


FACULTY OF MEDICINE
AND HEALTH SCIENCES

Department of Internal Medicine

Neurophysiological aspects of speech perception and production in stuttering

Sarah Vanhoutte

° 25 December 1987, Ghent

Promotor: prof. dr. Patrick Santens

Co-promotor: prof. dr. John Van Borsel

Thesis submitted to fulfil the requirements for the degree of

Doctor in Social Health Sciences:

Logopedics & Audiology

2015

Board of examiners

Prof. dr. Kristiane Van Lierde (chair)

Prof. dr. Roger Ingham

Prof. dr. Robert Hartsuiker

Prof. dr. Alfred Meurs

Prof. dr. Arnaud Szmalec

Prof. dr. Rudy Van Coster

dr. Kurt Eggers

Neurophysiological aspects of speech perception and production in stuttering

Ghent University | Department of Internal Medicine

De Pintelaan 185 (1K12A)

9000 Ghent | Belgium

sarah.vanhoutte@ugent.be | sarahvanhoutte@live.be

The greatest glory in living lies not in never falling,

but in rising every time we fall.

Nelson Mandela

Voor mijn peter en mijn oma

TABLE OF CONTENTS

LIST OF PUBLICATIONS

This doctoral thesis is based on the following articles published in or submitted to international peer-reviewed journals:

Vanhoutte, S., Strobbe, G., van Mierlo, P., Cosyns, M., Batens, K., Corthals, P., De Letter, M., Van Borsel, J., Santens, P. (2015). **Early lexico-semantic modulation of motor related areas during action and non-action verb processing.** *Journal of neurolinguistics* (34), 65-82 (Impact Factor 2014: 1.489)

Vanhoutte, S., Strobbe, G., van Mierlo, P., Cosyns, M., Batens, K., Corthals, P., De Letter, M., Santens, P., Van Borsel, J. (under review) **Timing and activation alterations of motor areas in stuttering: a silent single verb reading task.** *Brain and Language* (Impact Factor 2014: 3.215)

Vanhoutte, S., Van Borsel, J., Cosyns, M., Batens, K., van Mierlo, P., Hemelsoet, D., Van Roost, D., Corthals, P., De Letter, M., Santens, P. (2014). **CNV amplitude as a neural correlate for stuttering frequency: a case report of acquired stuttering.** *Neuropsychologia* (64), 349-59. (Impact Factor 2014: 3.302)

Vanhoutte, S., Santens, P., Cosyns, M., van Mierlo, P., Batens, K., Corthals, P., De Letter, M., Van Borsel, J. (2015). **Increased motor preparation activity during fluent single word production in developmental stuttering: a correlate for stuttering frequency and severity.** *Neuropsychologia* (75), 1-10 (Impact Factor 2014: 3.302)

Vanhoutte, S., Cosyns, M., van Mierlo, P., Batens, K., Corthals, P., De Letter, M., Van Borsel, J., Santens, P. (under review) **When will a stutter occur? The determining role of speech motor preparation.** *Neuropsychologia* (Impact Factor 2014: 3.302)

LIST OF ABBREVATIONS

µV	microvolt
%SS	percentage stuttered syllables
AAT	Aachen Aphasia Test
AF	arcuate fasciculus
Ag/AgCl	silver/silvercloride
ANOVA	analysis of variance
AVM	arteriovenous malformation
AWS	adults who stutter
BA	Brodmann area
BG	basal ganglia
BGTC	basal ganglia-thalamo-cortical
CB	cerebellum
CI	confidence interval
CMC	cortico-muscular coherence
CNV	contingent negative variation
CT	computerized tomography
CWS	children who stutter
d	days
dB	decibel
DIVA	Directions into Velocities and Articulators
DLPFC	dorsolateral prefrontal cortex
DS	developmental stuttering
DSI	diffusion spectrum imaging
DTI	diffusion tensor imaging
EEG	electro-encephalography
EMG	electromyography
ERP	event-related potential
FA	fractional anisotropy
fMRI	functional magnetic resonance imaging
FDG-PET	fluorine-18-fluorodeoxyglucose positron emission tomography
FS	fluent speakers
GFP	global field power

G-G	Greenhouse-Geisser
GODIVA	Gradient Order Directions into Velocities and Articulators
Hz	hertz
ICC	intraclass correlation coefficient
IFG	inferior frontal gyrus
ISI	interstimulus interval
kΩ	kilo-ohm
L	left
M	mean
M1	primary motor cortex
MEG	magneto-encephalography
MMN	mismatch negativity
MNI	Montreal Neurological Institute
MRI	magnetic resonance imaging
ms	milliseconds
NS	neurogenic stuttering
PALPA	Psycholinguistic Assessment of Language Processing in Aphasia
PD	Parkinson's disease
PET	positron emission tomography
PMC	premotor cortex
pre-SMA	pre-supplementary motor area
PWS	persons who stutter
r	correlation coefficient
R	right
R^2	coefficient of determination
RFO	right frontal operculum
R-locked	response-locked
ROI	region of interest
RT	reaction time
S1	warning stimulus
S2	imperative stimulus
SD	standard deviation
sec	second
SLF	superior longitudinal fasciculus
S-locked	stimulus-locked

SLP	speech language pathologist
SMA	supplementary motor area
SNR	signal-to-noise ratio
SPM	Statistical Parametric Mapping
SPSS	Statistical Package for Social Sciences
SSI	Stuttering Severity Instrument
SSM	speech sound map
STG	superior temporal gyrus
TMS	transcranial magnetic stimulation
TOI	time window of interest
vPMC	ventral premotor cortex
vs	versus
WM	white matter

SUMMARY

Stuttering is a speech disorder in which the smooth succession of speech sounds is interrupted by frequent blocks, prolongations and/or repetitions of sounds or syllables. When stuttering manifests itself for the first time during childhood, it is called developmental stuttering. When stuttering is of non-developmental origin, it is referred to as acquired stuttering. Acquired stuttering mostly derives from damage to the central nervous system which is called neurogenic stuttering. Neurologically, stuttering is characterized by alterations in cortical and subcortical brain regions related to speech motor planning, initiation, execution and monitoring.

Neurological research in stuttering contains a plethora of spatial neuroimaging studies (e.g. fMRI) but a dearth of neurophysiological studies, especially when it comes to speech motor control. However, fluent speech does not only require the appropriate amount of (de)activation of specific brain regions, it also needs a timely and precise coordination of these brain regions. Therefore, the present thesis aimed **to identify neurophysiological characteristics of speech motor control in stuttering** by the use of electro-encephalography.

First, **temporal coordination** of motor related activity during a visual **word recognition** task was assessed. Time points of motor related activity during hand action and non-action verb processing were compared in a group of fluent speakers and a group of adults with developmental stuttering. Secondly, **speech motor preparatory activity** preceding single word production was measured in real time by evoking a contingent negative variation (CNV) during a picture naming task. The CNV is an event-related potential reflecting motor preparatory activity in the basal ganglia-thalamo-cortical – loop. Speech motor preparation was compared between fluent speakers, and both fluent and stuttered words of stuttering speakers. Thirdly, although developmental and neurogenic stuttering are suggested to share common neural substrates, both types of stuttering were compared to assess whether this also accounts for speech motor preparatory activity. To that purpose, the same CNV picture naming task was performed in **a case of neurogenic stuttering**.

Timing of motor related activation was considerable altered in the stuttering group, even during a silent reading task without (speech) movement requirements. The time point of maximal motor difference between both verb types was delayed with 100 ms and showed a reversed activation pattern compared to that of fluent speakers. This reversal is hypothesized to encompass two different motor abnormalities: a general motor hyperactivation, presenting during non-action verb processing, and a specific hand motor deficit, causing decreased excitability of this region during

hand action verb processing. These findings confirm that temporal alterations in neural motor activations in stuttering are not restricted to overt speech production.

Secondly, speech motor preparatory activity generated by the basal ganglia-thalamo-cortical – loop was found to have a crucial role in stuttering. Not only has its amount of activation a determining role in the actual moment of a stutter, its activation seems also related to the underlying stuttering pathology. An important divergence between left and right hemisphere is seen in this respect. When motor preparatory activity in right basal ganglia-thalamo-cortical – loop is markedly increased, no stutter will occur. The more frequent and/or the more severe a person stutters, the higher this increase is or must be to enable fluent speech production. The lower the motor preparatory activity preceding a stutter in the left basal ganglia-thalamo-cortical – network, the more this person will stutter in general. As such, left basal ganglia-thalamo-cortical – loop is suggested to have a link with the stuttering pathology. These findings concur with a growing amount of studies stating that right hemisphere alterations are related to (successful) compensation strategies, while the left hemisphere would contain the primary cause of stuttering.

Thirdly, important differences emerged when comparing the findings concerning speech motor preparatory activity of the developmental stuttering group and the case with neurogenic stuttering. Roughly speaking, an increase in stuttering frequency was associated with an increase in CNV slope in the developmental stuttering group and a decrease in CNV in the case of neurogenic stuttering. Although neurogenic and developmental stuttering are believed to share common neural characteristics, these may be restricted to neuroanatomical findings. Both types of stuttering may show considerable variation in neurophysiological functioning, probably related to a difference in lesion localisation.

Finally, when findings of the present studies are placed within a broader framework, the importance of the motor loop of feedforward processing in stuttering is highlighted. All observed motor alterations presented without simultaneous deficits in feedback processing or without obvious inferences of language impairments. Overall, the present thesis evidences that neurophysiology is able to discover interesting and intriguing neural findings that may aid in unravelling the enigma of stuttering.

SAMENVATTING

Stotteren is een spraakstoornis waarbij de snelle opeenvolging van spraakklanken onderbroken is door het frequent voorkomen van blokkades, verlengingen en/of herhalingen van klanken of syllabes. Wanneer stotteren zich voor het eerst manifesteert tijdens de kindertijd, spreekt men van ontwikkelingsstotteren. Wanneer stotteren geen ontwikkelingsoorsprong heeft, spreekt men van verworven stotteren. Verworven stotteren komt het meest frequent voor na een letsel ter hoogte van het centrale zenuwstelsel. In dit geval spreekt men van neurogeen stotteren. Vanuit neurologisch standpunt wordt stotteren gekenmerkt door afwijkingen in zowel corticale als subcorticale structuren die betrokken zijn bij spraak motorische planning, initiatie, uitvoering en monitoring.
Neurologisch onderzoek in stotteren maakt voornamelijk gebruik van beeldvormingstechnieken. Zeker op het vlak van spraak motorische controle is het neurofysiologisch onderzoek bijzonder beperkt. Nochtans vereist vloeiende spraak niet alleen de gepaste (de)activatie van specifieke regio's in het brein, het vergt tevens een goed getimede coördinatie van deze hersenregio's. Het belangrijkste doel van deze thesis is het **uitbreiden van de neurofysiologisch kennis omtrent spraak motorische controle in stotteren** door gebruik te maken van elektro-encefalografie.

Ten eerste werd de **temporele coördinatie** van motorisch gerelateerde activiteit geëvalueerd tijdens een **spraak perceptie** taak. De tijdstippen waarop motorische activiteit optrad tijdens het stillezen van hand actie en niet-actie werkwoorden werd vergeleken tussen een groep vloeiende sprekers en een groep volwassenen met ontwikkelingsstotteren. Ten tweede werd de mate van **spraak motorische voorbereidingsactiviteit** bij één-woord-uitingen geëvalueerd. Hiervoor werd een contingent negative variation (CNV) uitgelokt aan de hand van een prent benoemtaak. De CNV is een geëvokeerde potentiaal die de mate van motorische voorbereiding reflecteert die gegeneerd wordt door het basale ganglia-thalamo-corticale circuit. De CNV werd gemeten voor vloeiende en gestotterde woorden en vergeleken met de CNV bij vloeiende sprekers. Ten derde werd dezelfde CNV prent benoemtaak uitgevoerd bij **een casus met neurogeen stotteren**. Alhoewel neurogeen en ontwikkelingsstotteren een gemeenschappelijke neurologische basis zouden hebben, werden beide types stotteren vergeleken om te onderzoeken of dit ook geldt voor spraak motorische voorbereiding.

De timing van motorisch gerelateerde activiteit bleek aanzienlijk anders te verlopen in stotteren, zelfs tijdens stillezen waarbij geen (spraak) bewegingen vereist zijn. Het tijdsstip waarop zich een maximaal motorisch verschil tussen beide werkwoorden voordeed, was met 100 ms vertraagd.

Hierbij werd tevens een omgekeerd activatiepatroon vastgesteld. Deze omkering zou het gevolg zijn van twee verschillende motorische afwijkingen: (1) een algemene motorische overactivatie, die zich voordoet bij het verwerken van de niet-actie werkwoorden, en (2) een specifiek deficit in de hand motore regio, waardoor er een verminderde excitatie optreedt van deze regio tijdens het verwerken van hand actie werkwoorden. Deze bevindingen bevestigen dat temporele veranderingen in motorische activiteit bij stotteren zich niet beperken tot spraak productie taken.

Ten tweede werd het belang van spraak motorische voorbereiding in het basale ganglia-thalamo-corticale netwerk aangetoond. Niet alleen blijkt de hoeveelheid activiteit een determinerende rol te spelen in het wel of niet optreden van een stotter, deze activiteit lijkt ook gerelateerd te zijn aan de onderliggende pathologie. Een belangrijk onderscheid tussen de linker en de rechter hemisfeer moet hierbij gemaakt worden. Wanneer spraak motorische voorbereiding in het rechter cortico-corticaal netwerk significant toeneemt, zal er geen stotter optreden. Hoe meer een persoon stottert, hoe hoger deze stijging is of moet zijn. Hoe lager de hoeveelheid motorische voorbereidingsactiviteit in het linker cortico-corticaal netwerk voordat een stotter optreedt, hoe meer deze persoon in het algemeen blijkt te stotteren. Dit suggereert een link met de onderliggende neuropathologie van stotteren. Deze resultaten bevestigen de hypothese dat bij stotteren veranderingen in de rechter hemisfeer gerelateerd zijn aan (succesvolle) compensatie strategieën terwijl veranderingen in de linker hemisfeer de primaire oorzaak van stotteren zouden omvatten.

Ten derde bleken er belangrijke verschillen te zijn op het vlak van spraak motorische voorbereiding tussen neurogeen en ontwikkelingsstotteren. Ruw gesteld werd een stijging in stotterfrequentie geassocieerd met een stijging in CNV bij de groep met ontwikkelingsstotteren en een daling in CNV bij de casus met neurogeen stotteren. De gesuggereerde gemeenschappelijke basis van neurogeen en ontwikkelingsstotteren lijkt zich te beperken tot neuroanatomische aspecten. Neurofysiologisch kunnen grote verschillen optreden die waarschijnlijk te wijten zijn aan een verschillende lokalisatie van het primaire letsel.

Wanneer de bevindingen van deze thesis in een groter kader worden geplaatst, wordt het belang van de ‘motor loop’ in feedforward verwerking beklemtoond. Alle geobserveerde motorische veranderingen treden op zonder simultane afwijkingen in feedback verwerking of zonder duidelijke interferentie van talige problemen. In het algemeen toont deze thesis aan dat neurofysiologisch onderzoek in stotteren een belangrijke bijdrage kan leveren tot het ontrafelen van het mysterie rond stotteren.

General introduction

Part I

Chapter 1

What is stuttering?

1. Definition

Stuttering is, according to the World Health Organization (WHO, 2007, F98.5), "*speech that is characterized by frequent* ***repetition or prolongation of sounds or syllables or words****, or by frequent* ***hesitations or pauses*** *that disrupt the rhythmic flow of speech. It should be classified as a disorder only if its severity is such as to markedly disturb the fluency of speech*".

Additionally, persons who stutter (PWS) may develop **secondary symptoms** in an attempt to overcome or avoid the primary speech characteristics described in the WHO definition. These secondary symptoms are learned behaviours and can be verbal (e.g. changes in pitch and/or loudness, incomplete phrases, synonyms, …) and non-verbal (e.g. frowning, eye blinks, …). Finally, stuttering can also evoke **negative emotions and cognitions** like fear, embarrassment, guilt, … As these may have a major impact on life, stuttering is often compared to an iceberg *(see figure 1)* in which the overt features (primary and secondary symptoms) are situated above the surface and the covert features (negative emotions and cognitions) below the surface (Bloodstein & Ratner, 2008; Guitar, 2006; Van Borsel, 2011).

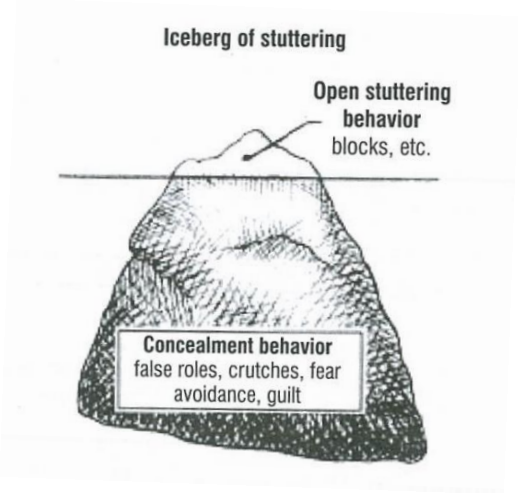

Figure 1: Dr. Joseph Sheehan's picture of the iceberg of stuttering showing the overt and covert features of stuttering. Source: https://www.mnsu.edu/comdis/kuster/TherapyWWW/intensive/sheehanclinic.html

2. Subtypes of stuttering

In most cases, stuttering is of developmental origin, manifesting itself for the first time during childhood and as such is called developmental stuttering (DS). When stuttering is of non-developmental origin, it is referred to as acquired stuttering (Van Borsel, 2014). Acquired stuttering can be divided in 4 subtypes depending on the aetiology: drug-induced, psychogenic, malingered and neurogenic stuttering (NS) (Van Borsel, 2011). In what follows, all types are described in more detail. As the present thesis concerns DS and NS, these subtypes will be highlighted.

In the following chapters, the term stuttering is used to refer to DS unless stated otherwise.

3. Developmental stuttering

3.1. Onset

Despite a few cases of stuttering emergence during the teens (e.g. Andrews & Harris, 1964; Meltzer, 1934; Preus, 1981), most studies do not report onset past the age of 9 years (e.g. Ohasi, 1977). About 95% of children who stutter (CWS) are found to have started stuttering by the age of 4 years (Yairi & Ambrose, 2005) with a mean age of onset at 33 months (for a review, see Yairi & Ambrose, 2013). Whereas onset has been described as mostly gradual for a long time, it now seems that a substantial amount of children (40 % – 53.2 %) experience a rather sudden onset (Buck et al., 2002; Yairi & Ambrose, 2005). Repetitions, of both syllables and single-syllable words, are the most frequently observed stuttering-like dysfluencies in early stuttering (Ambrose & Yairi, 1999; Van Riper, 1982) and are the prime speech characteristics that prompt identification of early stuttering by parents (e.g. Yairi, 1983). Prolongations usually appear somewhat later followed by blocks, though some children display prolongations and blocks already at or close to stuttering onset (Guitar, 2006).

3.2. Incidence, prevalence and natural recovery

Many CWS recover spontaneously without any treatment. Percentages vary from 68% to even as high as 96% (for a review, see Yairi & Ambrose, 2013). Several factors have been identified that increase the likelihood for spontaneous recovery (Bloodstein & Ratner, 2008; Guitar, 2006):

Factor	Associated with recovery
Gender	Being a girl
Age at onset	Earlier age at onset
Family history	No relatives who stutter or relatives that have recovered from stuttering
Linguistic skills	Higher receptive and expressive language skills, especially phonological skills

Both incidence and prevalence can vary greatly depending on the age range that is sampled. This is due to (1) the high percentage of natural recovery in young children mentioned above, and (2) a decrease in percentage of new onsets as the population included becomes older (Preus, 1981). Until now, an average life-span incidence of 5% and prevalence of 1% have generally been accepted (Bloodstein & Ratner, 2008). Recently, these numbers are suggested to be an under- and overestimation respectively. Concerning incidence, 4 out of 6 investigations performed since 2000 report an average of 8% or higher (Dworzynski et al., 2007; Felsenfield et al., 2000; Månsson, 2005; Reilly et al., 2009). Concerning prevalence, a 0.72% life-span prevalence was found with a considerably higher prevalence for pre-schoolers and early grades (1.4%) compared to adults (ages 21-50: 0.78%; ages 51+: 0.37%) (Craig et al., 2002). Stuttering is known to be a worldwide speech

disorder afflicting all races and probably all etnic/cultural groups (e.g. Ardila et al., 1994; Riaz et al., 2005).

