Neumann et al., 2003). The adult brain, on the contrary, is more likely to be less responsive to therapy. The aim of therapeutic intervention after adolescence is not normal fluency but rather successful management of stuttering. It implies stuttering modification and fluency shaping as well as psychological therapy (Chang, 2011; Guitar, 2014). From a therapeutic perspective, the left hemisphere is seen as crucial for successful management of stuttering, i.e the left hemisphere should regain its activity and not pass over duties to the right homologue (Kell et al., 2009). De Nil and colleagues (2003) found evidence of neural activation changes following fluency-inducing therapy. In particular, they reported on attenuated right-hemisphere over-activity and a shift towards more activity in the left-hemisphere regions following fluency inducing treatment. However, neuronal activation was not entirely normalized at follow-up. Normally, a stabilized therapeutic outcome requires repeated sessions with therapy (Kell et al., 2009). Giraud and colleagues (2008) reported on negative correlation between the basal ganglia activity and severity of dysfluency as a result of stuttering therapy. Hence, the fact that the correlation between the PWS' scores on the WASSP and their levels of mI was positive (more severe stuttering = high mI level) could be explained by that the PWS that participated in the study have all on some point gone through therapy for their stuttering. Unfortunately, we don't have an individual history of the therapy for each participant, so this argument remains a speculation.

We tried to supplement the above findings with the additional correlation analysis fMRI (ROI)/^{1}H-MRS to investigate the relationship between the neurotransmitter mI and the BOLD response from the regions of interest (pars opercularis, pars triangularis and pars orbitalis). We wanted to look at a complex interplay between the level of mI in the modulation of neuronal activation, where high or low concentration of the studied metabolites could be responsible for the local

effect during speech production activity. The findings did not show any correlation between local ml concentration in either left or right hemispheres and the strength of the BOLD response within the measured regions. Based on our results we concluded that there are no indications that interhemispheric concentration of mI might be directly related to local BOLD response in the IFG. However, we cannot rule out a remote effect on the cortical brain areas supporting auditory-motor integration for speech processing which we have not investigated.

Limitations of the study

In localized ^{1}H-MRS metabolites levels are often expressed as ratios, the so-called relative quantification rather than as absolute concentrations. Cr is considered the most stable brain metabolite (no change reported with age or variety of disease) and it makes it a common choice of metabolite in clinical practice for calculating metabolite peak ratios. For example, the ratios are less sensitive to relaxation properties of the signal and partial volume effects compared to absolute metabolite concentrations (de Graaf, 2007; Dramsdahl et al., 2011). However, the referencing should be done with caution since there have been detected deviant concentrations of Cr in certain pathological states as for example tumors or frontal lobe epilepsy. Hence, there is a chance that changes in the mI/Cr ratio may be a result of changes in the concentration of Cr. However, since we only see an effect for mI and not other metabolite ratios, it is unlikely that changes in Cr are driving this effect.

An other possible confounding factor is that the spectroscopy voxel covers a relatively large volume, and measures the total quantity of metabolite within that region – possibly incorporating tissue from other structures in the vicinity of the structure of interest, and without distinction between intracellular and extracellular metabolites (De Graaf, 2007).

The neurotransmitters glutamate (Glu) and glutamine (Gln) have similar chemical structures that lead to very similar spectra. Hence, at 3T field strength, their ^{1}H-MRS absorption peaks are partially overlapping. Using the PRESS sequence with TE=35 ms the separation between Glu and Gln becomes unreliable. One should keep this fact in mind when interpreting the results. The sum of these two metabolites may still be calculated with high accuracy, however optimizing the acquisition to improve distinction between the two would be beneficial.

Stuttering symptoms appear to be related to the use of language, where the PWS show consistency in a stuttered dysfluency. They stutter primary at syllable or word

initial position (Bloodstein & Ratner, 2008; Craig-McQuaide, Akram, Zrinzo, & Tripoliti, 2014). Increase in syntactic complexity also tends to enhance stuttering episodes (Perkins, Kent, & Curlee, 1991). An evident relationship between language and stuttering has influenced our choice of stimuli for the behavioral MRI. We used short, consonant-vowel DL-syllables with the intention to provoke less possible stuttering behavior in the scanner due to possible movement artifacts during articulation. As a result, none of the participants from the PWS group showed an overt stuttering during data acquisition. One may question such a methodological approach given the presence of co-articulation in the normal speech where the proceeding and the following phonemes often affect the way of pronunciation (Browman & Goldstein, 1992). Consequently, the fact that we did not detect any relationship between the neurotransmitter myo-inositol and the BOLD response from the regions of interest (i.e. IFG) may be explained by the choice of stimuli, where research on speech production may require more complex stimuli then those delivered by a version of DL-paradigm.