3.3. Gender

Although in general more boys are found to stutter than girls (on average 3:1), this ratio, similar to prevalence and incidence, varies according to the age range sampled. The younger the children, the smaller the ratio (Bloodstein & Ratner, 2008). It even approaches an equal distribution near stuttering onset (e.g. Månsson, 2005). For children aged 6-20 years, a male-to-female ratio of 4.6 was found in a large European study (Van Borsel et al., 2006). This increase in sex ratio is either the result of an increasing proportion of boys beginning to stutter at later ages (West, 1931) or by a larger amount of girls that recover (Yairi & Ambrose, 2005). Several explanations for the gender bias have been given varying from hormonal influences (Geschwind & Galaburda, 1985), environmental aspects (Johnson & Associates, 1959; Goldman, 1967), slower early language development in boys (West & Ansberry, 1968) to genetic factors (Kidd, 1984; Suresh et al., 2006).

3.4. Genetics

Several lines of evidence point to a genetic component in stuttering. Besides a higher incidence of stuttering in first degree relatives of PWS (20 – 74%) than in the general population (1.3 – 42%) (Andrews et al., 1991; Felsenfeld et al., 2000; Howie, 1981), also twin studies reveal considerably higher concordance levels[1] of stuttering in monozygotic (20 – 90%) compared with dizygotic twins (3 – 19%) (Kidd et al., 1981; Yairi et al., 1996). Recently, several candidate genes have been identified that possibly contribute to the transmission of stuttering in families (for a review, see Kraft & Yairi, 2012). Noteworthy is that none of the twin studies found a concordance of 100% suggesting that stuttering is not 100% gene-based. Overall, the current findings suggest that emergence of stuttering might include multiple genes and relies on additional factors like environmental influences (Ambrose et al., 1997; Ward, 2006; Yairi & Ambrose, 2013) which will impact neurodevelopment (Bloodstein & Ratner, 2008). Important environmental influences encompass e.g. other's reactions, family communication style, family expectations, stressful life events (Guitar, 2006).

3.5. Continuity hypothesis

The continuity hypothesis suggested that the difference between stuttering and normal nonfluency in young children is one of degree only. Heavy pressure on the child to speak would increase the nonfluency which would then be entitled as stuttering (Bloodstein, 1970). As such, the difference

[1] The presence of a given trait (in this case: stuttering) in both members of a pair of twins.

between normal nonfluency and stuttering was hypothesized to be quantitative and not qualitative. Currently, stuttering is more often addressed as qualitatively different from normal nonfluency (Bloodstein & Ratner, 2008).

4. Acquired stuttering

4.1. Drug-induced stuttering

Drug-induced (or pharmacogenic) stuttering refers to stuttering that originates as a side-effect of pharmacological agents (Van Borsel, 2014). A large variety of drugs affecting multiple neurotransmitter systems (cholinergic, dopaminergic, noradrenergic and serotonergic) have been found to induce stuttering (Brady, 1998). The clinical picture is very heterogeneous. While different drugs may elicit similar characteristics, one and the same drug may evoke different symptoms in different patients (Beck, 2000). In all reported cases, stuttering was resolved by discontinuing the offending drug (Brady, 1998).

4.2. Psychogenic stuttering

When the involuntary appearance of speech dysfluencies is related to a psychological problem, a prolonged period of stress, or an emotional trauma, it is referred to as psychogenic stuttering (Guitar, 2006; Van Borsel, 2014), previously called hysterical stuttering (Bluemel, 1935; Deal & Doro, 1987; Freund, 1966). It has sometimes been classified as a conversion reaction (i.e. a physical or behavioural expression of a psychological conflict) (Mahr & Leitz, 1992). Due to the varying clinical picture on both primary and secondary behaviours as well as on affective reactions towards the stuttering (Baumgartner, 1999; Guitar, 2006), the differential diagnosis with NS may be very challenging (Lundgren et al., 2010).

4.3. Malingered stuttering

In malingering, a person fabricates (pure malingering) or exaggerates (aggravation) symptoms of an illness or incapacity usually for some sort of personal gain (Van Borsel, 2014). Malingered stuttering is a rare condition that has only been reported in a forensic context (Bloodstein, 1988; Seery, 2005; Shirkey, 1987). Although it is clearly distinct from psychogenic stuttering, as the dysfluent speech is produced consciously and intentionally, the differential diagnosis may be very difficult (Van Borsel, 2014). At present, no sound test to detect malingered stuttering exists (Van Borsel, 2011).

4.4. Neurogenic stuttering

NS refers to stuttering deriving from damage to the central nervous system (Canter, 1971). It is the most common type of acquired stuttering and can arise following a wide variety of disorders of which stroke is the most common cause, followed by traumatic brain injury and neurodegenerative disorders like Parkinson's disease (Theys et al., 2008). NS usually refers to a first occurrence of stuttering in previously fluent individuals. Several alternative names for NS have been proposed (for an overview, see Van Borsel, 2014). 'Neurogenic stuttering' remains however the most frequently used term.

4.4.1. Incidence and gender ratio

As the main body of knowledge on NS is based on case descriptions, NS has previously been described as an uncommon disorder (Ludlow et al., 1987; Ringo & Dietrich, 1995). The findings of a systematic, one-year prospective study in stroke patients contradict this idea. In the acute phase, an incidence of 5.3% was found. 17 out of 319 stroke patients presented with more than 3% stuttering-like dysfluencies during either conversation, monologue or reading of a text. After 6 months, the stuttering persisted in half of them, i.e. 2.5% of all stroke patients (Theys et al, 2011).

NS irrespective of aetiology seems to occur more in men than in women (Bloodstein & Ratner, 2008). Gender ratios as high as 15:1 have been reported (Mazzucchi et al., 1981). Interestingly, Theys et al., (2011) found an equal male/female ratio in the acute phase following stroke which increased to 3:1 after 6 months. This finding cautiously suggests that female stroke patients are more likely to recover from NS.

4.4.2. Behavioural characteristics

Previously, NS has been suggested to differ from DS based on some typical speech and non-speech characteristics (Helm-Estabrooks, 1999):

Characteristic	**Typical for NS**
Primary speech symptoms	Nearly as frequent on grammatical as on substantive words Not only on initial syllables/sounds Relatively consistently across different speaking tasks
Secondary symptoms	Not associated with moments of dysfluency
Emotions and cognitions	The person may be annoyed but is not anxious

However, many case studies demonstrated that a substantial amount of NS patients do not conform these differential characteristics (e.g. Koller, 1983; Mowrer & Younts, 2001; Sahin et al., 2005; Van Borsel et al., 2003b). NS may even be more similar to DS than originally suggested (Theys et al., 2008;

Van Borsel & Taillieu, 2001). Reviewing the literature, also Van Borsel (1997) suggested that the clinical symptomatology does not enable a safe distinction between NS and DS.

4.4.3. Neurological characteristics

Case reports have described NS following damage in all cortical lobes as well as in basal ganglia, thalamus, cerebellum, brain stem and corpus callosum (for a review, see Van Borsel, 1997; De Nil et al., 2009). A recent group study revealed that stroke induced stuttering was associated with a left-sided cortico-basal ganglia-cortical network encompassing inferior frontal, superior temporal, and intraparietal cortex, as well as basal ganglia and their white matter interconnections through the superior longitudinal fasciculus and internal capsule (Theys et al., 2012). Many of these structures have been found to have a crucial role in DS as well *(see chapter 3)*, suggesting that NS and DS may share common neural characteristics (Theys et al., 2012).

Formerly, both types of stuttering were considered to be two different entities (e.g. Helm-Estabrooks, 1999; Ringo & Dietrich, 1995). The observed overlap in behavioural and neurological characteristics triggers the question whether DS and NS really are two distinct types of stuttering.

Chapter 2

Neuroanatomical organization of fluent speech production

Speech production is the result of a complex interaction between linguistic, motor, auditory and somatosensory processes involving many cortical and subcortical brain structures. Models on speech production belong either to a psycholinguistic tradition, which focuses on higher-level linguistic processing (e.g. Dell, 1986; Indefrey & Levelt, 2004), or to a motor control tradition which concentrates on lower-level articulatory control (e.g. Directions into Velocities of Articulators (DIVA) model by Guenther, 2006; Gradient Order DIVA (GODIVA) model by Bohland et al., 2010). The present thesis will focus on the motor part of speech production. Phonological representations are suggested to interface higher-level language centres and lower-level motor systems (Bohland et al., 2010). As such no detailed description of the linguistic processes preceding phonological encoding nor their neural correlates will be discussed.

A substantial part of what follows is based on the GODIVA model (Bohland et al., 2010), which addresses the selection, sequencing and initiation of speech movements, and on the DIVA model (Guenther, 2006), which addresses the acquisition and execution of sensorimotor speech programs. According to the DIVA model *(see figure 2)*, speech motor control encompasses a feedforward and a feedback control subsystem. In the feedforward system, speech production is realized by sending well-learned speech motor programs from speech motor planning to execution areas. The feedback system compares the expected and the actual sensory speech output and guides the articulators in case of mismatch.

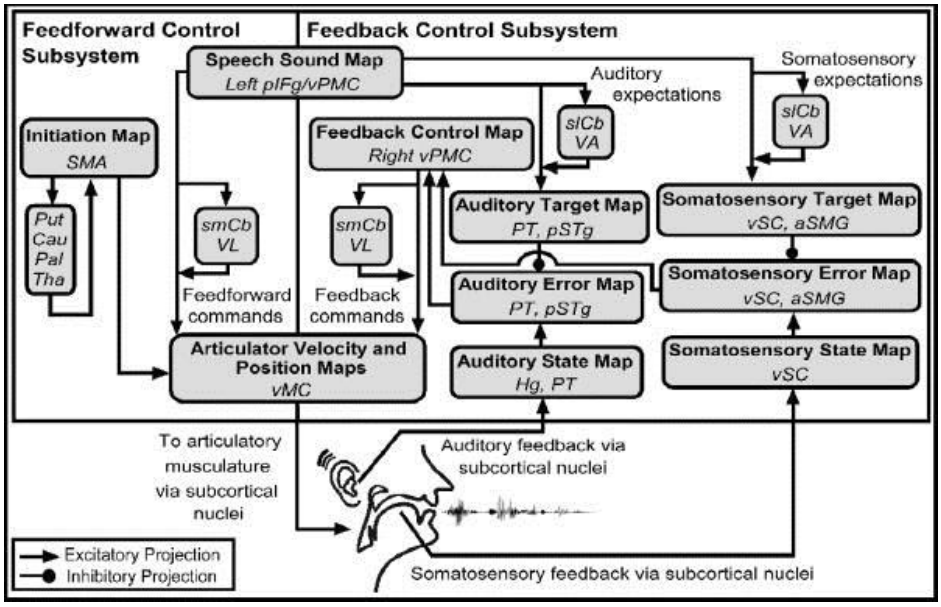

Figure 2: Schematic diagram of the DIVA neural network model obtained from Golfinopoulus et al., 2010. Abbreviations: aSMg = anterior supramarginal gyrus; Cau = caudate; Pal = pallidum; Hg = Heschl's gyrus; pIFg = posterior inferior frontal gyrus; pSTg= posterior superior temporal gyrus; PT = planum temporale; Put = Putamen; slCB = superior lateral cerebellum; smCB = superior medial cerebellum; SMA = supplementary motor area; Tha = thalamus; VA = ventral anterior nucleus of the cerebellum; VL = ventral lateral nucleus of the thalamus; vMC = ventral motor cortex; vPMC = ventral premotor cortex; vSC = ventral somatosensory cortex.

All cortical regions discussed in light of feedforward and feedback processing are shown in figure 3.

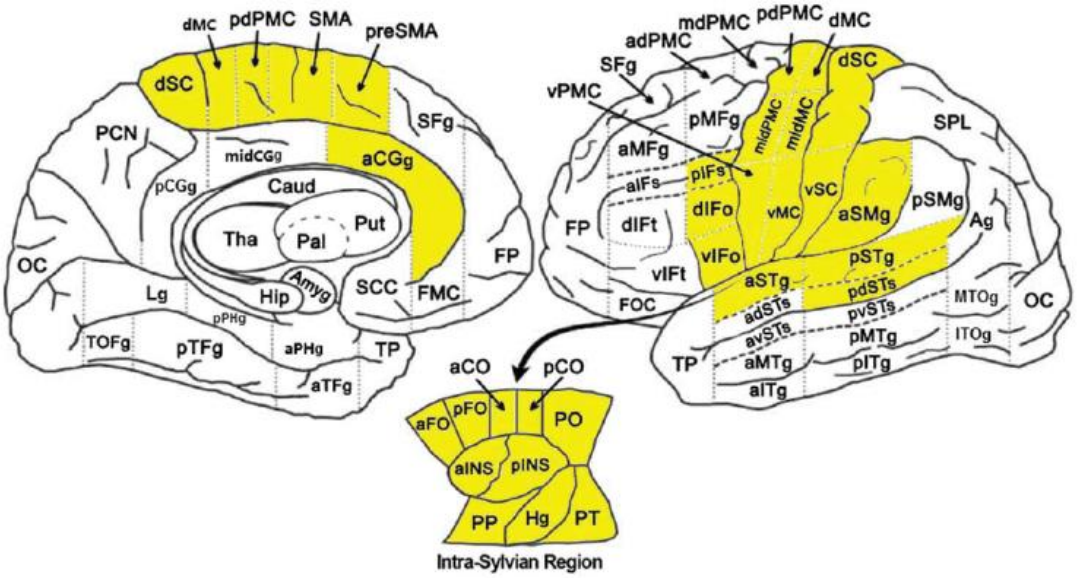

Figure 3: Schematic diagram of the brain showing in yellow the regions that provide major contributions to speech, both perception as production (obtained from Cai et al., 2014b).
Abbrevations referring to structures mentioned in the text: a = anterior; d = dorsal; p = posterior; v = ventral; Caud = caudatum; CGg = cingulate gyrus; IFO = inferior frontal operculum; Ifs = inferior frontal sulcus; MC = motor cortex; PMC = premotor cortex; preSMA = pre-supplementary motor area; Put = putamen; SC = sensory cortex; SMA = supplementary motor area; STg = superior temporal gyrus; Tha = thalamus

1. Feedforward processing

After retrieving the phonological codes from the left posterior superior temporal gyrus (STG) (Indefrey, 2011), **phonological encoding** can take place in pre-supplementary motor area (pre-SMA) and left posterior inferior frontal gyrus (IFG), i.e. dorsal pars opercularis (Brodmann Area (BA) 44, posterior part of Broca's area) (Bohland & Guenther, 2006; Indefrey & Levelt, 2004; Papoutsi et al., 2009; Price, 2009, 2012). Both regions are associated with hierarchical sequencing. While pre-SMA would contain cells that represent abstract syllable frames, the left dorsal pars opercularis would be more related to sequencing discrete units like phonemes (Bohland et al., 2010).

Next, these phonological words are transferred into **articulatory motor programs** (Indefrey & Levelt, 2004). For this purpose, the left ventral premotor cortex (vPMC) and/or the adjacent left ventral pars opercularis is/are activated (Papoutsi et al., 2009; Price, 2009, 2012). This region is suggested to contain the mental syllabary, as referred to by Levelt and Wheeldon (1994) or the Speech Sound Map (SSM), as referred to in the DIVA (Guenther, 2006) and GODIVA model (Bohland et al., 2010). The mental syllabary/SSM is a repository for articulatory scores for frequently used syllables and phonemes, with syllables being the most typical sound type represented. The best matching articulatory scores are selected and compiled so that sensorimotor planning can take place. The resulting speech motor programs are sent to the left primary motor cortex (M1) for **execution** (Guenther, 2006).

M1 is characterized by a dorso-ventral somatotopic organization for lip, jaw, vocal/laryngeal and tongue movements (Grabski et al., 2011). While left M1 is hypothesized to drive the execution of the motor programs, right M1 would become active once overt speech is initiated in order to aid in the online control of the articulators (Bohland & Guenther, 2006). Corticospinal and corticobulbar tracts transport the execution commands from M1 to the cranial and peripheral nerves that control the muscles involved in respiration, phonation and articulation (Santens & De Letter, 2010).

An important **subcortical contribution** during speech production is known as well. Although basal ganglia (BG) do not generate movements themselves, they select and enable them by coordinating signal flows throughout the cortical representations (Bohland et al., 2010). Several basal ganglia-thalamo-cortical (BGTC) loops exist (Alexander & Crutcher, 1990). According to the GODIVA model (Bohland et al., 2010; Civier et al., 2013), two BGTC-loops are involved in speech production. The planning loop interferes during phonological encoding and involves the caudate nucleus and the ventral anterior thalamus. The motor loop interferes during motor execution and passes activity from the SMA proper via left putamen and ventrolateral thalamus into M1. BGTC-loops are important for biasing cortical competition in favour of the appropriate response and for the properly timed initiation and release of the speech motor programs (Cunnington et al., 1996; Mink, 1996; Price, 2012). Anterior cingulate cortex would aid in the suppression of inappropriate responses (Price, 2009, 2012).

Also the cerebellum (CB) is suggested to provide precisely timed motor commands (Bohland & Guenther, 2006). CB receives a copy of the feedforward command from the premotor areas and projects information back to M1 (Guenther, 2006). CB is hypothesized to subserve the online concatenation of syllable-sized motor programs into fast, smooth and rhythmically organized larger units such as words and phrases (Ackermann, 2008; Price, 2012).

2. Feedback processing

Another copy of the feedforward speech motor command is sent to the auditory and somatosensory areas. This duplicate, called **the efference copy**, contains the intended sensory outcome of the speech motor command which is compared to the actual outcome as registered by the sensory cortical areas (Hickok, 2012; Golfinopoulus et al., 2010). In case of discrepancy, corrective motor commands are sent back to the motor areas (Guenther, 2006). In case of a direct match, activity in auditory cortex is suppressed. This mechanism is termed speech-induced auditory suppression (Christoffels et al., 2007; Curio et al., 2000; Houde et al., 2002; Numminen et al., 1999; Tourville et al., 2008). A similar somatosensory suppression might exist in conformity with the motor induced somatosensory suppression observed in limb movement research (Blakemore et al., 1998; Miall & Wolpert, 1996; Wolpert et al., 1995).

Feedback processing is very important during speech acquisition and development. Every mismatch results in corrective motor commands that update the articulatory score saved in the mental syllabary/SSM. By consequence, feedforward commands become more accurate and less mismatches occur. Eventually, feedforward commands are sufficient and speech production will rely more heavily on the feedforward than on the feedback subsystem (Guenther & Vladusich, 2012).

In addition to this external monitoring loop, there is also an internal loop in which an inner phonological plan is sent to the speech comprehension system. This monitoring loop is specified in psycholinguistic models of speech production (e.g. Levelt et al., 1999).

3. Cortico-cortical communication

To transfer information from one cortical area to another, cortico-cortical white matter (WM) bundles are necessary. For speech production, four important tracts have been identified that are part of the so called 'dorsal stream' which connects frontal with temporal and parietal regions. They encompass the arcuate fasciculus (AF), which directly connects frontal with temporal cortex, and three superior longitudinal fasciculi (SLF II, SLF III, SLF-tp) which pass through the parietal cortex (Friederici & Gierhan, 2012). A detailed overview of their connections is depicted in figure 4. Because these tracts interconnect frontal motor areas (IFG, PMC, M1) with posterior temporo-parietal areas (STG, middle temporal gyrus, supramarginal gyrus, angular gyrus, inferior parietal lobule), they highly support sensorimotor integration (Friederici & Gierhan, 2012; Gierhan, 2013).