Our findings did not show any correlation between local mI concentration in either left or right hemispheres and the strength of the BOLD response within the IFG. One may speculate on whether for example right overactivation in the IFG characteristic for PWS is a state or a trait effect. Maybe, an enhanced activation of the right frontal operculum, the right homolog of Broca's area, is a case only when the stuttering takes place. However, following Ingham and colleagues (2000) the occurrence of overt stuttering may not be necessary to observe the neuronal activation associated with stuttering.

Because the data sample has unequal number of male and female participants in PWS group (13 male and 3 female), gender differences within the stuttering group should be interpreted with caution. This unequal number reflects the fact that more males than females stutter (3:1) (Bloodstein, 1995; Yairi & Ambrose, 2013). One could claim that this dataset support the notion of higher rate of spontaneous recovery in females during early development. Such a discrepancy in sex ratios negatively impacts the internal validity. It may however, strengthen the external validity by making the sample more representative for gender distribution. In order to make statements about the whole population of PWS we would have needed a higher number of participants. The control sample size of 27 participants was also much bigger than the PWS sample of 16 participants, probably causing a mismatch of the

variance between the PWS and control groups. This makes the results from the PWS group more susceptible to individual differences. In order to check whether the main group effect on the level of myo-inositol in the IFG could possibly be influenced by gender differences (to control for sex difference between and within groups as a confounding factor) we performed a test that did not detect any gender contribution to the result.

The main concern with self-reports, such as the WASSP is the credibility. Self-perception might be shaped by self-consciousness, true self-image, self-deception and memory. The advantage of self-report is that the people who stutter are the best-qualified witnesses to their own stuttering. They have access to the thoughts, feelings and sensations connected to their dysfluency that is inaccessible for the therapist. People usually invest more time and effort when reporting on their own disability profile, which ensures greater validity. However, even if the participants are doing their best on reporting insightful and factual information, their replies are usually inaccurate due to individual response bias (Paulhus & Vazire, 2008).

Future directions

A direction for further studies could be to look at the connectivity pattern in the brain as a function of neurotransmitter concentration. We already know that PWS have unbalanced dopamine uptake both in cortical and subcortical areas of the brain and that insufficient release of dopamine would lead to a general inhibition of movements and impulses that might cause a dysfluency in speech (Alm, 2004; Wu et al., 1997). It may be beneficial for the field of stuttering to move beyond the classic dopamine hypothesis and look into how other neurotransmitters modulate the activation observed at the fMRI imaging by using MR spectroscopy.

The fact that we found the main effect of hemispheric side on levels of NAA and Glx in the IFG but no side x group interaction leads us to the assumption that the groups do not differ in the concentration of the above named neurotransmitters. The theories on aberrant pattern of cerebral hemispheric dominance, i.e. reduced left hemisphere dominance for language processing and overactivation of right hemispheric motor and pre-motor areas might benefit from including measurements of gamma-Aminobutyric acid (GABA) a key inhibitory neurotransmitter in addition to Glx. That would contribute to a fuller picture regarding the excitation/inhibition balance in the brain. We believe that more studies mapping the pathways of neurotransmitters and their interaction with other transmitters in persons who stutter

would help to further reveal the underlying neurobiology of the disorder.

Conclusion

Stuttering is a disorder that impacts the fluency of speech hence, impedes the capacity to communicate effectively. Decades of research on the topic have produced an extensive amount of data but the mechanism behind the symptoms associated with stuttering is still not clear. Our findings confirm the close relationship between biochemistry and stuttering and suggest the role of the neurotransmitter myo-inositol as a marker of deficient myelogenesis. However, the claim that the improper formation of the myelin sheath during brain development may cause disruption in functional connectivity between the areas responsible for fluent speech production awaits further research in bigger and more matched datasets.