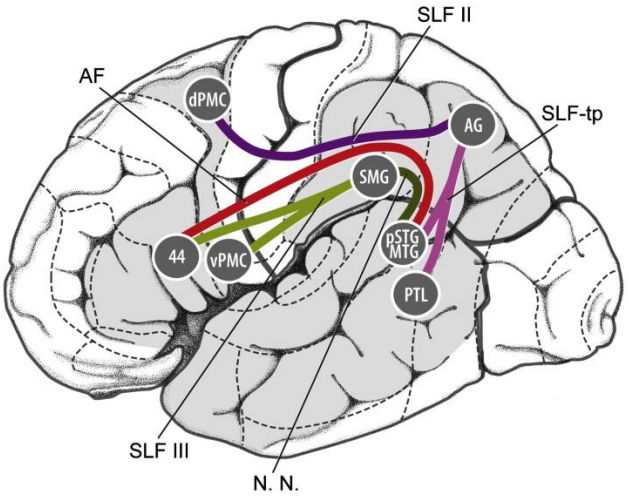

Figure 4: Construction and schematic illustration of the dorsal fiber tracts that form the SLF and AF (obtained from Gierhan, 2013).
Abbrevations: AF (red) = arcuate fasciculus; SLF II (purple), SLF III (light green), SLF-tp (pink) = second/third/temporoparietal component of superior longitudinal fascicle. AG = angular gyrus, dPMC = dorsal premotor cortex, N. N. = nomen nescio, pSTG/MTG = posterior superior temporal gyrus/middle temporal gyrus, PTL = posterior temporal lobe, SMG = supramarginal gyrus, vPMC = ventral premotor cortex, 44 = BA 44.

Chapter 3

Structural and functional neural alterations in developmental stuttering

Despite decades of research, the enigma of stuttering has still not been unraveled entirely. There is, however, compelling evidence that DS arises from genetic determinants *(see chapter 1, 3.4. Genetics)* affecting neurodevelopment during childhood (Bloodstein & Ratner, 2008).

Just as fluent speech, stuttering has mainly been approached from two different traditions: a psycholinguistic and a motor control tradition. It is an ongoing debate whether stuttering is a language and/or a motor disorder (Kent, 2000). In this thesis, a method is used that approaches stuttering as a deficit in speech motor control. More information on the psycholinguistic theories can be found in Bloodstein and Ratner (2008). In what follows, the main neural findings related to speech motor control will be addressed. A wide variety of anatomical and functional neural abnormalities have been found in PWS suggestive of an impaired dynamic interaction among cortical and subcortical systems supporting speech motor planning, initiation, execution and monitoring.

1. Cortical findings

1.1. Motor hyperactivation

The first neural signature of stuttering involves the abnormal engagement of the frontal motor areas. Overactivation of M1, SMA and cingulate motor area are frequently reported (for a meta-analysis, see Brown et al., 2005). Additional overactivations have been described in pre-SMA, IFG and PMC (e.g. Chang et al., 2009; De Nil et al., 2008; Fox et al., 2000; Loucks et al., 2011; Lu et al., 2010b). Deactivations are reported as well though mostly in left motor areas (Belyk et al., 2014; Neumann et al., 2003; Preibisch et al., 2003; Watkins et al., 2008). Overt speech is not a prerequisite to find these motor abnormalities. Also during perception tasks, PWS overactivate motor-speech planning and execution areas (De Nil et al., 2000, 2001, 2003; Liotti et al., 2010). Thus, even without the requirement of overt speech, PWS strongly emphasize articulatory processes (De Nil et al., 2003).

The implications of these motor abnormalities depend on the areas that are involved. The alterations in IFG and vPMC are assumed to be related to deficits in sending **feedforward commands** to primary motor and auditory regions to execute and monitor speech (Brown et al., 2005; Chang et al., 2011; Giraud et al., 2008). The hyperactivation in M1 seems to represent a lack of coordination in the **cortical control of the articulators and the larynx** (Belyk et al., 2014). As SMA and pre-SMA are important cortical input and projection areas of the subcortical BG, their overactivation is linked with an impairment in BGTC- loops (Belyk et al., 2014) causing **timing and/or automaticity deficits** *(see 2. Subcortical findings).*

Dysfunctional forward modelling implies that other movements but speech may be affected too. Indeed, adults who stutter (AWS) have difficulties in motor skills unrelated to speech. Both non-speech orofacial and vocal tract gestures as well as upper limb movements show alterations in neural

control. Non-speech oral gestures (e.g. cough, sigh, kiss, …) evoke similar neural differences between AWS and fluent speakers (FS) as speech production (Chang et al., 2009). In addition, AWS have an imbalanced functional lateralization of the control of finger tapping (Morgan et al., 2008; Neef et al., 2011) and an abnormal excitability in hand motor representations (Busan et al., 2011).

Based on these observations, some authors proposed that DS is a **general motor disorder** involving the entire motor system (Chang et al., 2009; Neef et al., 2011). Stuttering would then only be a symptom of a subtle and complex motor disorder that becomes evident during speech control due to its dynamic complexity (Busan et al., 2011). Speech is, however, also proposed to have evolved from hand gesture control (Corballis, 2002; Rizzolatti & Arbib, 1998). If true, a link between hand and mouth motor areas might have remained, explaining the subtle deficiencies in manual tasks. In this view, DS is primarily a speech motor disorder with secondary hand motor deficits (Saltuklaroglu et al., 2009).

1.2. Auditory hypoactivation

A second neural signature of stuttering is a reduced auditory activation in left (De Nil et al., 2008; Watkins et al., 2008) or bilateral STG (Brown et al., 2005). Although auditory processing in itself seems to be altered in PWS for tones (Hampton & Weber-Fox, 2008) and speech stimuli (Corbera et al., 2005; Jansson-Verkasalo et al., 2014), it is especially the simultaneous auditory cortex hypoactivation and speech-motor cortex hyperactivation that has been theorized and examined. These studies undoubtedly show that the interaction between auditory and motor cortices is abnormal during speech production (e.g. Braun et al., 1997; Chang et al., 2009; Fox et al., 1996; Watkins et al., 2008). It remains to be determined, however, which part(s) of the auditory-motor integration is(are) altered (Belyk et al., 2014).

An anthology of some current theories shows that evidence is available for impairments in both feedforward and feedback modelling. Concerning feedback processing, one hypothesis suggests that auditory errors are inefficiently detected (Jansson-Verkasalo et al., 2014), while another hypothesis posits that auditory errors are correctly identified but incorrectly translated into motor corrective responses (Cai et al., 2012). Timing related explanations exist as well. In PWS, the rapid integration of auditory information with ongoing motor planning and control is impaired (Cai et al., 2014a). During speech acquisition and development, this default might impair the creation of stable and accurate internal speech sound representations (Beal et al., 2010, 2011).

Problems in feedforward processing have been hypothesized as well *(see 1.1. Motor hyperactivation).* These defaults would cause an overreliance on feedback processes (Civier et al., 2010; Max et al., 2004). According to Brown et al., (2005), the overactivation of the motor cortex

results in an increased efference copy signal which overly inhibits the auditory cortex activity. This hypothesis is contradicted by the findings of Beal et al., (2010, 2011) who reported a normal speech-induced suppression of the auditory cortex during vowel production in AWS as well as in CWS.

1.3. Impaired white matter connectivity

The distributed nature of the above mentioned functional differences suggests that anatomical abnormalities in stuttering may not be limited to specific cortical damage. Indeed, deviations in WM pathways that connect cortical areas involved in speech motor control and monitoring have frequently been described though large spatial variation exists among these studies *(see figure 5).*

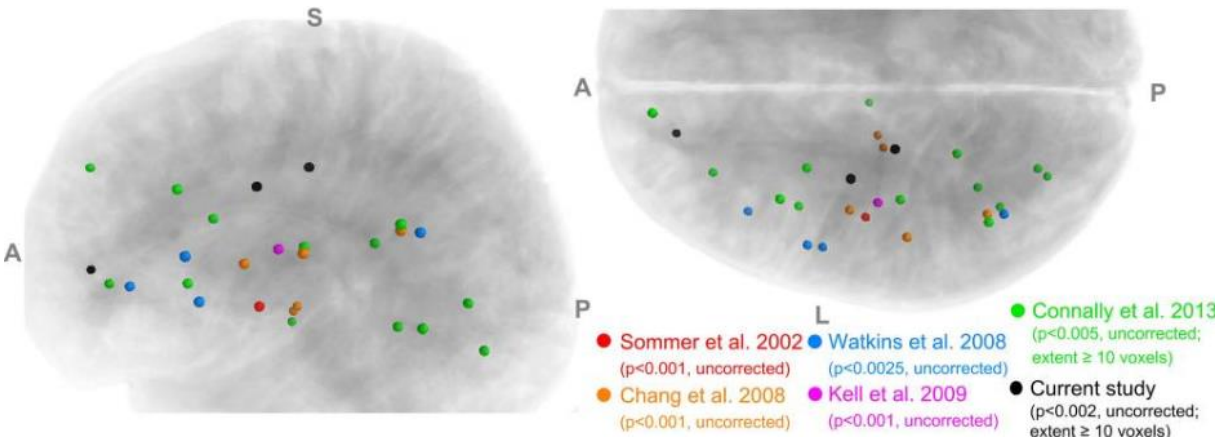

Figure 5: A summary of voxels identified with significantly lower fractional anisotropy (FA) in PWS than in FS reported in 6 studies (obtained from Cai et al., 2014b). Only the left hemisphere is depicted. Left panel: left view; Right panel: superior view. This figure illustrates the large spatial variation of the FA reductions across different studies. Abbrevations: A = anterior, L = lateral, P = posterior, S = superior.

The most consistent finding is a reduced WM density, as measured by fractional anisotropy (FA) based on diffusion tensor imaging (DTI), in the region of the left ventral sensorimotor cortex (also referred to as left Rolandic operculum). This region is located caudally to Broca's area and close to the M1 representations of the articulators and the larynx (Chang et al., 2008; Connally et al., 2014; Cykowski et al., 2010; Sommer et al., 2002; Watkins et al., 2008). As several WM bundles pass through this area, the FA decreases might reflect a disruption in one or more of the following pathways:

(1) The largest candidates are the **SLF and AF**. Because these long-range WM tracts interconnect frontal motor areas with posterior temporo-parietal areas, they are of critical importance for integrating motor plans and sensory feedback during speech production (Gierhan,

2013). As such, an AF/SLF impairment provides a structural correlate for the inefficient auditory-motor integration in stuttering (Connally et al., 2014; Cykowski et al., 2010; Watkins et al., 2008).

(2) This region also contains small, cortico-cortical u-fibres **interconnecting Broca's area, vPMC and M1** representations of the articulators (Connally et al., 2014). As posterior Broca and vPMC are suggested to store well-learned speech sensorimotor programs (Guenther, 2006), a defective connectivity with M1 may lead to inefficient readout of the selected speech motor programs (Cai et al., 2014b; Chang et al., 2011; Neef et al., 2015; Sommer et al., 2002). A disruption in this pathway thus provides a structural correlate for the hypothesized weakened feedforward system in stuttering (Civier et al., 2010; Max et al., 2004).

(3) Also the **corticobulbar tract** is located here, carrying upper motor neurons from M1 to the pons where the cranial nerves supporting orofacial movements are innervated (Chang et al., 2008; Connally et al., 2014).

(4) The decreased WM density might also hamper **cortico-striatal connectivity** (Civier et al., 2013) interrupting the BGTC-loops *(see 2. Subcortical findings).*

Unfortunately, due to inherent limitations of DTI and FA, the contribution of any specific WM tract cannot be distinguished (Cieslak et al., 2015). Diffusion spectrum imaging (DSI) allows to overcome these limitations (Shin et al., 2012; Wedeen et al., 2008). Using DSI, abnormalities were also found in AF, though in different regions than previously reported. A decrease in streamlines was observed in left AF, connecting the insula and IFG, and in right AF, connecting inferior temporal gyrus and supramarginal gyrus (Cieslak et al., 2015).
Some other WM bundles that are altered in PWS include corticospinal tract (e.g. Kronfeld-Duenias et al., 2014), corpus callosum (e.g. Choo et al., 2011), and the newly identified frontal aslant tract, connecting IFG with SMA and pre-SMA (Kronfeld-Duenias et al., 2014).

1.4. Cause? Consequence? Compensation?

Because DS starts during childhood, neuroanatomical growth and maturation in CWS may follow an abnormal trajectory (Beal et al., 2013; Chang, 2011). Structural anomalies will cause functional alterations which on their turn may further affect brain networks across development. The SLF for example is known to develop up to adolescence (Giorgio et al., 2008; Paus, 1999). Moreover, the brain will try to overcome these deficiencies. Neural adaptations and compensatory processes may also shape structural development (Chang et al., 2015). As a result, the neural activity and morphology pattern observed in adults is a combination of the cause of stuttering on the one hand and the consequence of lifelong stuttering and compensation strategies on the other hand. It is an

ongoing discussion which neural anomalies are related to the cause and which to consequence/compensation. Particularly the relative role of left and right hemisphere has been addressed.

Although not generally believed (e.g. Connally et al., 2014; Kronfeld-Duenias et al., 2014; Watkins et al., 2008), **left hemisphere** abnormalities are more often associated with the basis of stuttering. Especially the abnormalities in left inferior frontal regions have been mentioned in this respect. A reduction in grey matter volume of left IFG has been found to correlate positively with stuttering severity and to be independent from recovery (Kell et al., 2009). Its activation has also been described to remain reduced after successful therapy, despite the normalization of other abnormal activations due to this therapy (Neumann et al., 2003). These findings suggest that the left inferior frontal region is closely related to the origin of stuttering. Indeed, many structural imaging studies proposed the reduction in the density of the underlying WM as the core deficit of stuttering (Chang et al., 2008; Cykowski et al., 2010; Sommer et al., 2002; Watkins et al., 2008). This reduction has been reported to correlate positively with stuttering severity (Cai et al., 2014b).
The consistently reported overactivation in **right IFG** (also referred to as right frontal operculum) (Brown et al., 2005) is hypothesized to compensate for the planning deficits in its left homologous area as it appears to be positively correlated with speech fluency (Lu et al., 2010b; Preibisch et al., 2003). Right IFG is involved in inhibiting speech acts that are generated in the left IFG (Xue et al., 2008) and would only interfere when left IFG experiences problems (Lu et al., 2010a).

Another concept in this regard is **'state' versus 'trait' stuttering**. While 'trait' stuttering refers to fluent speech in PWS, 'state' stuttering encompasses episodes of stuttered speech. Recent meta-analyses showed that trait and state stuttering are associated with large neural differences e.g. dysfluent speech seems related to overactivation of (bilateral) SMA and underactivation of right primary auditory cortex while fluent speech would be linked with overactivation of (right) pre-SMA and underactivation of left primary auditory cortex (Belyk et al., 2014; Budde et al., 2014). Moreover, stuttering frequency/severity correlates with different neural activations than fluent syllable rate (Fox et al., 2000; Ingham et al., 2000, 2004). Unfortunately, most studies refer to stuttered speech when stutters are embedded in otherwise fluent speech (Braun et al., 1997; Fox et al., 2000, Ingham et al., 2004; Toyomura et al., 2011). Making a clear distinction between 100% stuttered and 100% fluent speech might elucidate which brain deficit(s) is/are associated with stutters and how the brain overcomes a stutter or functions when there is no stutter.

2. Subcortical findings

2.1. Basal ganglia

PWS generally show BG alterations. Aberrant activation patterns have been described in several BG nuclei during a variety of tasks (e.g. Braun et al., 1997; Chang et al., 2009; Ingham et al., 2004; Kell et al., 2009; Loucks et al., 2011; Watkins et al., 2008). These activations are found to normalize under fluency enhancing conditions (Toyomura et al., 2011, 2015) or to be affected by therapy (Neumann et al., 2003, 2005). A decrease in left putamen activity has even been suggested to be predictive of successful treatment progress (Ingham et al., 2013). Moreover, activity in BG correlates positively with stuttering severity/frequency measures (Braun et al., 1997; Giraud et al., 2008; Ingham et al., 2012; Kell et al., 2009).

Besides alterations in BG nuclei, also connectivity abnormalities have been described in the BGTC-loops connecting BG with cortical areas involved in speech motor planning, execution and monitoring (Chang et al., 2011; Lu et al., 2010a, 2010b). The exact consequence of these BGTC-loop dysfunctions is not yet clear. While some authors suggest it impairs sequence performance by hampering the timed selection and initiation of motor segments (Alm, 2004; Civier et al., 2013), others hypothesize it results in deficient sequence learning and automaticity development (Smits-Bandstra & De Nil, 2007). As BG are known to modulate activity in left motor and temporal cortices (Alexander et al., 1986), BG dysfunctions might affect auditory-motor synchronization as well (Hove et al., 2013). Finally, the BGTC-network, especially on the right, also plays a crucial role in motor response inhibition (Boehler et al., 2010; Xue et al., 2008), known to be altered in CWS (Eggers et al., 2013).

A third confirmation for BG involvement in stuttering comes from research on dopamine, an important neurotransmitter in the BGTC-loops. Several studies associate stuttering, at least in part, to a hyperdopaminergic state (Maguire et al., 2004). A small positron emission tomography (PET) study performed in 3 AWS observed elevated dopaminergic activity in several limbic structures (Wu et al., 1997). While dopamine antagonists typically reduce dysfluencies (Lavid et al., 1999; Maguire et al., 2000), dopamine agonists worsen stuttering (Anderson et al., 1999; Movsessian et al., 2005). Moreover, a strong positive correlation has been observed between the increase in dysfluencies and the total cumulative dose of dopaminergic medication in Parkinson's disease (Tykalova et al., 2015).

2.2. Cerebellum

The overactivation of the CB is the third and last neural signature of stuttering according to the meta-analysis of Brown et al., (2005). Even during silent reading, cerebellar activity is increased in AWS (De Nil et al., 2003; Van Borsel et al., 2003a). As this overactivation correlates negatively with stuttering frequency (Ingham et al., 2012) and decreases to normal levels following therapy (De Nil et al., 2001; Lu et al., 2012; Toyomura et al., 2015), it is likely related to compensation (De Nil et al., 2008; Etchell

et al., 2014; Watkins et al., 2008). Because the cerebellum contributes to timing and coordination of sensorimotor actions, this increased activation probably reflects increased speech motor control (De Nil et al., 2001).

2.3. Internally versus externally timed movements

PWS show a striking distinction between internally and externally triggered events. During tasks on response inhibition for example, CWS are as efficient as (to even better than) nonstuttering children when the inhibition is exogenously triggered but impaired when the inhibition is endogenously triggered (Eggers, 2012). A similar distinction is seen during speech: stutters only occur during self-paced speech, whereas speaking in unison with an external factor (e.g. another person, a metronome beat) improves fluency (Bloodstein & Ratner, 2008). The difference between internally and externally guided speech is hypothesized to be related to the reciprocal loops between BG and CB with cortical structures supporting motor control (see chapter 2). These loops would work in harmony to produce fine-grained timed initiation of speech movements (Alm, 2004). While the BGTC-loop would operate during internally timed movements, the cortico-cerebellar network would utilize external timing cues to sequence movements (Cunnington et al., 2002; Taniwaki et al., 2006). As self-paced speech is an internally timed movement, stuttering is suggested to result from dysfunctions in the BGTC-loop. Induced fluency conditions would engage the cortico-cerebellar network and override the defective BGTC-loop by providing external timing cues (Alm, 2004; Etchell et al., 2014).