We believe that knowledge of changes in neurotransmitter levels and their *in vivo* detection may add useful information to the pathophysiology of stuttering, to monitor disease progression and patients' response to the therapy in a more objective manner.

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Attachment 1

Table 1

Scores for SSI-3 and WASSP

Subject	SSI-3	SSI-3 classification	WASSP Stuttering behavior	WASSP Thoughts about stuttering	WASSP Feelings about stuttering	WASSP Avoidance due to stuttering	Disa st
101	18	Mild	3.50	2.00	2.80	1.75	
102	23	Mild	5.88	6.30	7.00	5.75	
103	22	Mild	3.88	3.67	5.40	2.50	
104	26	Moderate	4.00	4.00	5.00	2.25	
105	9	Very mild	4.38	4.67	2.40	3.50	
106	10	Very mild	4.88	2.00	1.80	3.00	
107	23	Mild	3.38	6.33	5.80	4.25	
108	9	Very mild	3.50	3.00	2.80	2.75	
109	34	Severe	5.25	2.67	2.20	2.00	
130	20	Mild	4.60	5.30	5.40	3.50	
131	21	Mild	3.75	4.00	4.80	3.75	
132	6	Very mild	2.37	1.00	1.40	2.50	
133	32	Severe	2.50	2.00	1.80	1.50	
134	13	Very mild	2.50	2.00	1.80	1.50	
136	23	Mild	4.60	2.00	2.60	1.50	
137	17	Very mild	4.37	3.66	2.40	2.50	

Attachment 2

HELSE BERGEN

INFORMERT SAMTYKKE TIL fMRI-UNDERSØKELSER

Hva er MRI og fMRI?

MRI (Magnetic Resonance Imaging) er en metode hvor det tas bilder av kroppen uten bruk av røntgenstråler. Teknikken er basert på at radiobølger, fremstilt i et magnetfelt, treffer kroppen og sender signaler tilbake (ekkosignaler). Signalene blir oppfanget av en antenne, og omdannet til MR bilder på en datamaskin. fMRI (functional Magnetic Resonance Imaging) er en variant av MRI-metoden hvor endringer i blodgjennomstrømningen i hjernen ved mental anstrengelse registreres.

Hvordan forløper undersøkelsen?

Undersøkelsen kan variere noe avhengig av spørsmålsstilling og apparattype. Felles er at du ligger på en benk som kjøres inn i MR-maskinens magnetfelt. Det er helt avgjørende at du ligger helt rolig med hele kroppen under opptakene. Under opptakene hører du kraftig støy. Dette er normalt og kommer fra selve maskinen.

Er det begrensninger?

Det er enkelte personer som ikke kan delta i fMRI undersøkelser. Besvar følgende spørsmål, for å unngå mulige risikoer:

- Har du operert inn metallgjenstander i kroppen (f. eks. pacemaker, hofteprotese, eller elektroder)?
- Har du metallsplinter i øynene eller andre steder i kroppen?
- Har du tannregulering?
- Har du vært operert i hodet, øynene, ørene eller hjertet?
- Er du gravid?
- Har du fobi for trange rom?

Er det noen komplikasjoner?

Undersøkelsen er vanligvis uten komplikasjoner. Så langt vi vet i dag er der ingen risiko eller bivirkninger knyttet til fMRI undersøkelser, så lenge du overholder de sikkerhetsinstruksjoner som blir gitt før undersøkelsen. Tatovering eller makeup kan imidlertid gi hudirritasjoner.

Oppdagelse av eventuell sykdom eller andre funn under fMRI undersøkelse

En fMRI undersøkelse er per i dag ikke egnet som grunnlag for oppdagelse av eventuelle sykdommer. fMRI blir primært brukt i forskning, og er ikke en metode for klinisk diagnostikk. Personalet som gjennomfører fMRI-undersøkelsene har vanligvis ikke formell kompetanse i radiologisk diagnostikk. Alle MR-bildene vil imidlertid rutinemessig bli gransket av medisinsk ansvarlig lege/nevroradiolog. Om det oppdages noe som kan representere sykdom, vil medisinsk ansvarlig personale kontakte deg og spørre deg om du ønsker videre utredning ved og/eller henvisning til respektiv avdeling ved Haukeland Universitetssykehus eller annet sykehus.