3. Linking cortical and subcortical findings

Overall, DS is associated with deficient connectivity and aberrant interhemispheric integration among neural circuits that underlie forward modelling, auditory-motor integration and precise timing of movements. Due to neural plasticity, structural anomalies may affect neuroanatomical development by causing new or exacerbating existing alterations. Therefore, it is difficult to determine whether cortical or subcortical anomalies are the common basis for stuttering. Two major hypotheses exist:

3.1. Cortical hypothesis

The decrease in WM density below the left ventral sensorimotor cortex is believed by many to be the primary cause of stuttering (e.g. Cai et al., 2014b; Chang et al., 2008; Cykowski et al., 2010; Sommer et al., 2002; Watkins et al., 2008). This decrease hampers motor related functions in inferior frontal regions. Consequently, left IFG/motor cortex fails to send sufficient and correct input to BG which are, on their turn, unable to project correct timing information to their cortical projection areas. This will further negatively impact cortical functions and interactions in IFG, M1 and posterior areas (Alm,

2004; Giraud et al., 2008; Lu et al., 2010a). This view has long been supported by many as no structural anomalies were found in BG in PWS (Kell et al., 2009).

3.2. Subcortical hypothesis

Recently, however, structural alterations have been described in BG (Beal et al., 2013) and their connections (Chang & Zhu, 2013). As these disruptions are already present in childhood, they may impact speech motor learning (Toyomura et al., 2015). BGTC connectivity deficits may disrupt the timing of motor sequences which may result in aberrant auditory-motor matching (Hove et al., 2013). With development, discrepancies in auditory-motor matching may aggravate, causing increased effort and compensatory strategies. These strategies might, on their turn, drive structural and functional neuroplastic changes in cortical auditory and motor areas and their connections (Chang & Zhu, 2013). Adaptations and compensations are likely to be individual-specific which could lead to variable changes in WM development (Chang et al., 2015). As such, the inconsistencies observed in some cortical findings might fit in with this hypothesis. Even 'the most consistent structural anomaly', i.e. the reduced WM density below left Rolandic operculum, shows quite a large spatial variation across different studies (see Cai et al., 2014b).

Overall, **no consensus** has been achieved on which alteration might provide the primary cause of stuttering. However, should there be solely one common neural deficit in all PWS and for all stuttering symptoms or is there rather a final common pathway? Toyomura et al., (2015) posited that neural deficits in subcortical structures may not be the sole cause of stuttering, but one of many. Indeed, several authors suggested there might be subtypes in stuttering (for a review, see Yairi, 2007). Moreover, different dysfluencies (e.g. blocks/prolongations versus sound/syllable repetitions) have been proposed to be associated with different neural deficits (Civier et al., 2013). Jiang et al., (2012) succeeded to differentiate more and less typical stuttering symptoms based on brain activity.

Chapter 4

Neurophysiology:

General aspects and state-of-the-art in developmental stuttering

1. General aspects

Chapter 2 and 3 reported on neural functioning in terms of spatial localization. Apart from relying on large neural circuits, speech production is also a rapid and dynamic motor process. It takes only 600 ms to produce a word, from conceptual formulation to articulation (Levelt, 2004; Sahin et al., 2009). FS are able to produce six to nine syllables per second, which is faster than any other form of discrete motor behaviour (Kent, 2000). Thus, these large neural circuits must respond in a timely, precise and sequential manner to ensure fluent speech production (Ludlow & Loucks, 2003). Because neuroimaging techniques like positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have poor temporal resolution, they are unable to resolve temporal events occurring over periods shorter than several seconds. In order to evaluate timing, order of activation and dynamic interactions of different brain regions, neurophysiological tools such as electro-encephalography (EEG) and magneto-encephalography (MEG) can be used. They enable non-invasive measurement of cognitive processes with millisecond precision. EEG is used in the studies presented in the current thesis.

1.1. What is EEG?

EEG is a non-invasive technique which measures the electrical activity of the brain over time. Electrodes are placed on the scalp on fixed positions following an internationally accepted standard, the so called 10-20 system (Jasper, 1958) (*see figure 6*). The first EEG was recorded in 1924 by Hans Berger. Because the EEG reflects thousands of simultaneously ongoing brain processes, it is impossible to identify an individual neurocognitive process in the pure EEG signal. For this purpose, an event-related potential (ERP) should be evoked (Handy, 2005).

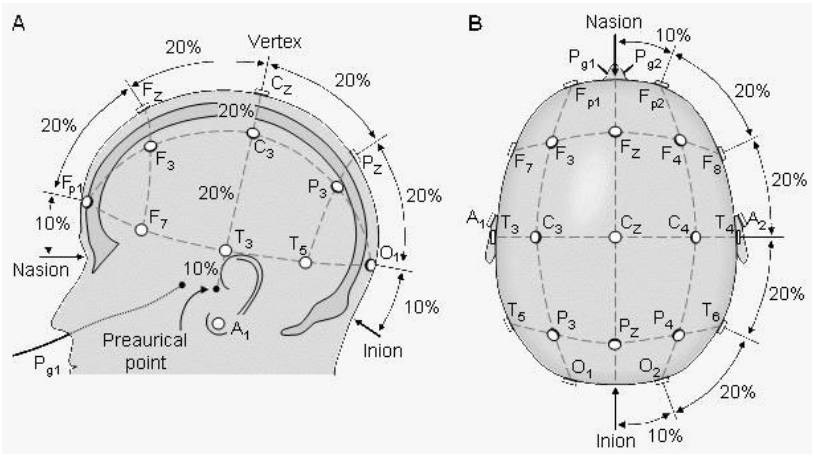

Figure 6: Illustration of the 10 – 20 system for electrode positions for EEG recordings.
Source: http://www.bem.fi/book/13/13.htm

1.2. What is an ERP?

A particular stimulus (e.g. a word, a picture, a sound, …) will elicit a stereotype, electrical response in the brain. This response is very small compared to the surrounding brain activity. Therefore, several similar stimuli should be presented to evoke this particular response an equal amount of times *(see figure 7).* By averaging all the responses, the surrounding brain activity is averaged out and the relevant waveform remains. This waveform is called the ERP. **An ERP is thus a time-locked electrical brain potential that reflects the average neural activity related to a certain sensory, motor or cognitive process.** By examining the ERP, the underlying process that is represented by the ERP can be evaluated. Its latency (timing of activation), amplitude (amount of neurons that participate), and scalp distribution (possible location in the brain) can be assessed (Handy, 2005; Luck, 2005) *(see figure 8).*

In neurochemical terms, an ERP reflects the postsynaptic potential of the neurons involved in the brain process. Each neuron forms a dipole due to a negativity at the dendrites and a positivity at the cell body. These dipoles will summate and result in a recordable ERP at the scalp if they occur at approximately the same time across thousands or even millions of spatially aligned neurons. The orientation of the dipole together with the position of the electrode at the scalp will determine the polarity (positive or negative) of the ERP (Luck, 2005).

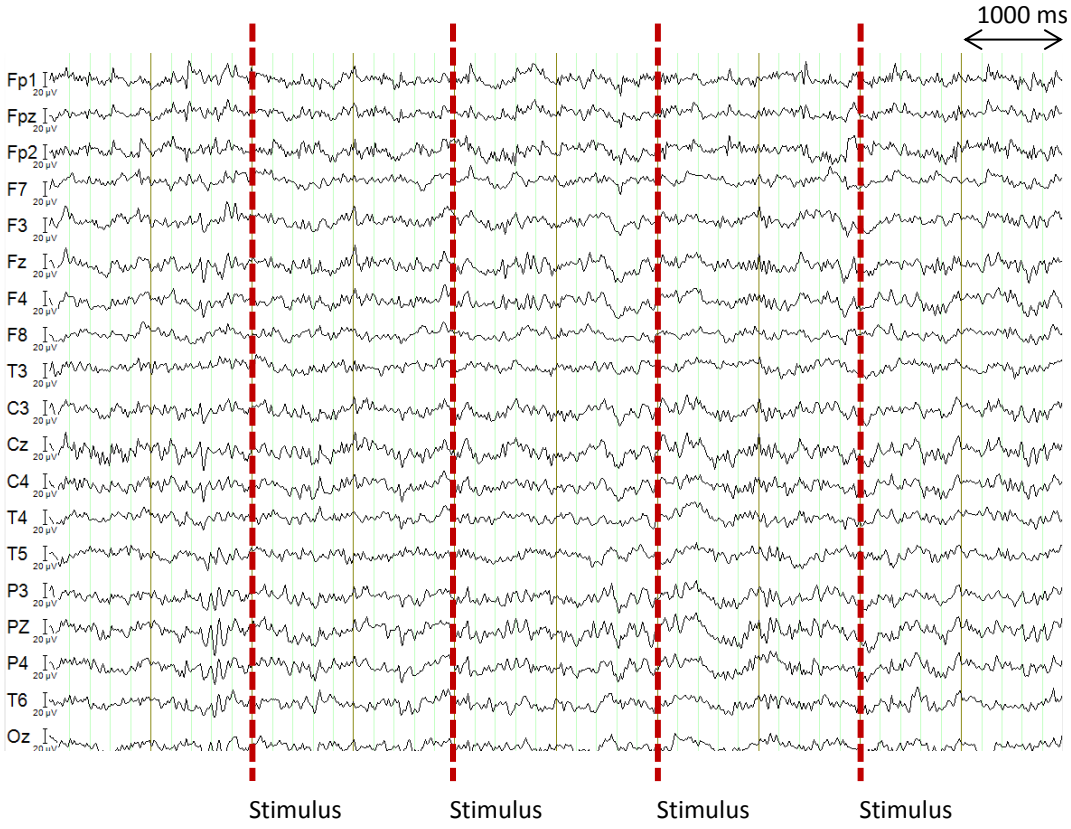

Figure 7: Illustrative example of a continuous EEG registration in which stimuli are presented at regular times (red dotted line).

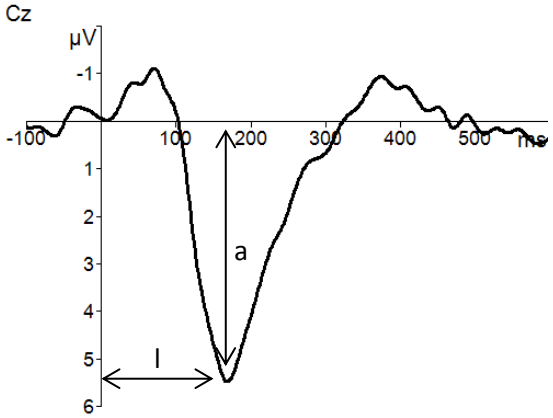

Figure 8: ERP at Cz, the electrode above the vertex. After averaging all EEG responses time-locked to the presentation of the stimuli, surrounding brain activity and noise are suppressed and the relevant waveform remains. This waveform, called the ERP, can be analysed for a = amplitude, l = latency.

1.3. What is source reconstruction?

Ideally, both temporal and spatial information is obtained about the neurocognitive process of interest. Source localization (or source reconstruction) refers to a number of non-invasive source imaging techniques that allow an estimation of the source of the electrical brain activity by use of algorithms. As source localisation is based on EEG data, it provides spatial information on a millisecond time basis. Several studies that applied EEG source imaging techniques have revealed interesting results for speech related tasks (e.g. Egorova et al., 2013; Möhring et al., 2014). These studies clearly evidence the validity of source imaging techniques and how they can clarify spatiotemporal aspects of speech related processes.

2. State-of-the-art in developmental stuttering

Although quite some EEG studies have been performed in PWS since the very first one in 1936 by Travis and Knott, neurophysiological studies focusing on speech motor control in stuttering are extremely scarce.

Neurophysiological studies of the previous century can mainly be divided in two groups. A first group concentrated on standard, clinical EEG analysis. While some reported essentially normal findings (e.g. Busse & Clark, 1957; Graham, 1966), others found large percentages of PWS with pathological indications in the EEG tracings, e.g. epileptic changes, maturation defects, diffuse dysrhythmias (e.g. Okasha et al., 1974; Sayles, 1971). A second group of studies used EEG to evaluate hemispheric lateralization in light of the Cerebral Dominance theory[2]. Most studies confirmed a higher reliance on

[2] The Cerebral Dominance Theory proposed by Orton and Travis (see Travis, 1931) suggests that PWS do not display the normal left over right hemisphere dominance for speech production. By consequence, both hemispheres will not function synchronically, which was suggested to be necessary for fluent speech production as the speech muscles are bilaterally innervated. As a result, speech dysfluencies would appear.

right hemisphere areas during a variety of linguistic tasks (e.g. Boberg et al., 1983; Douglas, 1943; Knott & Tjossem, 1943; Moore et al., 1982; Wells & Moore, 1990). Over the last 15 years, EEG research in stuttering became more characterized by ERP studies. These particularly focused on language (e.g. Maxfield et al., 2010, 2011, 2014; Weber-Fox & Hampton, 2008; Weber-Fox et al., 2008, 2013) and auditory (e.g. Corbera et al., 2005; Hampton & Weber-Fox, 2008; Jansson-Verkasalo et al., 2014; Kaganovich et al., 2010; Özcan et al., 2009) processing and revealed promising results.

Concerning speech motor control, some older and, to our knowledge, only one recent ERP study have been performed. The older reports all used a **contingent negative variation (CNV)** paradigm. The CNV is the first cognitive ERP described (Walter et al., 1964). It is a slow, negative potential that would primarily represent motor preparation (Bender et al., 2004; Bares et al., 2007). The first CNV reports in stuttering focused on hemispheric lateralization, consistent with the spirit of that time (Pinsky & McAdam, 1980; Zimmermann & Knott, 1974). No significant results were reported but 'large inter- and intrahemispheric variability, …, that is greater in, …, stutterers than in normal speakers' (Zimmermann & Knott, 1974, p604). Two later studies performed by Prescott and Andrews (1984) and Prescott (1988) indicated some minor differences between AWS and FS. AWS showed larger CNV amplitudes than FS preceding the production of familiar words. As familiar words are highly practiced words and therefore very likely to be completely preprogrammed, AWS were suggested to have difficulties in establishing efficient motor programs (Prescott, 1988) *(see figure 9).*

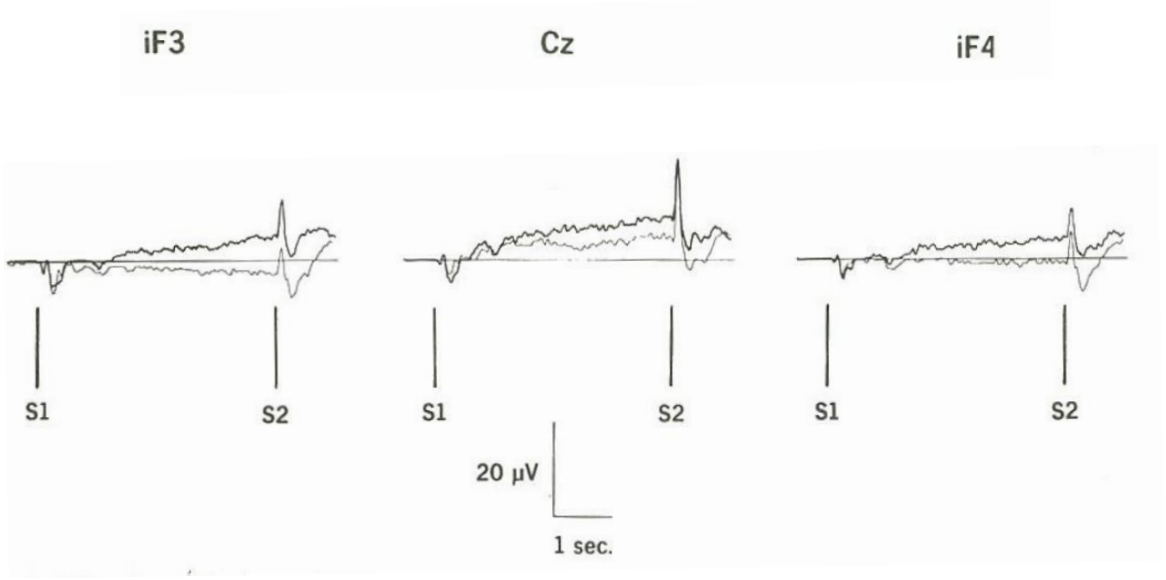

Figure 9: The increased CNV amplitude observed in AWS (dark grey) as compared to FS (light grey) (obtained from Prescott, 1988). The CNV is a slow negative potential occurring in between two successive stimuli (S1 and S2). The part of the CNV that reflects motor preparation is situated just before the second stimulus (S2). iF3 and iF4: electrodes situated over left and right inferior frontal sites, Cz: electrode situated above the vertex.

The only recent ERP study that described motor related aspects concerned an auditory vowel perception task (Liotti et al., 2010). AWS showed an abnormal early (from 50 to 60 ms post-stimulus onset) speech-motor activation in the right hemisphere. Other evidence for differences in neural timing of the speech motor system comes from two MEG[3] studies. Salmelin et al., (2000) observed, during a single word reading task, that the normal activation sequence of articulatory planning followed by motor execution is not present in stuttering. AWS first activated the left motor cortex followed by a delayed activation of the left inferior frontal region. They appear to initiate motor programs before preparing the articulatory code. Biermann-Ruben et al., (2005) found different timing in left and right motor related activations during a sentence production task. A very early (95 to 145 ms post-stimulus onset) activation of left inferior frontal cortex and an additional, late (from 315 ms post-stimulus onset onwards) activation of the right Rolandic operculum was observed. A third and final MEG study observed a decreased preparatory activity in or close to bilateral motor cortex preceding overt word reading (Walla et al., 2004).

As in most experimental settings, AWS spoke mainly fluent and as such, all above described results of the EEG and MEG studies are based on fluent speech production. To our knowledge, one case report has been published which presents electrophysiological information preceding purely stuttered speech (blocks), as compared to purely fluent speech (Sowman et al., 2012). By use of MEG, activation preceding visually cued vowel production was evaluated in a 24-year-old right-handed female. From 300 to 600 ms post-stimulus onset, blocks were associated with a reduced engagement of left orbitofrontal and inferior frontal cortices. In later stages, from 600 to 800 ms post-stimulus onset, these areas showed increased activation preceding blocks. The findings of this case report highlight that depending on the time window, other (even reversed) activation patterns can be observed.

In sum, neurophysiological research focusing on speech motor aspects is very scarce despite evidence from these few reports that AWS activate speech motor regions in a different temporal sequence than FS. Moreover, electrophysiological research on language and auditory processing shows that valuable ERP results can be obtained in stuttering.

[3] MEG is a non-invasive neurophysiological technique that measures the magnetic fields generated by neuronal activity of the brain. It combines excellent temporal with good spatial resolution.

Chapter 5

Research aims

The stuttering literature contains a plethora of spatial neuroimaging studies (e.g. fMRI) but a dearth of neurophysiological studies, particularly when it comes to evaluating motor speech related processes. There is, however, clear evidence for alterations in the neural timing of speech motor regions. The major aim of this thesis is to **identify neurophysiological characteristics of speech motor control** in stuttering in order to contribute to the neural understanding of this speech disorder. Only visual tasks were used to exclude influences from auditory deficits as it remains to be determined which aspects of auditory processing and/or auditory-motor integration are altered.
The following research aims were formulated:

As motor areas are found to contribute to speech perception as well, our first aim was to evaluate **temporal coordination and sequencing of motor related activity during a visual word recognition task**. A well-known task from the action literature was used: silent reading of action verbs. The selected action verbs denoted movements performed with hands and/or arms as PWS are suggested to have an altered neural control of upper limb movements too. The timing of motor related activations was first evaluated by use of source reconstruction in a group of healthy FS *(chapter 6)* and subsequently compared to a group of AWS *(chapter 7).*

EEG also allows examining specific processing stages in real time by use of ERP analysis. An important motor related ERP is the CNV which primarily reflects motor preparation. Our second aim was to elicit a CNV by use of a picture naming task to evaluate **speech motor preparatory activity preceding overt single word production in real time**.

A) First, we aimed to measure the amount of speech motor preparatory activity in AWS with DS. For this purpose, the CNV preceding fluently uttered words in AWS was compared to the CNV of a group of FS *(chapter 9).*

B) Secondly, we aimed to elucidate whether or not the observed alterations in motor preparation were related to successful compensation strategies. Therefore, the CNV preceding stuttered words (in AWS) was compared to the CNV preceding fluent words of FS and AWS *(chapter 10).* By comparing 100% stuttered and 100% fluent speech, a distinction can be made between neural deficits associated with stutters and neural alterations related to successful compensation strategies.

C) Thirdly, because PWS are known to show considerable intra-individual variation in stuttering severity and frequency, we aimed to explore a possible relationship between speech motor preparation and stuttering frequency. For this purpose, the CNV task was administered in a case of NS at four points in time associated with differences in stuttering frequency *(chapter 8)*.

D) Fourthly, although DS and NS are suggested to share common neural substrates, we aimed to assess whether this also accounts for speech motor preparatory activity. The results of the DS group *(chapter 9)* were compared to the results of the NS case-report *(chapter 8)*.

Table I: Chapter overview including research aims, participant variables and paradigms used.

Chapter	Purpose	Participants					Paradigm
		N	Age (M ± SD)	Gender (M/F)	Handedness (Right/left)	Stuttering Severity (SSI-4)	
Ch. 6	To evaluate the timing of motor related activations in FS during visual word recognition	30 FS	30.2 ± 10.6	22/8	30/0	/	Visual word recognition: - 50 hand/arm action verbs (e.g. to knead) - 50 abstract non-action verbs (e.g. to believe)
Ch. 7	To compare the timing of motor related activations during visual word recognition between FS and AWS with DS	30 FS	30.2 ± 10.6	22/8	30/0	/	Visual word recognition: - 50 hand/arm action verbs (e.g. to knead) - 50 abstract non-action verbs (e.g. to believe)
		30 AWS	30.9 ± 11.8	22/8	30/0	very mild: 9 mild: 9 moderate: 3 severe: 6 very severe: 3	
Ch. 8	To explore a relationship between speech motor preparation and stuttering frequency	Single-case of NS	28	F	Right	Varying, evaluated at different points in time	Single word production: - 110 black and white pictures
Ch. 9	To measure and compare speech motor preparation preceding fluent words in FS and AWS with DS	35 FS	28.9 ± 10.6	24/11	32/3	/	Single word production: - 110 black and white pictures
		25 AWS	29.9 ± 9.2	19/6	21/4	very mild: 10 mild: 7 moderate: 3 severe: 3 very severe: 2	
Ch. 10	To measure speech motor preparation preceding stuttered words to elucidate whether the observed alteration in ch. 9 is a compensation strategy	*In addition to the participants of chapter 9:*					Single word production: - 110 black and white pictures
		7 AWS	33.4 ± 14.8	4/3	6/1	mild: 3 severe: 3 very severe: 1	

Publications

Part II

Chapter 6

Early lexico-semantic modulation of motor related areas during action and non-action verb processing

Vanhoutte Sarah, Strobbe Gregor, van Mierlo Pieter, Cosyns Marjan, Batens Katja

Corthals Paul, De Letter Miet, Van Borsel John, Santens Patrick

Journal of Neurolinguistics (34), 65-82

Abstract

Although action verb processing deficits have been described in diseases affecting the motor system, research on temporal processing in this area has not been reported. In this study, action and non-action verb processing was contrasted in healthy volunteers using electro-encephalography. These data may serve as a control condition for further research in motor disorders. Latency and amplitude evaluations as well as source reconstruction were applied on event-related potentials. Action verbs evoked higher activation in bilateral sensorimotor areas from 155 to 174 ms and in bilateral dorsolateral prefrontal cortex (DLPFC) from 219 to 238 ms. Hand action verb processing activates the motor programmes of the actions the verbs refer to. This seems not restricted to the core (pre)motor cortical areas of the brain. A broad motor brain network is hypothesized to be involved. While sensorimotor activation seems essential for action verb understanding, this cannot be concluded for DLPFC activation.

Keywords

motor cortex, dorsolateral prefrontal cortex, action verb, abstract verb, semantic processing, lexical access

1. Introduction

Substantial research has been conducted on the perception of action related linguistic material such as action verbs. Besides the classic language areas, also the premotor and primary motor cortex are reported to be involved in the processing of action-related words and sentences. Moreover, this processing appears to occur in a somatotopic way. Action verbs related to face, arm or leg movements elicit the strongest activation close to the cortical motor representation of the face, hands or legs respectively (Aziz-Zadeh et al., 2006; Boulenger et al., 2009; Buccino et al., 2005; Hauk et al., 2004; Kemmerer et al., 2008; Pulvermüller et al., 2001, 2005; Raposo et al., 2009; Repetto et al., 2013; Shtyrov et al., 2004; Tettamanti et al., 2005). Although this somatotopical activation is not always found (Arévalo et al., 2012; Postle et al., 2013), a review by Kemmerer and Gonzalez-Castillo (2010) showed surprising consistencies among different labs and languages.

Unfortunately, the underlying mechanism responsible for the motor activation remains a contentious issue because conflicting results are found on the processing stage during which this motor activation occurs. Some studies revealed somatotopic motor activation after auditory and visual single word presentation from 130 to 170 ms (Pulvermüller et al., 2005; Shtyrov et al., 2004) and from 210 to 230 ms (Hauk & Pulvermüller, 2004b) respectively. In addition, visually presented action words appear to interfere with a reaching movement already within 200 ms after word onset (Boulenger et al., 2006). Within the first 200 to 250 ms after word presentation, essential lexical and semantic processes are known to occur (Federmeier & Kutas, 2001; Hauk et al., 2012; Penolazzi et al., 2007). Thus, actions and action semantics related to words apparently share cognitive and neural resources. This is in line with theories of embodied cognition which state that all concepts are (partly) modality dependent and are grounded in neural action and perception systems (e.g. Barsalou, 1999; Dove, 2009). Consequently, motor areas are suggested to be involved in lexical access (Hauk et al., 2008). By contrast, other studies found a much later motor cortex modulation around 500 ms post stimulus onset (Oliveri et al., 2004; Papeo et al., 2009). At this stage, post-conceptual processes of word recognition occur (Marinkovic et al., 2003). Motor strip activation would then follow the identification of the action concept, instead of being part of it. This 'spreading activation' occurs because the word's concept is associated with the motor system controlling the respective action (Hickok, 2010) or because of mental imagery (Tomasino et al., 2008).

A recent study conducted by Moseley et al., (2013) used excellent equipment to elucidate which processing stage is involved. Passive reading of written words was found to evoke maximal brain responses at 150 ms post-stimulus onset. Besides widespread activity in perisylvian regions for all words, inferior frontal gyrus and precentral cortex were significantly more engaged during action compared to abstract word processing. Thus, category-specific semantics seem to be represented in the neural systems for perception and action. As these regions were activated within the first 200

ms, this representation seems essential for concept understanding. Unfortunately, while the action words were mostly verbs, the abstract words were a conglomeration of both nouns and verbs. Although grammatical class in itself does not have an influence on the organization of knowledge in the brain (Vigliocco et al., 2011), electrophysiological differences between verbs and nouns have been reported (Kellenbach et al., 2002; Osterhout et al., 1997). Thus, a possible lexical/grammatical confound cannot be excluded to have influenced the results.

In sum, there is no consensus on the function, timing and necessity of motor cortex activation during action related word processing. Diseases affecting the motor system might help in clarifying this issue. If the motor cortex contributes to word understanding, action verb processing deficits should occur in patients with disturbances of their motor system. Indeed, a large variety of pathologies have been shown to evoke disturbances in action verb processing: motor neuron disease (Bak & Hodges, 1999, 2004; Bak et al., 2001; Grossman et al., 2008), progressive supranuclear palsy (Bak et al., 2006; Daniele et al., 1994), frontotemporal dementia (Cappa et al., 1998), aphasia (Saygin et al., 2004), apraxia in chronic stroke patients (Buxbaum & Saffran, 2002), lesions in the right frontal area (Neininger & Pulvermüller, 2003), and Parkinson's disease (Fernandino et al., 2012). Unfortunately, most of these studies contrasted action verbs with non-action *nouns.* As mentioned above in relation to the Moseley et al., (2013) study, electrophysiological differences between verbs and nouns have been reported (Kellenbach et al., 2002; Osterhout et al., 1997). In addition, verbs are inherently more difficult than nouns because of more complex semantic and syntactic constraints (for a review, Druks, 2002). Therefore, these action verb processing deficits might rather be related to grammatical than to semantic aspects. Moreover, no temporal information on action linguistic processing in motor pathologies is available. To our knowledge, all studies reported behavioural and neuroimaging data with good spatial, but poor temporal resolution like e.g. fMRI. However, by applying neurophysiological tools such as electro-encephalography (EEG), one could elucidate which processing stage is affected in these motor pathologies and consequently, which processing stage relies (partly) on motor related brain areas.

Therefore, the present study aimed at evaluating motor related brain activations during action verb processing in motor pathologies by use of EEG. All action verbs denoted movements performed with hand and/or arms to evoke focalized activity in motor cortex. To overcome a grammatical class confound, these action verbs should be contrasted with another group of verbs. As contrast condition, non-action verbs were chosen instead of action verbs related to another body part because these verbs require no or only limited motor involvement. Variability in disease severity will cause variability in motor cortex deficiency. If the control condition would rely on motor cortex activity, variability in its processing would occur as well. A control condition should however provide

a reliable comparison for the measure of interest. If the control condition varies, no straightforward conclusion can be made about the measure of interest.

To our knowledge, no EEG research has been performed in which action verbs were contrasted with a group of only non-action verbs, not even in healthy populations. Therefore, the task was first administered in a group of healthy control participants. These data are presented in the present study. They will be used as a control condition for further experiments in patient populations with motor disorders. Therefore, an accessible method to use in a clinical setting was developed.

2. Methods

2.1. Participants

30 (male/female: 22/8) healthy, right-handed (Oldfield, 1971) volunteers (mean age ± standard deviation: 30.2 ± 10.6; age range: 18 – 57) were included in this study. They were all monolingual native speakers of Dutch and reported no history of hearing complaints, dyslexia or other speech-language problems, neurological or psychiatric disorders, and presented with normal or corrected-to-normal vision. None of them was on psycho-active drugs. All participants gave their written informed consent in accordance with the declaration of Helsinki. This study was approved by the local ethics committee.

2.2. Neurophysiological assessment

2.2.1. Stimuli

50 action and 50 non-action verbs were selected from WordGen (Duyck et al., 2004), based on the CELEX database (Baayen et al., 1995). To evoke focalized activity in sensorimotor cortices, all action verbs referred to hand and/or arm movements (e.g. to knead, to sew). The non-action verbs were abstract verbs unrelated to actions or body parts (e.g. to believe, to tolerate). A list of all stimuli items is provided in appendix A. Both verb classes were as closely matched as possible on several psycholinguistic and lexical characteristics as to minimize their possible impact in early neurophysiological processing (Dambacher et al., 2006; Federmeier & Kutas, 2001; Hauk & Pulvermüller, 2004a; Hauk et al., 2006a, 2006b, 2012; Penolazzi et al., 2007; Takashima et al., 2001). An overview of these features can be found in table I.

Semantic relatedness between verbs and body parts was determined in a pre-test by 11 native speakers of Dutch who did not participate in the EEG study. These body areas included (1) head (head/face/mouth), (2) arms (arms/hands/fingers), and (3) legs (legs/feet/toes). All verbs were scored in relation to these 3 body areas using a 5 point-scale ranging from 1, labelled “highly unrelated”, to 5, labelled “highly related”. Word imageability was estimated as well, following the same procedure. The question to be rated was: “how easily does this word evoke an image?” with 1

labelled “not at all” and 5 labelled “very easily”. The only body part that was supposed to be associated with half of the verbs was ‘arms’. This might have been noted by the participants and biased their scoring. Therefore, 30 leg and 30 head verbs were added in this pre-test. As these leg and head verbs only served as distractors, they were not specifically matched on psycholinguistic features to the verbs of the experimental set. They were randomly chosen from WordGen (Duyck et al., 2004) with as only requirement being related to legs/head respectively.

The arm action verbs were significantly more imaginable and more linked to arms than the non-action verbs. In addition, arm action verbs were more associated with arms than with legs and head. A similar finding was seen for the distractor verbs: leg and head verbs were significantly more linked with legs and head respectively compared to other body parts and compared to the arm action and non-action verbs of the experimental set (Mann – Whitney U test: $p < 0.001$ for all comparisons).

Table I: Summary of stimuli characteristics.
Mean ± SD is displayed. The p-value of the Mann-Whitney U test comparing action and non-action verbs is shown in the right column. For action verbs, the mutual comparison of the semantic relatedness scores for different body parts is shown on the left.

Feature		*Action verbs*	*Non-action verbs*	*P-value*
Word length				
Letters		7.0 ± 1.3	6.9 ± 1.3	0.77
Syllables		2.2 ± 0.4	2.3 ± 0.5	0.36
Word frequency		1.4 ± 0.6	1.6 ± 0.6	0.18
Bigramfrequency		12771 ± 3037	13811 ± 3129	0.06
Orthogr. neighborhood size		4.3 ± 4.0	4.5 ± 4.2	0.90
Imageability		4.5 ± 0.2	2.4 ± 0.6	***< 0.001***
Head relatedness	***< 0.001***[a]	1.5 ± 0.5	1.7 ± 0.8	
Arm relatedness	***< 0.001***[b]	4.9 ± 0.1	1.4 ± 0.4	***< 0.001***[c]
Leg relatedness		1.6 ± 0.5	1.2 ± 0.2	

[a] Arm action verbs are significantly more related to arms than to head
[b] Arm action verbs are significantly more related to arms than to legs
[c] Arm action verbs are significantly more related to arms than the non-action verbs

2.2.2. Procedure

All arm action verbs and non-action verbs were presented in their infinitive form as single words to minimize the interference of syntactic processes. They were shown in black letters (font: Calibri; size: 96) on a white background in the middle of a computer screen that was placed one meter in front of the participant. Stimuli were randomly presented with a stimulus frequency of 0.7Hz (+/- 1428 ms). No blank screen was shown in between successive stimuli. Participants were instructed to read each of the words mentally and to avoid overt articulation or any other kind of orofacial movement. To optimize EEG quality, they were encouraged to reduce eye-blinks as much as possible.

2.2.3. Data acquisition and analysis

EEG data were collected with Neuron-Spectrum-5 (4EPM) registration software (Neurosoft, Moscow, Russia). 21 Ag/AgCl electrodes were placed on the scalp according to the international 10/20 system (Fp1, Fpz, Fp2, F7, F3, Fz, F4, F8, C3, Cz, C4, T3, T5, T4, T6, P3, Pz, P4, O1, Oz, O2). Additional reference and ground electrodes were placed on the earlobes and forehead respectively. Neurophysiological data were recorded at a sampling rate of 500 Hz (0.01-75Hz band-pass filter). Impedance of each electrode was kept below 5kΩ.

Off-line EEG analysis was performed using BrainVision Analyzer 2 (Brain Products, Munich, Germany). After additional filtering (0.5-30 Hz band-pass filter, Notch filter 50 Hz), eye artefacts were excluded using Independent Component Analysis. Two components were removed (eye blinks; left-right eye movement) based on inspection of the components' spatial distribution (Mennes et al., 2010; Joyce et al., 2004). Next, the continuous EEG data were segmented into epochs of 1100 ms, starting 100 ms prior to stimulus onset, and baseline corrected to this pre-stimulus interval. Trials with voltage variations larger than 100 µV were manually rejected. By averaging over corresponding epochs, event-related potentials (ERPs) were computed for every subject, electrode, and verb category. ERP participant averages were then grand-averaged across participants for both verb classes separately.

2.2.4. ERP analysis

Visual presentation of single words typically evokes an ordered succession of 6 peaks. Whereas P1, N2 and P3 are known to be related to primary visual and visual attention processes (e.g. Di Russo et al., 2001; Folstein & Van Petten, 2008; Polich, 2007) N1, P2 and N400 (partly) reflect linguistic processes (e.g. Dambacher et al., 2006; Duncan et al., 2009; Tarkiainen et al., 1999). Therefore, only the latter were subjected to further analyses. Peak latency and mean amplitude were determined for both verbs separately. Peaks were semi-automatically determined as the local maximum within 50 – 200 ms for N1, 100 – 250 ms for P2 and 300 – 500 ms for N400. Mean amplitude was computed for the following time windows: N1 (95 – 135 ms), P2 (160 – 210 ms) and N400 (300 – 450 ms). These windows were chosen based on the grand averaged waveforms and previous research (Duncan et al., 2009; Weber-Fox, 2001). To investigate the topographical distribution of the peaks while keeping the amount of data limited, subsets of adjacent electrodes were taken together.

As P2 was observed over the entire scalp, nine clusters with average amplitude/latency of adjacent electrodes were calculated: anterior left (F7, F3), anterior midline (Fz), anterior right (F4, F8), central left (T3, C3), central midline (Cz), central right (C4, T4), posterior left (T5, P3), posterior midline (Pz), and posterior right (P4, T6). Also for the N400, the same nine clusters were created with one exception: posterior left and right did not include T5 and T6 respectively, as no N400 was seen over

these electrodes. Since N1 was only seen over posterior regions, one left (T5/P3/O1), one midline (Pz/Oz) and one right (T6/P4/O2) subset was created.

Statistical analysis was performed in IBM SPSS Statistics 19.0. Latency and amplitude were analysed using repeated measures ANOVAs with three within-subject factors for P2 and N400: hemisphere (left, midline, right), region (anterior, central, posterior) and verbs (action, non-action). As N1 only occurred over posterior sites, the factor 'region' was not included in its analysis. To evaluate whether the assumption of homogeneity of covariance was met, Mauchly's Test of Sphericity was computed for all factors with more than one degree of freedom in the numerator. If the assumption was violated ($\alpha \leq 0.05$), Greenhouse-Geisser (G-G) adjusted p-values were used to determine significance. Significance values were set at $\alpha \leq 0.05$ for main and interaction effects. All further pairwise comparisons were Bonferroni corrected.

2.2.5. Source reconstruction

The Statistical Parametric Mapping 8 software package (SPM 8: Welcome Department of Cognitive Neurology, University College, London, United Kingdom) implemented in MATLAB (the MathWorks, Inc., Massachusetts, USA) was used for EEG source reconstruction. To limit the number of comparison, a sensor-space analysis was first performed to search for time points at which maximal differences between action and non-action verbs occurred. In the sensor-space analysis, the ERP data from 0 to 380 ms of every participant was converted into 3D images for every verb category separately. This time window encompasses early and late time points described in similar previous research at which significant sensorimotor activations during action verb processing occurred (Hauk & Pulvermüller, 2004b, Moseley et al., 2013; Pulvermüller et al., 2001). These images were generated by constructing 2D, 64 x 64 pixels resolution, scalp maps for each time point (using interpolation to estimate the activation between the electrodes) and by stacking the scalp maps over peristimulus time, resulting in [64 x 64 x number of time points]-images (Litvak et al., 2011). These images were statistically evaluated by paired t-tests. F-contrasts were calculated to test for differences of either direction between action and non-action verbs.

The multiple sparse priors (MSP) algorithm (Friston et al., 2008) was used to reconstruct the source activity for every subject and verb category. A 3-layered scalp-skull-brain template head model matched to the MNI template was implemented for which the default electrode positions were used. 8196 dipoles were assumed on a template cortical surface mesh and the "bemcp" method (BEM) implemented in FieldTrip (Oostenveld et al., 2011) was used to calculate the forward model. 3D images containing the evoked energy of the reconstructed activity for every subject and verb class were generated in a time window centred around the significant time point(s) from the sensor-space analysis (Litvak et al., 2011). If the time point occurred before 250 ms post-stimulus onset, a time

window of 20 ms was chosen, because processes that take place within the first 250 ms are mostly characterized by short-lived transient activations. If the time point occurred after 250 ms, a window of 40 ms was chosen. Using these images, second level analysis was performed to identify the most significant source areas over subjects and verb class. F-contrasts were calculated by performing paired t-tests for the main effect of verb. As motor related activity was the primary focus of this test, only significant results in frontal and parietal lobe were explored. Because the amount of comparisons was already largely reduced by the sensor-analysis and by limiting the analysis to fronto-parietal areas, p-value was set at 0.05 at the source level. The resulting MNI coordinates holding significant activation differences between verb class, were explored by means of the traditional Brodmann categorization and by means of the SPM Anatomy toolbox developed by Eickhoff et al. (2007). Despite the seemingly limited spatial resolution inherent to less dense EEG recordings, low-density recordings have been established to provide an accurate estimate of ERP generators and to be sufficient to fully describe the variance of an ERP data set when compared to high-density recordings (Kayser & Tenke, 2006).