Hva må du være forsiktig med?

Metalldeler som kommer inn i magnetfeltet kan føre til skader. Derfor må du legge fra deg følgende gjenstander før du går inn på undersøkelsesrommet:

- ur, briller, øreringer og andre smykker
- bankkort med magnetstripe (blir avmagnetisert)
- metalldeler på påkledning (f.eks. beltespenner)
- mynter, kulepenner, nøkler eller andre metalldeler (f.eks. hårnål)
- tannprotese
- høreapparat

Hvilken fordeler er det for meg som deltaker om jeg skulle delta?

Som økonomisk kompensasjon for din deltakelse så utbetales det 200 kroner kontant direkte til deg umiddelbart etter du har fullført eksperimentet. Du må kvittere under for å ha mottatt disse pengene. Deltagelse i eksperimentet vil samtidig gi erfaring med det å være forsøksperson som kan være nyttig for å forstå resultater i forskningsrapporter.

Hvilke rettigheter og forpliktelser har jeg som deltaker?

- Deltakelse er frivillig og krever samtykke hvor du som deltaker må gjøre deg kjent med dette informasjonsskrivet og undertegne samtykke om du skulle ønske å delta. Du må også fylle ut og levere de to spørreskjemaene som legges ved dette informasjonsskrivet.
- Som deltaker i forskningsprosjektet har du ingen forpliktelser.
- Du kan når som helst trekke deg fra prosjektet uten å oppgi grunn. Alt data vi har samlet fra deg vil ikke brukes og bli slettet. Det vil ikke ha noen konsekvenser for deg å trekke deg fra prosjektet.
- Hvis vi ikke skulle kunne bruke noe av den data vi samler inn fra deg til prosjektet, så vil alt data fra deg bli slettet. Men fullfører du prosjektet vil du likevel bli betalt og få skriftlig tilbakemelding etter forskningsprosjektet er avsluttet.
- Fullfører du eksperimentet så vil du få 200 kroner i kompensasjon for deltakelse som vil bli utbetalt til deg direkte etter fullført eksperiment. Du vil også få muntlig tilbakemelding umiddelbart etter fullført fMRI eksperiment og skriftlig tilbakemelding om forskningsprosjektets resultater og konklusjon etter forskningsprosjektet er avsluttet. Prosjektet vil senest være avsluttet 2013.
- Vi forbeholder oss retten til å ikke inkludere deg i prosjektet eller delta i eksperimentet om du ikke oppfyller noen av de kriterier for deltakelse som vi har tidligere beskrevet.

Hvem er ansvarlige for prosjektet og hvordan kan de kontaktes?

Prosjektleder med overordnet ansvar for prosjektet er:
Professor Kenneth Hugdahl,
Biologisk og Medisinsk Psykologi, Psykologisk Fakultet ved Universitetet i Bergen.
BB-bygget, Johannes Lie vei 91, 5009 Bergen
Telefon: 55586277 E-Post: hugdahl@psybp.uib.no

Ansvarlig for gjennomføring av prosjektet er:
Professor Kenneth Hugdahl

Medarbeidere:
Mastergradsstudenter/forskningteknikere/stipendiater/postdoktorer.

For å kunne delta så må du lese dette informasjonsskrivet og gjøre deg kjent med innholdet. Du må også fylle ut og levere spørreskjemaet som følger med dette informasjonsskrivet. Alt informasjon og personopplysninger fra deg vil holdes konfidensielt gjennom hele forskningsprosjektet. Etter forskningsprosjektet er avsluttet (senest 2013) vil alle personopplysninger om deg bli slettet mens dine fMRI opptak fra eksperimentet vil lagres med et anonymt referansenummer.

Din deltakelse er frivillig og krever samtykke.

Jeg har mottatt skriftlig og muntlig informasjon og har gjort meg kjent med dette informasjonsskrivet og gir samtykke til å delta i prosjektet.