As the pre- and post-central gyrus was the main region of interest (ROI), an additional source analysis was performed on the earliest time point with prominent activity above this region. For this purpose, the Global Field Power (GFP) was calculated for each verb class separately over all (pre)frontal and parietal sensors for all participants. GFP illustrates the time course of the overall signal strength of the ERPs. Based on the maps of current estimates that were made for each peak of the GFP, the earliest time point with clear activity over pre- and post-central gyrus was identified. Similar second level analysis was performed on a time window centred around this time point. Again, depending on whether the time point occurred before or after 250 ms post-stimulus onset, a time window of 20 or 40 ms respectively was chosen. F-contrasts were calculated by performing paired t-tests for the main effect of verb. A mask was applied so only activity in pre- and post-central gyrus (BA 4, 6, 3, 1 , 2 and 43) was evaluated. Because only one ROI was included, the criterion for significance could be set at $\alpha = 0.05$.

3. Results

3.1. ERP analysis

The waveforms in figure 1 are characterized by a series of components. At posterior electrodes, a very early negative peak around 40 ms was immediately followed by a P1, peaking at around 70 ms, and an N1, peaking at around 115 ms. At anterior sites, the P1 was reversed evoking a negative peak around 70 ms. No equivalent of the posterior N1 was seen. Next, a P2 could be observed over the entire scalp. This wave reached its maximum around 180 ms. The subsequent N2 (peak: 225 ms) and P3 (broad wave around 300 ms) were quite small and could only be seen over occipital and T5/T6

electrode sites. Finally, a large N400 occurred, most clearly pronounced over anterior electrodes. Although this component has a protracted morphology, a peak could be described at around 360 ms. Topographic EEG maps for N1, P2 and N400 can be seen in figure 2.

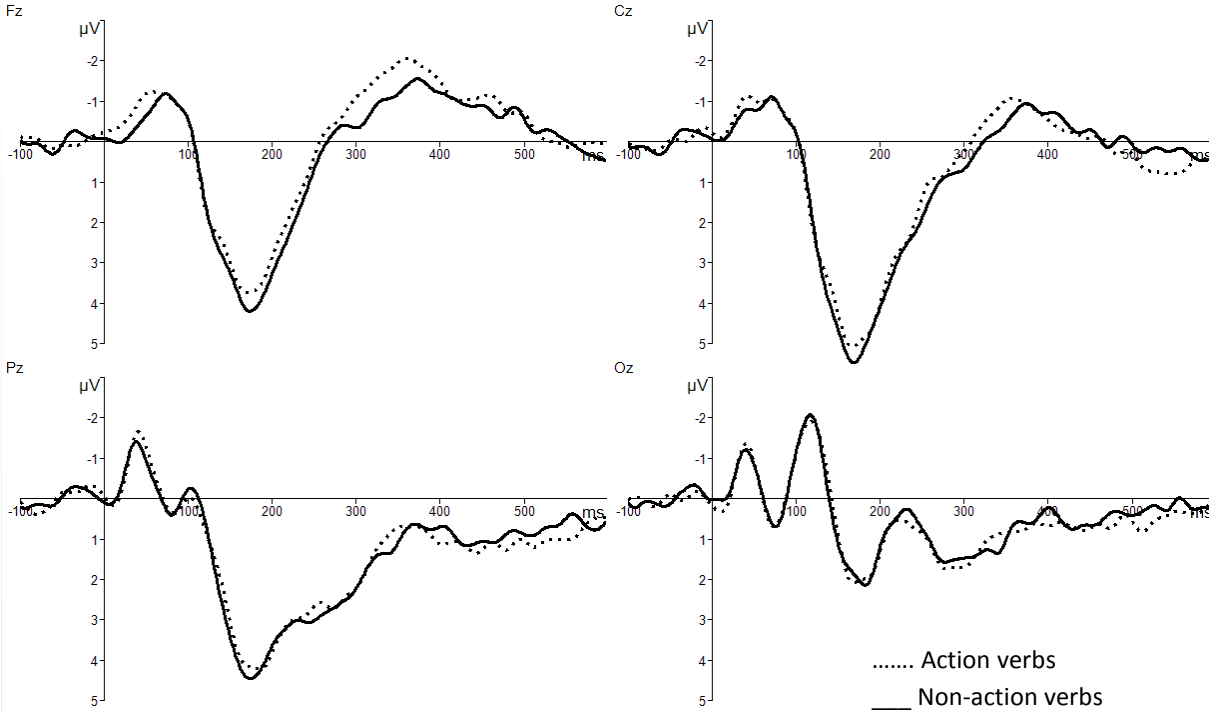

Figure 1: Grand average for action and non-action verbs separately at midline electrodes.
The 100 ms baseline and the first 600 ms of stimulus processing are depicted. Negative is plotted upwards. The x-axis represents latency (ms), the y-axis represents amplitude (µV).

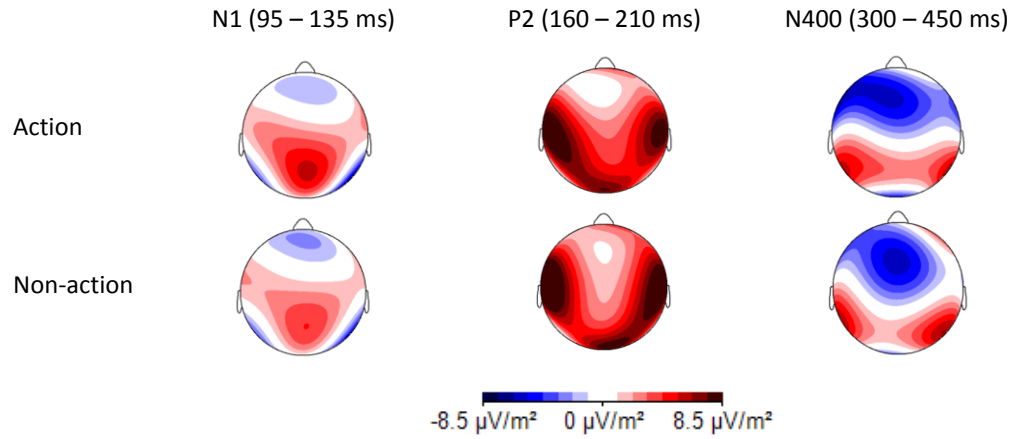

Figure 2: Topographic EEG maps of N1, P2 and N400.

Statistical analysis revealed a significant interaction of Verb*Region for the N400 amplitude ($F(2,58)= 3.43$; $\varepsilon= 0.69$; G-G $p= 0.05$). Action verbs showed a larger N400 than non-action verbs over anterior regions ($p= 0.007$). No other significant main or interaction effect of the factor Verb was found for either peak. Furthermore, some distributional amplitude variations were observed. The largest

amplitude for the P2 was seen over midline and central electrodes, especially at Cz (Region*Hemisphere: F(4,116)= 3.28; ε= 0.80; G-G p= 0.02) and for the N400 over left and anterior electrodes (Region*Hemisphere: F(4,116)= 6.01; ε= 0.67; G-G p= 0.001). The N1 appeared to be smallest (Hemisphere: F(2,58)= 4.62; p= 0.01) and earliest (Hemisphere: F(2,58)= 4.13; p= 0.02) over midline electrodes.

3.2. Source reconstruction

Detailed results of the source reconstruction are shown in table II. The sensor-space analysis found maximal differences between action and non-action verbs at 228 ms post-stimulus onset (F(1,29)= 20.96, p < 0.0001). As this time point occurred within the first 250 ms after stimulus presentation, second level source analysis was performed on an epoch of 20 ms centred around this peak (219 – 238 ms). Statistical analysis revealed a significant stronger cluster of activation during action compared to non-action verb processing located in dorsolateral prefrontal cortex (DLPFC) in left (p= 0.011) and right (p= 0.011) hemisphere (see figure 3).

Table II: Significant results of the source reconstruction for both time windows.
Reported are the coordinates of local maxima in MNI space which are part of larger clusters as well as the number of voxels per cluster (2 mm x 2 mm x 2 mm). A description of the region that contains these coordinates (based on both macroscopical parcellation and BA labelling) is added. The last column shows which verb type evoked most energy.

Time interval	*Macroscopic anatomical name*	*BA*	*Coordinates (MNI)*			*P value*	*Extent (voxel)*	*Highest activation*
			x	*y*	*z*			
155 – 174	R precentral gyrus	BA 6	18	-24	68	0.026	158	Action verb
	L precentral gyrus	BA 4	-14	-28	71	0.027	110	Action verb
219 – 238	R middle frontal gyrus	BA 9	32	28	42	0.011	164	Action verb
	L middle frontal gyrus	BA 9	-26	26	35	0.011	222	Action verb

Figure 4 shows the GFP and the map of current estimates for action and non-action verbs separately. The earliest prominent peak activity over pre- and post-central areas occurred at 165 ms for both action and non-action verbs. The subsequent analysis was performed from 155 to 174 ms. During this time window, source reconstruction of the grand averages revealed for both verbs widespread activation in perisylvian regions, including superior temporal cortex and inferior frontal gyrus, supramarginal gyrus, pre- and postcentral gyrus. Also prominent activity was seen in occipital, inferior temporal and fusiform gyrus (see figure 5). Both left and right hemisphere showed similar patterns of activity. Statistical analysis only focused on the sensorimotor cortex (pre- and postcentral gyrus). Indeed, a prominent cluster of activation was located in this region. Action verbs evoked significantly more activation than non-action verbs in left (p= 0.027) and right (p= 0.026) hemisphere.

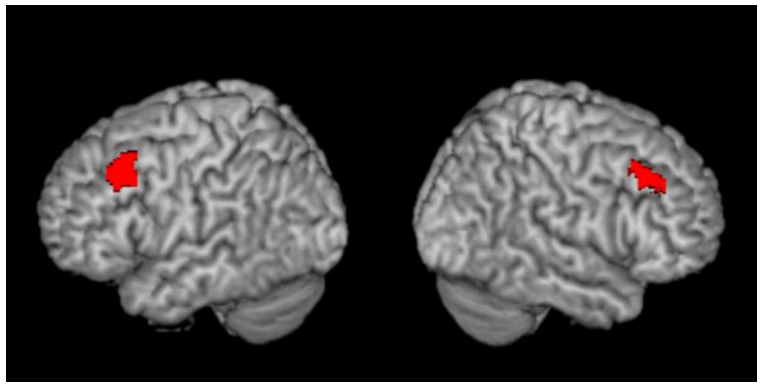

Figure 3: Result of the sensor-space analysis.
Source reconstruction for the statistical significant difference between action and non-action verbs in the time window from 219 to 238 ms. An activation focus located in bilateral DLPFC can clearly be identified.

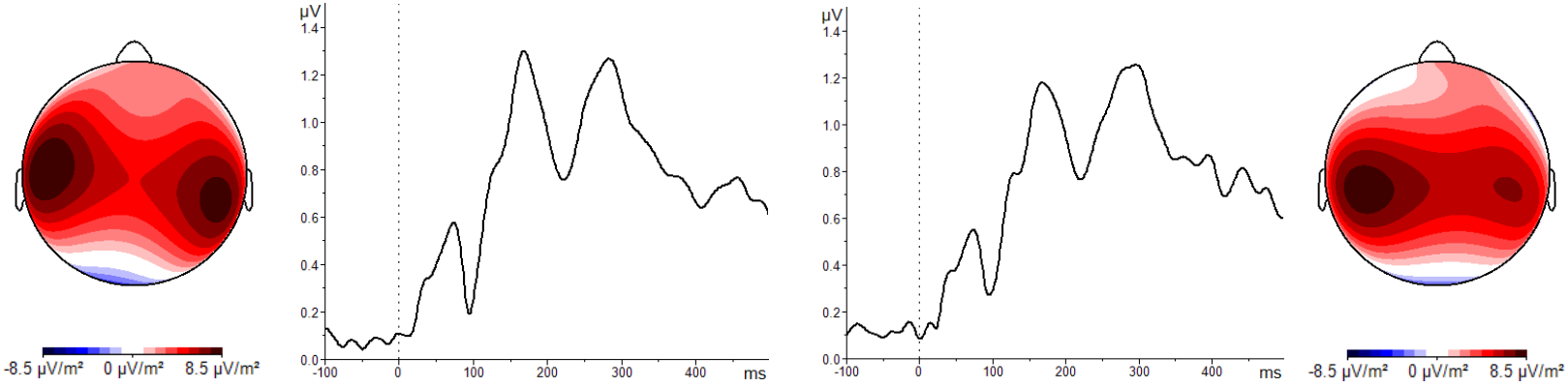

Figure 4: GFP calculated over all (pre)frontal and parietal sensors for all participants.
Non-action verbs are shown on the left, action verbs on the right. The current estimates map at 165 ms post-stimulus onset is presented as well. This is the earliest time point with clear activity over pre- and post-central gyrus.

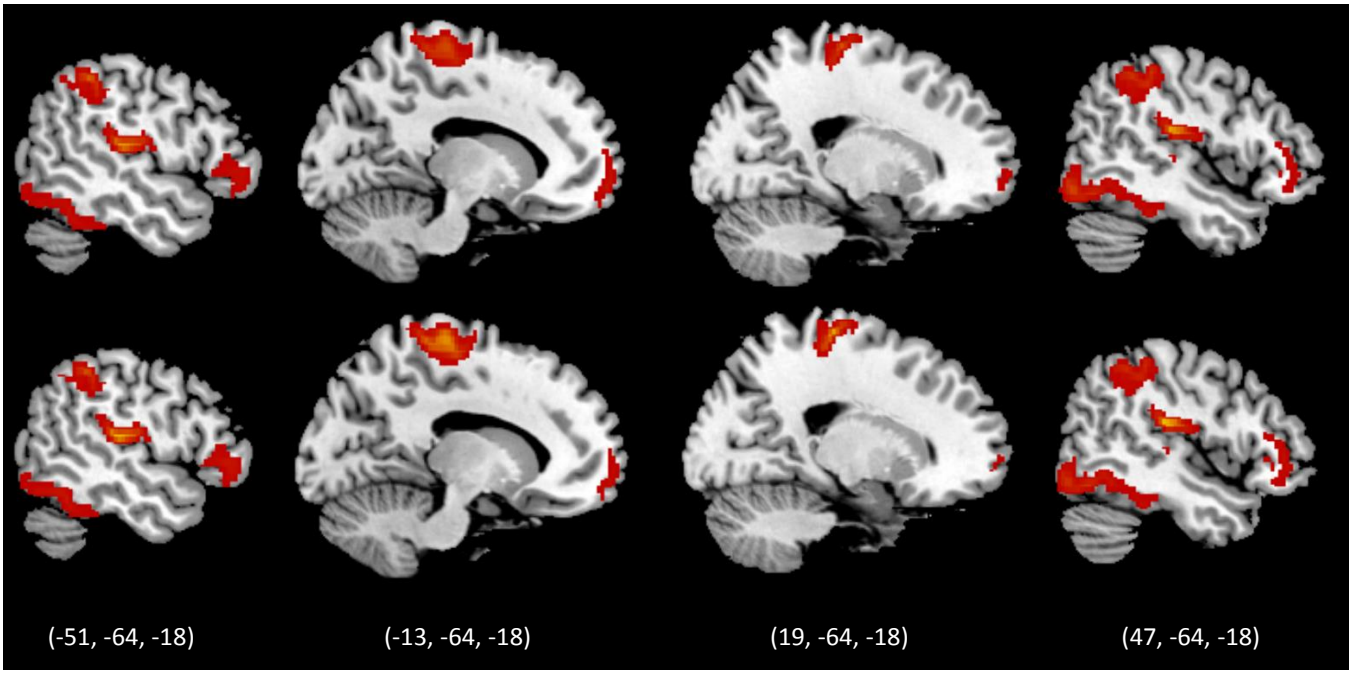

Figure 5: Source reconstruction of the grand average ERP's from 155 to 174 ms.
Four sagittal slices are shown for non-action verbs (top diagram) and action verbs (bottom diagram). The most prominent activation clusters (> 2 standard deviations calculated over the whole volume of reconstructed activity) are depicted in red. The two slices on the left are located in the left hemisphere, the two slices on the right are located in the right hemisphere. Corresponding MNI coordinates are shown underneath the pictures (x, y, z).

4. Discussion

The present study aimed at evaluating the time point(s) of motor related activity in the brain during hand action verb processing. To exclude a possible grammatical class confound, these verbs were contrasted with non-action verbs, i.e. verbs not related to a certain body part or movement. Following previous research, motor related lexico-semantic differences between verbs were not expected in the raw ERP signal (Hauk & Pulvermüller, 2004b; Pulvermüller et al., 2001). Indeed, no statistical difference arose between action and non-action verbs within 300 ms based on the ERP waveforms. Therefore, source reconstruction was performed in two time windows centred around two well-defined time points. Based on the GFP, the earliest, most prominent peak activity over pre- and post-central areas for both verb classes was identified around 165 ms. A sensor-space analysis searched for the time point with maximal difference between action and non-action verbs: 228 ms.

4.1. Motor related activation during action verb processing

An early semantic category effect was observed in bilateral sensorimotor areas. In line with a similar study (Moseley et al., 2013), both action and non-action verb processing evoke the most prominent activation peak at 165 ms post-stimulus onset. Besides a clear activation in sensorimotor cortices, a widespread bilateral activity was observed in core linguistic brain regions, like superior temporal

gyrus and inferior frontal gyrus, and in other brain areas partly related to linguistic processing, like fusiform gyrus and supramarginal gyrus. Statistical analysis revealed a significantly higher activation focus in sensorimotor areas during action than during non-action verb processing from 155 to 174 ms. Action verb perception is confirmed to trigger the sensorimotor areas responsible for the motor action the verb refers to. Action verbs and motor action seem to share neuronal representations (Hauk et al., 2004).

The present findings suggest that these neuronal representations contribute essential information to hand action verb processing. The significant sensorimotor difference is observed within 200 ms post-stimulus onset, a time window in which essential lexico-semantic information for concept understanding is retrieved (Federmeier & Kutas, 2001; Hauk et al., 2012; Penolazzi et al., 2007). Moreover, linguistic regions known to be involved in lexical access and semantic retrieval are simultaneously activated (Binder et al., 2009). Finally, since both stimulus categories only included verbs, grammatical class will not have confounded the results. The simultaneous activation of sensorimotor regions and other linguistic regions illustrates the functional links that are suggested to exist between the cortical systems for language and action (Pulvermüller, 2005). This is in line with moderate theories of embodied cognition which state that concepts are not only represented in specific language brain areas, but are also modality dependent and grounded in neural action and perception systems (e.g. Barsalou, 1999; Dove, 2009). In sum, these results provide evidence for an early, automatic and functionally relevant role of sensorimotor activation in lexico-semantic processing of hand action verbs.

About 50 ms later, action and non-action verb processing evoked a maximal difference in brain activity. From 219 to 238 ms, bilateral DLPFC was significantly more engaged during action than during non-action verb processing. DLPFC can be seen as a higher order motor region of the brain as it plays an important role in the cognitive control of motor behaviour (e.g. Funahashi, 2001; Miller, 2000; Miller & Cohen, 2001; Tanji & Hoshi, 2001). It receives motor information from both cortical and subcortical motor related brain structures and integrates them for motor control and action planning (Hoshi, 2006). Its involvement in language processing is however not new (Binder et al., 2009; Jeon et al., 2009). Moreover, a comparable study found a right prefrontal activation in the same time range. From 210 to 230 ms, hand action verbs evoked higher activations in dorsal DLPFC than leg action verbs (Hauk & Pulvermüller, 2004b). Thus, DLPFC is suggested to contribute to hand action verb processing around this time point. Motor activation during hand action verb processing does not seem to be restricted to the core (pre)motor cortical areas of the brain, but a broad motor brain network is hypothesized to be involved.

Whether DLPFC activation is necessary for action verb understanding cannot be concluded. From 200 to 300 ms onwards, brain activation can be influenced by conscious processes (Dehaene & Changeux,

2011). Since DLPFC activation occurred on the border of this time range, the present data do not allow a straightforward conclusion. Future studies with the present task in motor pathologies might address this issue.

4.2. Concreteness/imageability effects

The present action verbs are significantly more imaginable than the non-action verbs. This argument is often used to posit that motor related activations during action verb processing are rather related to mental imagery and concreteness effects (Postle et al., 2013; Tomasino et al., 2008). Although especially concreteness cannot entirely be excluded to have influenced the present results, several arguments are in favour for the embodied cognition point of view.

Explicit mental imagery can be excluded because this also involves posterior brain regions which were not found to be more engaged during action verb processing (Willems et al., 2009). Furthermore, effects of mental imagery are reported to occur from 300 (Gullick et al., 2013), 500 (West & Holcomb, 2000), or even 700 ms (Welcome et al., 2011) onwards.

A 'concreteness effect' is however present in the N400: action verbs evoke a significantly larger N400 than non-action verbs over anterior brain regions which is compatible with concrete words evoking larger N400 amplitudes than abstract words (e.g. Gullick et al., 2013). The N400 is generally accepted to reflect semantic processing, more specifically it represents the integration of different kinds of information in large scale networks (Hauk et al., 2012; Kutas & Federmeier, 2000). The larger N400 for action verbs would be related to a larger amount of neural correlates that need to be integrated (Xiao et al., 2012). Notwithstanding a concreteness effect can be seen, it does not seem responsible for the difference in activation. Post-lexical processing is suggested to be necessary to elicit concreteness effects (West & Holcomb, 2000). Indeed, concreteness is typically found to modulate ERP results from 250 to 300 ms onwards (Barber et al., 2013; Kanske & Kotz, 2007; Palazova et al., 2013), just as in the current study. By contrast, sensorimotor and DLPFC activity occurred earlier, at 165 and 228 ms respectively. Moreover, from 250 ms onwards, no significant difference between both verb classes appeared in the source reconstruction. Thus, no significant difference in brain activation was found in a time window where a concreteness effect is (1) generally found in previous research, and (2) also found in the present N400 results. Consequently, a concreteness influence is very unlikely to occur in preceding time windows (Kanske & Kotz, 2007; West & Holcomb, 2000). Moreover, the verbs used in the Hauk and Pulvermüller (2004b) study, that observed a comparable result, did not significantly differ in concreteness/imageability. Thus even with similar concreteness scores, hand action verbs elicited higher DLPFC activity than other verbs.

4.3. Bilateral motor related activation

The significantly higher activation during action verb processing was observed in left and right hemisphere for both DLPFC and sensorimotor cortex. The laterality of activation during action word processing has been suggested to be determined by language dominance and/or handedness (Hauk & Pulvermüller, 2011; Willems et al., 2010). Although only right handed participants were included, both uni- and bimanual words were presented which may be responsible for the observed bilateral activity. In general, hand action verbs are more often reported to activate left and right motor areas than action verbs related to other body parts (Hauk & Pulvermüller, 2004b; Pulvermüller et al., 2001; Raposo et al., 2009; Rüschemeyer et al., 2007; Tettamanti et al., 2005).

4.4. Sensorimotor involvement in non-action verb processing

The source reconstruction of the grand average of the non-action verbs also showed an important sensorimotor activity from 155 to 174 ms. Thus, even abstract verbs not related to a certain body part or body movement evoke some activity in sensorimotor cortex. Considering the early time point of activation and the simultaneous activation of brain regions involved in lexical access and semantic retrieval, this activity seems to be related to lexico-semantic processing as well.

Sensorimotor contribution in lexico-semantic processing of abstract verbs is subject for discussion. While some theories claim that only linguistic brain areas contribute to abstract verb processing, others propose a reliance on modal (perception and action related) information as well (e.g. Borghi & Cimatti, 2010; Fodor, 1998; Louwerse & Jeuniaux, 2010; Paivio, 1986). Most neuroimaging studies report activations in language related areas like inferior frontal and middle temporal gyrus (Wang et al., 2010). However, some studies also found limited sensorimotor activity (Rodriguez-Ferreiro et al., 2010; Sakreida et al., 2013). In a recent fMRI study, strong haemodynamic responses to abstract emotion words were observed in face- and arm-related motor regions. The authors concluded that these abstract words evoked precentral activity because they refer to body internal states. As face and arms are used to express emotions, these brain regions are activated when the corresponding words are perceived (Moseley et al., 2012). Thus, depending on the semantic associations of a word, sensorimotor brain regions can be activated during abstract word processing. The present non-action verbs were primarily used as contrast condition and were not controlled for strict semantic features. They were a conglomeration of words, not characterized by one strong semantic association. Therefore, the diverse semantic networks across non-action items might have resulted in some sensorimotor engagement that is however weaker than the one evoked by the action verbs (Moseley et al., 2013).

Other researchers link the sensorimotor activity to the acquisition modality of abstract words. When a child obtains language, the meaning of an abstract word has to be explained linguistically. This

would be a bodily experience as well which might lead to face-related motor activity during abstract word processing (Borghi et al., 2011; Scorolli et al., 2012). This research group also reports arm-related sensorimotor activity during abstract word processing in a later post-lexical stage (Scorolli et al., 2012).

4.5. Early ERP results

As mentioned above, no statistical differences arose between action and non-action verbs within 300 ms based on the ERP waveforms. Latency and amplitude of N1 and P2 were similar for both verb classes. Besides reflecting early sensory processes, both peaks show important amplitude modulations by lexical and psycholinguistic features. As N1 is related to visual word form processes (Brem et al., 2006; Salmelin, 2007; Tarkiainen et al., 1999), its amplitude is affected by psycholinguistic features like word length and neighbourhood size (Hauk & Pulvermüller, 2004a; Hauk et al., 2006a, 2006b). The exact function of the P2 remains unresolved. However, recent studies link it with early lexico-semantic and –syntactic processes as its amplitude is modulated by word frequency and grammatical class (Dambacher et al., 2006; Palazova et al., 2013; Takashima et al., 2001). Since stimuli were carefully controlled for these confounds and both stimuli types were verbs, no statistical difference in amplitude was expected.

4.6. Additional considerations

The strength of EEG research concerns its excellent temporal resolution. The major aim of this study was to clarify the time points at which significant differences can be found between action and non-action verbs. To interpret these timing results, one should have a look at their spatial characteristics as well. For this purpose, the source reconstruction was performed. Spatial resolution of EEG research is however limited and therefore, its results should be interpreted with caution. To meet this limited spatial resolution, no fine grained analyses nor interpretations were performed (e.g. making a distinction between ventral and dorsal DLPFC) though this might have provided valuable information (Hoshi, 2006).

Two concerns might arise regarding the participants: (1) large age range, and (2) unequal number of men and women. Because age is suggested to influence information processing rate (Cerella, 1985; Salthouse, 1991) and language differences between men and women have (inconsistently) been described (Gölgeli et al., 1999; Swink & Stuart, 2012; Wirth et al., 2007), additional statistical analyses were performed to evaluate a possible impact of age and gender on the present results. For age, all analyses were performed again without the oldest 6 participants (older than 1 SD above the mean age of the entire group). As these results mirrored the original results entirely, the large age range can be concluded to not have influenced the present findings.

A possible gender effect was explored by using a linear mixed model approach as this technique is preferred if data are not balanced (Field, 2009). Although men and women showed slight differences in the ERP analysis, these differences were rather small as there were no significant results when men and women were compared to each other. For the source reconstruction, both genders evoked similar results in the areas of interest. These findings do not support the suggestion that the results of the original group are biased by the results of the men.

5. Conclusion

Single verb processing evoked the most prominent peak activity at 165 ms after word presentation. Besides a clear activation in several linguistic brain regions, also bilateral sensorimotor cortex was engaged. This sensorimotor activation was significantly higher during action than during non-action verb processing from 155 to 174 ms. This result is suggested to be a word-specific semantic difference as (1) a grammatical class confound can be excluded (2) it occurs within 200 ms in which essential lexico-semantic information is known to be retrieved, and (3) brain regions involved in lexical access and semantic retrieval are simultaneously activated. From 219 to 238 ms, action compared to non-action verbs evoked significantly higher DLPFC activations which is a higher order motor brain region involved in action planning.

Hand action verbs thus seem to activate the motor programmes of the actions the verbs refer to. This is not restricted to the core (pre)motor cortical areas of the brain. A broad motor brain network is hypothesized to be involved. While the sensorimotor activation appears to be automatic and necessary for action verb understanding, this cannot be concluded for DLPFC activation. Nevertheless, the present results are in line with theories of embodied cognition which state that concepts are represented in specific language brain areas ànd in neural action and perception systems. Future EEG research in disorders affecting the motor system may contribute to our understanding of motor related brain activations during hand action verb processing.

Appendix A

List of all stimuli items: hand- and armrelated action verbs on the left, non-action verbs on the right.

Action verbs		**Non-action verbs**	
aaien	*to stroke*	bedriegen	*to cheat*
aangeven	*to hand*	behoren	*to belong*
aanraken	*to touch*	beloven	*to promise*
boetseren	*to mould*	blijken	*to turn out*
boksen	*to box*	dempen	*to muffle*
borduren	*to embroider*	dulden	*to tolerate*
borstelen	*to brush*	dunken	*to deem*
breien	*to knit*	durven	*to dare*
duwen	*to push*	eisen	*to demand*
gieten	*to pour*	ergeren	*to annoy*
gooien	*to throw*	flitsen	*to flash*
grijpen	*to grab*	geloven	*to believe*
haken	*to crochet*	genieten	*to enjoy*
kammen	*to comb*	gokken	*to gamble*
klappen	*to clap*	gunnen	*to grant*
kloppen	*to knock*	haten	*to hate*
kneden	*to knead*	hopen	*to hope*
knijpen	*to pinch*	huren	*to rent*
knippen	*to cut (with a scissor)*	kiezen	*to choose*
krabben	*to scratch*	kwellen	*to agonize*
masseren	*to massage*	lenen	*to lend*
naaien	*to sew*	liegen	*to lie, as in tell a lie*
pakken	*to grasp*	melden	*to report*
plukken	*to pick*	menen	*to mean*
roeren	*to stir*	missen	*to miss*
schetsen	*to sketch*	mogen	*to may*
schilderen	*to paint*	onthouden	*to remember*
schillen	*to peel*	opletten	*to pay attention*
schrijven	*to write*	pleiten	*to plead*
schrobben	*to scrub*	raden	*to guess*
schuren	*to sand*	rijmen	*to rhyme*
slaan	*to hit*	riskeren	*to risk*
smeren	*to butter*	roesten	*to rust*
smijten	*to fling*	schamen	*to be ashamed*
snijden	*to cut (with a knife)*	schatten	*to estimate*
stempelen	*to stamp*	scheiden	*to separate*
strelen	*to caress*	schijnen	*to shine*
strijken	*to iron*	spijten	*to regret*
tekenen	*to draw*	stralen	*to shine*
tikken	*to tap*	treiteren	*to torment*
timmeren	*to hammer*	treuren	*to grieve*
trekken	*to pull*	twijfelen	*to doubt*
typen	*to type*	verlossen	*to release*
vangen	*to catch*	verstaan	*to understand*
vasthouden	*to hold*	verwachten	*to expect*
werpen	*to cast*	verzinnen	*to make up*
wijzen	*to point*	verzoeken	*to request*
wrijven	*to rub*	vrezen	*to fear*
wuiven	*to wave*	wachten	*to wait*
zwaaien	*to wave*	wensen	*to wish*

Chapter 7

Timing and activation alterations of motor areas in stuttering: a silent single verb reading task

Vanhoutte Sarah, Strobbe Gregor, van Mierlo Pieter, Cosyns Marjan, Batens Katja

Corthals Paul, De Letter Miet, Van Borsel John, Santens Patrick

Brain and Language, under review

Abstract

Temporal aspects of motor activations in stuttering were evaluated during a perception task that triggers neural hand motor representations without interference of (speech) movement execution or auditory processing. Brain activity of 30 adults with developmental stuttering was registered by use of an electro-encephalogram during silent reading of hand action and non-action verbs. Latency and amplitude evaluations as well as source reconstruction were applied on event-related potentials. These results were compared to previous findings of fluent speakers (Vanhoutte et al., 2015b).

Temporal aspects of motor activations are considerably altered. The maximal motor difference between both verbs was delayed with about 100 ms and showed a reversed activation pattern: non-action verbs showed more sensorimotor activation than hand action verbs. This reversal is hypothesized to encompass two different activation patterns: a general motor hyperactivation and a specific hand motor deficit. Neural motor abnormalities in stuttering are confirmed not to require (speech) movement execution.

Keywords

speech perception, motor, action verb, hand motor, timing, temporal, stuttering

1. Introduction

Overt speech production can be seen as a complex form of movement which requires a dynamic, precise and timely coordination of a large brain network. Consequently, disruptions in the fluent production of speech may not only relate to dysfunctions in specific brain regions, but may also be linked to dynamic alterations in the temporal coordination of these specific brain regions.

Stuttering is one such possible disruption of the fluency of speech. Stuttering is a speech disorder in which the smooth succession of speech sounds is repeatedly interrupted by blocks, prolongations and/or repetitions of sounds or syllables (Bloodstein & Ratner, 2008). When stuttering begins during childhood, typically before the age of 4 years (Yairi & Ambrose, 2005), it is called developmental stuttering (Bloodstein & Ratner, 2008). Although stuttering may resolve in a substantial amount of children, still 4% to 32% of them will continue to stutter into adulthood (for a review, see Yairi & Ambrose, 2013). Neurologically, stuttering is characterized by alterations in cortical and subcortical brain regions related to speech motor planning, initiation, execution and monitoring (Chang et al., 2009; Ingham et al., 2012; Lu et al., 2010a; Neef et al., 2015; Watkins et al., 2008). Typically, a hyperactivation in cortical motor areas and the cerebellum is seen, either bilaterally or lateralized to the right hemisphere (for a meta-analysis, see Belyk et al., 2014; Brown et al., 2005; Budde et al., 2014). These overactivations even present without overt speech demands suggesting that adults who stutter (AWS) tend to recruit more neural resources for accomplishing even simple speech related tasks. During silent reading, increased activations have been reported in bilateral inferior frontal gyrus, left anterior cingulate cortex, right precentral cortex and right cerebellum (De Nil et al., 2000, 2001, 2003).

Studies evaluating temporal aspects of these speech motor activations are very scarce. Most neurological research in stuttering focuses on spatial evaluations by use of neuroimaging tools like functional Magnetic Resonance Imaging. In addition, the majority of neurophysiological research, using electro-encephalography (EEG) and magneto-encephalography (MEG) which have excellent temporal resolution and are therefore very suitable for timing related evaluations, focuses on language (e.g. Maxfield et al., 2010, 2011, 2014; Weber-Fox and Hampton, 2008; Weber-Fox et al., 2008, 2013) and auditory (e.g. Corbera et al., 2005; Hampton and Weber-Fox, 2008; Jansson-Verkasalo et al., 2014; Kaganovich et al., 2010; Özcan et al., 2009) processing. The few studies that have been performed provide, however, clear evidence for altered timing of motor related activations in stuttering. Salmelin et al., (2000) observed in AWS, during a single word reading task, an advanced activation of left motor cortex, related to motor execution, and a delayed activation of left inferior frontal region, related to articulatory planning. AWS thus appear to initiate motor programs before preparing the articulatory code. Biermann-Ruben et al., (2005) found temporal alterations in left and right motor activations during a sentence production task. A very early (95 to

145 ms post-stimulus onset) activation of left inferior frontal cortex and an additional, late (from 315 ms post-stimulus onset onwards) activation of the right Rolandic operculum was described. Also temporal alterations in motor activations even present without overt speech demands. Liotti et al., (2010) reported an abnormal early (20 to 80 ms post-stimulus onset) right-sided speech-motor activation. As this was accompanied by a hypoactivation in right auditory regions, the authors attributed their findings to an aberrant auditory-motor integration.

Although auditory related deficits are often reported in stuttering (Cai et al., 2012; Chang et al., 2009; Fox et al., 1996; Hampton and Weber-Fox, 2008; Jansson-Verkasalo et al., 2014; Watkins et al., 2008), it remains to be determined which aspects are altered (Belyk et al., 2014). Moreover, aberrant auditory processing seems no prerequisite for motor alterations to occur as motor hyperactivations are also present during silent reading tasks (De Nil et al., 2000, 2001, 2003). Therefore, the present study aimed at evaluating temporal coordination of motor related activations without a possible influence of aberrant auditory processing. For this purpose, a well-known task from the action literature was chosen: silent reading of action verbs.

Perception of action verbs activates, besides the classic language areas, also motor areas like premotor and primary motor cortex. These activations appear to follow a somatotopical organization. Action verbs related to face, arm or leg movements elicit the strongest activation close to the cortical motor representation of the face, arms or legs respectively (e.g. Hauk et al., 2004; Mosely et al., 2013). The time point(s) of these activations reflect(s) the processing stage(s) to which the motor areas contribute. In a previous study from our laboratory, perception of hand action verbs was contrasted with perception of non-action verbs, i.e. abstracts verbs unrelated to action or body parts, in a group of healthy fluent speakers (FS) (Vanhoutte et al., 2015b). Action verbs elicited significantly higher activation in bilateral sensorimotor cortex from 155 to 174 ms. This early sensorimotor activation is suggested to contribute to early lexico-semantic processing of the action verbs as (1) it occurs within 200 ms in which essential lexico-semantic information is known to be retrieved (Federmeier & Kutas, 2001; Hauk et al., 2012; Penolazzi et al., 2007) and (2) core linguistic brain regions involved in lexical access and semantic retrieval like inferior frontal gyrus (Binder et al., 2009) are simultaneously activated. These findings confirm previous reports that showed similar early motor related activations during action verb processing (Hauk & Pulvermüller, 2004b; Pulvermüller et al., 2005; Shtyrov et al., 2004) and are in line with theories of embodied cognition which state that all concepts are grounded in neural action and perception systems (e.g. Barsalou, 1999; Dove, 2009).

An additional sensor-space analysis revealed that the maximal difference in brain activity between action and non-action verb processing was situated around 228 ms in bilateral DLPFC (Vanhoutte et al., 2015b). As DLPFC is considered to be a higher order motor region involved in motor control and

action planning (Tanji & Hoshi, 2001; Hoshi, 2006), motor activations during hand action verb processing do not seem to be restricted to the core (pre)motor cortical areas, but may involve a broad motor brain network.

In the present study, the same task is applied in a group of AWS and compared to the previously obtained results of the FS. It is hypothesized that perception of the hand action verbs results in disturbed motor recruitment because hand action verbs spark the hand motor representations in the brain. Motor alterations in stuttering are known to extend to non-speech movements like finger and hand movements. Besides behavioural deficits (Bishop et al., 1991; Smits-Bandstra et al., 2006; Webster, 1997; Webster and Ryan, 1991), also neural dysfunctions have been reported like an imbalanced lateralization (Morgan et al., 2008; Neef et al., 2011) and a decreased excitability (Busan et al., 2011) during manual tasks. As a result, a decreased motor activation during hand action verb processing is hypothesized to occur. In sum, the present study aimed to evaluate motor related activations during a perception task that triggers hand motor representation in the brain, without interference of overt speech requirements, any other movement or aberrant auditory processing. The primary focus was put on temporal aspects of these motor activations.