Dato: _____

Navn (blokkbokstaver): _____

Signatur: _____

Attachment 3

UNIVERSITETET I BERGEN
Regional komité for medisinsk og helsefaglig forskningsetikk, Vest-Norge (REK Vest)

Karsten Specht
Avdeling for biologisk og medisinsk psykologi
Det psykologiske fakultet
Universitetet i Bergen

Deres ref	Vår ref	Dato
	2008/11273-ØYSV	06.10.2008

Ad. prosjekt: Auditorisk persepsjon, språklateralisering og stamming. Et multimodal studie som kombinerer atferdsundersøkelse med funksjonelle hjerneavbildningsteknikker (212.08)

Det vises til din søknad om godkjenning av forskningsprosjekt, datert 11.09.08.

Komiteen behandlet søknaden i møte den 25.09.08.

De regionale komiteene for medisinsk og helsefaglig forskningsetikk foretar sin forskningsetiske vurdering med hjemmel i Forskningsetikklovens § 4. Saksbehandlingen følger Forvaltningsloven.

Komiteen har følgende merknad. I denne studien ønsker en kun å inkludere menn. Ut fra forskningsetiske retningslinjer skal begge kjønn inkluderes der det ikke foreligger en akseptabel begrunnelse for at et av kjønnene kan utelukkes. I dette tilfellet mener komiteen at begrunnelsen ikke gir grunnlag for å utarbeide en forskningsprotokoll som kun tar hensyn til menn. Stamming rammer også kvinner og resultatene vil derfor være av betydning for begge kjønn. Komiteen ber om at kvinner inkluderes, eventuelt som en egen gruppe slik at resultatene kan sammenlignes.

Vedtak:
Prosjektet godkjennes på vilkår av at ovennevnte merknader tas til følge.

Komiteenes vedtak etter Forskningsetikklovens § 4 kan påklages (jfr. forvaltningsloven § 28) til Den nasjonale forskningsetiske komité for medisin og helsefag. Klagen skal sendes REK-Vest (jfr. forvaltningsloven § 32). Klagefristen er tre uker fra den dagen du mottar dette brevet (jfr. forvaltningsloven § 29).

Komiteen ber om å få tilsendt sluttrapport evt. trykt publikasjon for studien når dette foreligger.

Vennlig hilsen

Jon Lekven
leder

Anne Berit Ølmheim
førstekonsulent

Attachment 4

REK
REGIONALE KOMITEER FOR MEDISINSK OG HELSEFAGLIG FORSKNINGSETIKK

Region:	Saksbehandler:	Telefon:	Vår dato:	Vår referanse:
REK vest	Arne Salbu	55978498	18.08.2011	2011/1560/REK Vest
			Deres dato:	Deres referanse:
			16.08.2011	

Vår referanse må oppgis ved alle henvendelser

Karsten Specht
Department of Biological and Medical Psychology,
fMRI-Group, Jonas Lies vei 91

2011/1560 Auditorisk persepsjon, språklateralisering og stamming. Et multimodal studie som kombinere atferdsundersøkelse med funksjonelle hjerneavbilningsteknikken.

Vi viser til innsendt prosjektendringsskjema for ovennevnte studie mottatt 16.08.2011.

Endringene innebærer følgende:
Ny prosjektmedarbider.

Endring av prosjektstart og prosjektslutt

Prosjektstart	01.11.2008
Prosjektslutt	30.06.2012

Søknaden er vurdert på fullmakt av sekretariatet.

Det søkes om forlengelse av prosjektperioden til sommer 2012, grunnet forsinkelse i forbindelse med rekeruttering av ny masterstudent til å stå for gjennomføringen.

En har ingen innvendinger.

Vedtak

Prosjektendringen godkjennes i samsvar med forelagt søknad.

Vi ber om at alle henvendelser sendes inn via vår saksportal: http://helseforskning.etikkom.no ellerpå e-post til: post@helseforskning.etikkom.no.

Vennligst oppgi vårt referansenummer i korrespondansen.

Med vennlig hilsen,

Arne Salbu (sign.)
rådgiver

Norsk samfunnsvitenskapelig datatjeneste AS
NORWEGIAN SOCIAL SCIENCE DATA SERVICES

Harald Hårfagres gate 29
N-5007 Bergen
Norway
Tel: +47-55 58 21 17
Fax: +47-55 58 96 50
nsd@nsd.uib.no
www.nsd.uib.no
Org.nr. 985 321 884

Karsten Specht
Avdeling for biologisk og medisinsk psykologi
Universitetet i Bergen
Jonas Liesvei 91
5009 BERGEN

Vår dato: 17.12.2008 Vår ref: 20282 / 2 / PB Deres dato: Deres ref:

TILRÅDING AV BEHANDLING AV PERSONOPPLYSNINGER

Vi viser til melding om behandling av personopplysninger, mottatt 20.10.2008. Meldingen gjelder prosjektet:

20282	*Auditorisk persepsjon, språklateralisering og stamming. En multimodal studie som kombinerer atferdsundersøkelse med funksjonelle hjerneavbildningsteknikker*
Behandlingsansvarlig	*Universitetet i Bergen, ved institusjonens øverste leder*
Daglig ansvarlig	*Karsten Specht*

Personvernombudet har vurdert prosjektet, og finner at behandlingen av personopplysninger vil være regulert av § 7-27 i personopplysningsforskriften. Personvernombudet tilrår at prosjektet gjennomføres.

Personvernombudets tilråding forutsetter at prosjektet gjennomføres i tråd med opplysningene gitt i meldeskjemaet, korrespondanse med ombudet, eventuelle kommentarer samt personopplysningsloven/-helseregisterloven med forskrifter. Behandlingen av personopplysninger kan settes i gang.

Det gjøres oppmerksom på at det skal gis ny melding dersom behandlingen endres i forhold til de opplysninger som ligger til grunn for personvernombudets vurdering. Endringsmeldinger gis via et eget skjema, http://www.nsd.uib.no/personvern/forsk_stud/skjema.html. Det skal også gis melding etter tre år dersom prosjektet fortsatt pågår. Meldinger skal skje skriftlig til ombudet.

Personvernombudet har lagt ut opplysninger om prosjektet i en offentlig database, http://www.nsd.uib.no/personvern/prosjektoversikt.jsp.

Personvernombudet vil ved prosjektets avslutning, 31.10.2011, rette en henvendelse angående status for behandlingen av personopplysninger.

Vennlig hilsen

Bjørn Henrichsen

Pernilla Bollman

Kontaktperson: Pernilla Bollman tlf: 55 58 24 10
Vedlegg: Prosjektvurdering

Personvernombudet for forskning

Prosjektvurdering - Kommentar

20282

Hele prosjektet består av tre deler og skal sammenligne språklateralisering og organisering av språkfunksjoner i hjernen mellom stammere og kontrollpersoner. Norske, voksne og høyrehendte menn som stammer skal inngå i forsøksgruppen og skal bli målt med Bergen dikotisk lyttetest (Studie 1), funksjonell magnet resonans tomografi (fMRI) (Studie 2), og event-relatert potensiale (ERP) (Studie 3). Målsetning i alle tre studier er å finne sammenhenger mellom grad av stamming målt med SSI-3 og språklateralisering, målt med dikotisk lyttetest, fMRI, og ERP. Det er forventet at studiene vil vise en korrelasjon mellom SSI-3 målinger, som objektive målinger av grad av stamming, og grad av språklateralisering. Deltakerne, som ikke nødvendigvis må være de samme i de tre delene, vil bli rekruttert gjennom Voksenopplæringen i Bergen sentrum, StatPed-Vest, og Norsk Interesseforening for Stamme (NIFS).

Kontrollpersoner som skal inngå i prosjektet hentes til dels fra databasen for Bergen dikotisk lyttetest, og rekrutteres blant studenter og gjennom annonser og annet. Det forutsettes at opplysninger om kontrollpersoner som hentes fra nevnt database utleveres til forsker i anonym form dersom det ikke er mulig å innhente et samtykke til bruk av opplysningene. I anonym form innebærer uten koblingsnøkkel som viser til en navneliste og uten at indirekte identifiserbare opplysninger inngår.

I prosjektet vil man innhente og behandle sensitive personopplysninger om helseforhold (jf. personopplysningsloven § 2 pkt. 8 c). Behandlingen av personopplysninger kan foretas med hjemmel i personopplysningsloven §§ 8 første ledd (samtykke), 9 a.

Ombudet ønsker å gjøre oppmerksom på at bruk av en manuell navneliste med kode/koblingsnøkkel som knytter deltakernes navn til datamaterialet ikke innebærer at materialet er anonymt. Datamaterialet vil bli anonymisert senest 31.10.2011 ved at koblingsnøkkel fjernes og at alle indirekte personidentifiserende bakgrunns- og situasjonsopplysninger slettes eller endres (grovkategoriseres). Video- og lydopptak slettes.

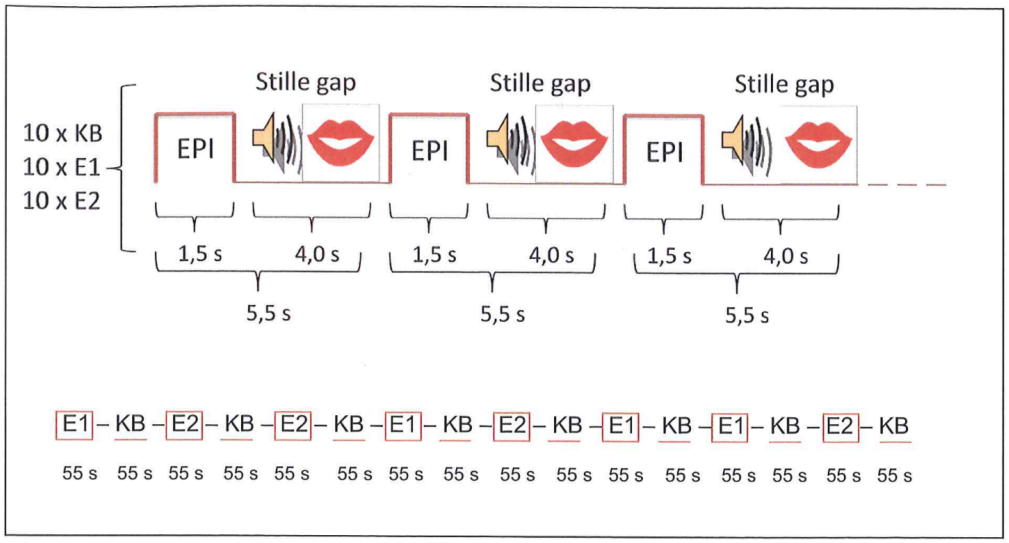
En masterstudent er involvert i prosjektet. Fra ombudets side oppfattes studenten som en medarbeider i prosjektet, og ombudet legger til grunn at det er avklart med fakultetet at studenten ikke melder inn sitt eget mastergradsprosjekt på selvstendig grunnlag.

Informasjonsskriv til deltakere i studie 1 (dikotisk lyttetest) vurderes som tilfredsstillende forutsatt at endelig anonymiseringsdato 31.10.2011 tilføyes.

Informasjonsskriv til studie 2 (fMRI) fremstår som en praktisk gjennomgang av selve MRI-undersøkelsen og sier ingenting om forskningsprosjektet, dets formål, hva som skal skje med datamaterialet, hvem som har tilgang til dette, frivillighet, trekkmuligheter mv. Det anbefales meget sterkt at man forfatter et skriv etter den mal som studie 1 benytter. Skrivet om selve MRI-undersøkelsen kan eventuelt presenteres i tillegg.

Informasjonsskriv tl studie 3 (ERP) krever en innholdsmessig gjennomgang og fjerning av gjentakelser. Det nevnes bl.a. tre ganger at man vil motta 200 kr i kompensasjon. Videre sies det at prosjektet avsluttes i mai 2009, noe som ikke stemmer overens med opplysninger i meldeskjema. Opplysningen om at erfaring som forsøksperson kan komme godt med om man har tenkt å jobbe med forskning eller pasienter i fremtiden passer eventuelt å presentere for studenter som deltar som kontrollpersoner i studien, men er neppe relevant for øvrige deltakere. I samtykkeslippen står det at "opptak fra eksperiment vil lagres med et anonymt referansenummer". Her må "anonymt" fjernes, samt at det skal redegjøres for i selve informasjonsskrivet hvor, hvor lenge og hvordan opptakene skal arkiveres. Det skal oppgis hvor man skal henvende seg for å få opptak og andre opplysninger slettet hvis ønskelig. Det anbefales meget sterkt, også når det gjelder dette skrivet, at man ser på skrivet til studie 1 og formulerer et tilsvarende skriv for studie 3. Opplysninger om formål, behandling av personopplysninger mv. mangler.

Attachment 5



Figur 1. fMRI-DL designet