2. Methods

2.1. Participants

30 AWS, with persistent developmental stuttering, (mean age ± standard deviation: 30.9 ± 11.8; age range: 18 – 57; male/female: 22/8) were included and compared to the 30 healthy, right-handed FS (mean age ± standard deviation: 30.2 ± 10.6; age range: 18 – 57; male/female: 22/8) included in Vanhoutte et al., (2015b). Both groups did not significantly differ in age (Mann-Whitney U test: p= 0.863). All AWS had already followed one or more treatments of variable duration and intensity. They were right-handed (Oldfield, 1971), monolingual native speakers of Dutch, reported no history of hearing complaints, dyslexia or other speech-language problems, neurological or psychiatric disorders, and presented with normal or corrected-to-normal vision. None of them was on psycho-active drugs. All participants gave their written informed consent in accordance with the declaration of Helsinki. This study was approved by the local ethics committee.

2.2. Speech assessment

To collect speech samples, participants engaged in a conversation with the investigator about work/school/hobby and performed a reading task. These samples were videotaped using a Canon ACV HD (1920 x 1080) camera and audiotaped in PRAAT, a free software program for acoustical analysis (Boersma and Weenink, Phonetic Sciences, University of Amsterdam, Amsterdam, The Netherlands) using a Samsung CU01 microphone placed 50 cm in front of the participant.

Speech samples were analysed for percentage stuttered syllables (%SS) and stuttering severity. %SS was counted following the principles of the Stuttering Measurement System (Ingham and Ingham, 2011), stuttering severity was judged by means of the Stuttering Severity Instrument, fourth edition (SSI-4; Riley, 2008). Stuttering was diagnosed by a certified speech-language pathologist based on % SS (>3%) and/or the presence of significant speech-related struggle behaviour. Stuttering severity varied considerably: 9 AWS presented with very mild, 9 with mild, 3 with moderate, 6 with severe and 3 with very severe stuttering.

All samples were scored off-line. 20% of samples were re-evaluated by a second rater (MC) to assess interrater reliability. Both raters are speech therapists specialized in stuttering. An intraclass correlation coefficient (ICC) was calculated for overall percentile score on SSI-4 (Riley, 2008), %SS for reading and %SS for conversation. ICC's of 96.3; 99.7 and 99.5 % respectively were obtained, which ensured excellent agreement.

2.3. Neurophysiological assessment

The same task as in Vanhoutte et al., (2015b) was used. To be able to compare the results of the AWS with the previous results obtained in the FS, the same analyses were performed.

2.3.1. Stimuli

50 action and 50 non-action verbs were selected from WordGen (Duyck et al., 2004), based on the CELEX database (Baayen et al., 1995). To evoke focalized activity in sensorimotor cortices, all action verbs referred to hand and/or arm movements (e.g. to knead, to sew). The non-action verbs were abstract verbs unrelated to actions or body parts (e.g. to believe, to tolerate). Both verb classes were as closely matched as possible on several psycholinguistic and lexical characteristics as to minimize their possible impact in early neurophysiological processing (Dambacher et al., 2006; Federmeier & Kutas, 2001; Hauk & Pulvermüller, 2004a; Hauk et al., 2006a, 2006b, 2012; Penolazzi et al., 2007; Takashima et al., 2001).

Semantic relatedness between verbs and body parts was determined in a pre-test by 11 native speakers of Dutch who did not participate in the EEG study. These body areas included (1) head (head/face/mouth) (2) arms (arms/hands/fingers) and (3) legs (legs/feet/toes). Word imageability was estimated as well (for a detailed description, see Vanhoutte et al., 2015b). The action verbs were significantly more imaginable and more linked to arms than the non-action verbs. In addition, arm action verbs were significantly more associated with arms than with legs and head (Mann Whitney U test: $p < 0.001$ for all comparisons). An overview of all these features can be found in table I.

Table I: Summary of stimuli characteristics.
Mean ± SD is displayed. The p-value of the Mann-Whitney U test comparing action and non-action verbs is shown in the right column. For action verbs, the mutual comparison of the semantic relatedness scores for different body parts is shown on the left.

Feature		*Action verbs*	*Non-action verbs*	*P-value*
Word length				
Letters		7.0 ± 1.3	6.9 ± 1.3	0.77
Syllables		2.2 ± 0.4	2.3 ± 0.5	0.36
Word frequency		1.4 ± 0.6	1.6 ± 0.6	0.18
Bigramfrequency		12771 ± 3037	13811 ± 3129	0.06
Orthogr. neighborhood size		4.3 ± 4.0	4.5 ± 4.2	0.90
Imageability		4.5 ± 0.2	2.4 ± 0.6	***< 0.001***
Head relatedness	***< 0.001***[a]	1.5 ± 0.5	1.7 ± 0.8	
Arm relatedness	***< 0.001***[b]	4.9 ± 0.1	1.4 ± 0.4	***< 0.001***[c]
Leg relatedness		1.6 ± 0.5	1.2 ± 0.2	

[a] Arm action verbs are significantly more related to arms than to head
[b] Arm action verbs are significantly more related to arms than to legs
[c] Arm action verbs are significantly more related to arms than the non-action verbs

2.3.2. Procedure

All verbs were presented in their infinitive form as single words in order to minimize the interference of syntactic processes. They were shown in black letters (font: Calibri; size: 96) on a white background in the middle of a computer screen that was placed one meter in front of the participant. Stimuli were randomly presented with a stimulus frequency of 0.7Hz (+/- 1428 ms) and no interstimulus interval. Participants were instructed to read each of the words mentally and to avoid overt articulation or any other kind of orofacial movement. To optimize EEG quality, they were encouraged to reduce eye-blinks as much as possible.

2.3.3. Data acquisition and analysis

EEG data were collected with Neuron-Spectrum-5 (4EPM) registration software (Neurosoft, Moscow, Russia). 21 Ag/AgCl electrodes were placed on the scalp according to the international 10/20 system (Fp1, Fpz, Fp2, F7, F3, Fz, F4, F8, C3, Cz, C4, T3, T5, T4, T6, P3, Pz, P4, O1, Oz, O2). Additional reference and ground electrodes were placed on the earlobes and forehead respectively. Neurophysiological data were recorded at a sampling rate of 500 Hz (0.01-75Hz band-pass filter). Impedance of each electrode was kept below 5kΩ.

Off-line EEG analysis was performed using BrainVision Analyzer 2 (Brain Products, Munich, Germany). After additional filtering (0.5-30 Hz band-pass filter, Notch filter 50 Hz), eye artefacts were excluded using Independent Component Analysis. Two components were removed (eye blinks; left-right eye

movement) based on inspection of the components' spatial distribution (Mennes et al., 2010; Joyce et al., 2004). Next, the continuous EEG data were segmented into epochs of 1100 ms, starting 100 ms prior to stimulus onset, and baseline corrected to this pre-stimulus interval. Trials with voltage variations larger than 100 μV were manually rejected. By averaging over corresponding epochs, event-related potentials (ERPs) were computed for every subject, electrode, and verb category. ERP participant averages were then grand-averaged across participants for both groups and verb classes separately.

2.3.4. ERP analysis

Visual presentation of single words typically evokes an ordered succession of 6 peaks. Whereas P1, N2 and P3 are known to be related to primary visual and visual attention processes, (e.g. Di Russo et al., 2001; Folstein & Van Petten, 2008; Polich, 2007) N1, P2 and N400 (partly) reflect linguistic processes (e.g. Dambacher et al., 2006; Duncan et al., 2009; Tarkiainen et al., 1999). Therefore, only the latter were subjected to further analyses. Peak latency and mean amplitude were determined for both verbs separately. Peaks were semi-automatically determined as the local maximum within 50 – 200 ms for N1, 100 – 250 ms for P2 and 300 – 500 ms for N400. Mean amplitude was computed for the following time windows: N1 (95 – 135 ms), P2 (160 – 210 ms) and N400 (300 – 450 ms). These windows were chosen based on the grand averaged waveforms and previous research (Duncan et al., 2009; Weber-Fox, 2001). To investigate the topographical distribution of the peaks while keeping the amount of data limited, subsets of adjacent electrodes were taken together.

As P2 was observed over the entire scalp, nine clusters with average amplitude/latency of adjacent electrodes were calculated: anterior left (F7, F3), anterior midline (Fz), anterior right (F4, F8), central left (T3, C3), central midline (Cz), central right (C4, T4), posterior left (T5, P3), posterior midline (Pz), and posterior right (P4, T6). Also for the N400, the same nine clusters were created with one exception: posterior left and right did not include T5 and T6 respectively, as no N400 was seen over these electrodes. Since N1 was only seen over posterior regions, one left (T5/P3/O1), one midline (Pz/Oz) and one right (T6/P4/O2) subset was created.

Statistical analysis was performed in IBM SPSS Statistics 19.0. Latency and amplitude were analysed using repeated measures ANOVAs with one between-subject factor group (FS, AWS) and three within-subject factors for P2 and N400: hemisphere (left, midline, right), region (anterior, central, posterior) and verbs (action, non-action). As N1 only occurred over posterior sites, the factor 'region' was not included in its analysis. To evaluate whether the assumption of homogeneity of covariance was met, Mauchly's Test of Sphericity was computed for all factors with more than one degree of freedom in the numerator. If the assumption was violated ($\alpha \leq 0.05$), Greenhouse-Geisser (G-G)

adjusted p-values were used to determine significance. Significance values were set at $\alpha \leq 0.05$ for main and interaction effects. All further pairwise comparisons were Bonferroni corrected.

2.2.5. Source reconstruction

The Statistical Parametric Mapping 8 software package (SPM 8: Welcome Department of Cognitive Neurology, University College, London, United Kingdom) implemented in MATLAB (the MathWorks, Inc., Massachusetts, USA) was used for EEG source reconstruction. To limit the number of comparisons, a sensor-space analysis was first performed to search for time points at which maximal differences occurred between groups and verb types. In the sensor-space analysis, the ERP data from 0 – 380 ms of every participant was converted into 3D images for every verb category separately. This time window encompasses early and late time points described in similar previous research at which significant sensorimotor activations during action verb processing occurred (Hauk & Pulvermüller, 2004b; Moseley et al., 2013; Pulvermüller et al., 2001). These images were generated by constructing 2D, 64 x 64 pixels resolution, scalp maps for each time point (using interpolation to estimate the activation between the electrodes) and by stacking the scalp maps over peristimulus time, resulting in [64 x 64 x number of time points]-images (Litvak et al., 2011). These images were statistically evaluated by paired and two sample t-tests for the effect of verb type or group respectively. F-contrasts were calculated to test for differences of either direction.

The multiple sparse priors (MSP) algorithm (Friston et al., 2008) was used to reconstruct the source activity for every subject and verb category. A 3-layered scalp-skull-brain template head model matched to the MNI template was implemented for which the default electrode positions were used. 8196 dipoles were assumed on a template cortical surface mesh and the “bemcp” method (BEM) implemented in FieldTrip (Oostenveld et al., 2011) was used to calculate the forward model. 3D images containing the evoked energy of the reconstructed activity for every subject and verb class were generated in a time window centred around the significant time point(s) from the sensor-space analysis (Litvak et al., 2011). If the time point occurred before 250 ms post-stimulus onset, a time window of 20 ms was chosen, because processes that take place within the first 250 ms are mostly characterized by short-lived transient activations. If the time point occurred after 250 ms, a window of 40 ms was chosen. Using these images, second level analysis was performed to identify the most significant source areas over subjects and verb class. Within the same group, F-contrasts were calculated by performing paired t-tests for the main effect of verb. Between groups, F-contrasts were calculated by performing two sample t-tests for the main effect of group. As motor related activity was the primary focus of this test, only significant results in frontal and parietal lobe were explored. Because the amount of comparisons was already largely reduced by the sensor-analysis and by limiting the analysis to fronto-parietal areas, p-value was set at 0.05 at the source level. The resulting

MNI coordinates holding significant activation differences between verb class, were explored by means of the traditional Brodmann categorization and by means of the SPM Anatomy toolbox developed by Eickhoff et al. (2007). Despite the seemingly limited spatial resolution inherent to less dense EEG recordings, low-density recordings have been established to provide an accurate estimate of ERP generators and to be sufficient to fully describe the variance of an ERP data set when compared to high-density recordings (Kayser & Tenke, 2006).

As the pre- and post-central gyrus was the main region of interest (ROI), an additional source analysis was performed on the earliest time point with prominent activity above this region. For this purpose, the Global Field Power (GFP) was calculated for each verb class separately over all (pre)frontal and parietal sensors for all participants. GFP illustrates the time course of the overall signal strength of the ERPs. Based on the maps of current estimates that were made for each peak of the GFP, the earliest time point with clear activity over pre- and post-central gyrus was identified. Similar second level analysis was performed on a time window centred around this time point. Again, depending on whether the time point occurred before or after 250 ms post-stimulus onset, a time window of 20 or 40 ms respectively was chosen. As the same time window emerged in AWS as in FS (see 3.2.), F-contrasts were calculated by performing a full factorial general linear model with group (FS, AWS) as between-subject factor and verb (action, non-action) as within-subject factor. A mask was applied so only activity in pre- and post-central gyrus (BA 4, 6, 3, 1 ,2 and 43) was evaluated. Because only one ROI was included, the criterion for significance could be set at $\alpha = 0.05$.

3. Results

3.1. ERP analysis

Grand average waveforms for FS and AWS were very similar in morphology, as can be seen in figure 1. The waveforms are characterized by a series of components. At posterior electrodes, a very early negative peak around 40 ms was immediately followed by a P1, peaking at around 70 ms, and a N1, peaking at around 115 ms. At anterior sites, the P1 was reversed evoking a negative peak around 70 ms. No equivalent of the posterior N1 was seen. Next, a P2 could be observed over the entire scalp. This wave reached its maximum around 180 ms. The subsequent N2 (peak: 225 ms) and P3 (broad wave around 300 ms) were quite small and could only be seen over occipital and T5/T6 electrode sites. Finally, a large N400 occurred, most clearly pronounced over anterior electrodes. Although this component has a protracted morphology, a peak could be described at around 380 ms.

No significant difference between FS and AWS emerged for either peak. A significant main effect of the factor Verb was observed for the N400 amplitude ($F(1,58) = 8.71$; $p = 0.005$). Both FS and AWS showed a larger N400 for action than for non-action verbs. No other main or interaction effect of the factor Verb was found. Furthermore, some distributional amplitude variations were observed. The

largest amplitude for the P2 was seen over midline and central electrodes, especially at Cz (Region*Hemisphere: F(4,232)= 11.79; ε= 0.80; G-G p< 0.001) and for the N400 over left and anterior electrodes (Region*Hemisphere: F(4,232)= 15.57; ε= 0.75; G-G p< 0.001). The N1 appeared to be smallest (Hemisphere: F(2,116)= 9.40; p< 0.001) and earliest (Hemisphere: F(2,116)= 9.21; p< 0.001) over midline electrodes.

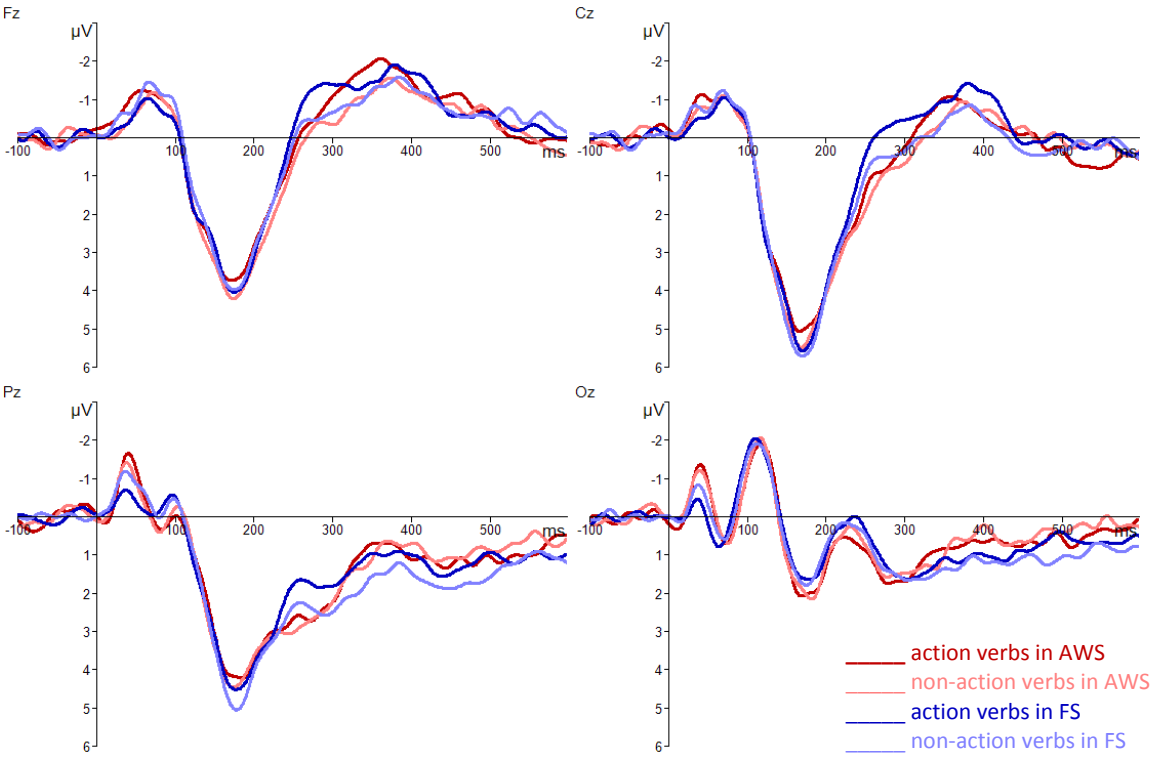

Figure 1: Grand average for action and non-action verbs separately for FS and AWS at midline electrodes. The 100 ms baseline and the first 600 ms of stimulus processing are depicted. Negative is plotted upwards. The x-axis represents latency (ms), the y-axis represents amplitude (µV).

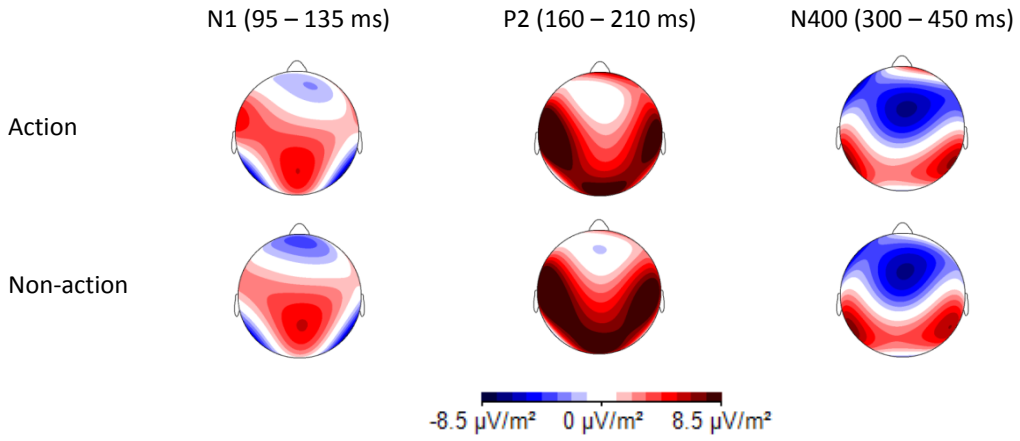

Figure 2: Topographic EEG maps of N1, P2 and N400 of the AWS.

3.2. Source reconstruction

Detailed results of the source reconstruction of both FS and AWS are shown in table II. As the results of the FS were already addressed in the introduction, they will not be repeated here.

The sensor-space analysis found a maximal difference in the stuttering group between action and non-action verbs at 332 ms ($F(1,29)= 19.69$; $p< 0.0001$). As this time point situated after 250 ms post-stimulus onset, second level source analysis was performed on an epoch of 40 ms centred around this peak (313 – 352 ms). Statistical analysis revealed a significant stronger cluster of activation in right ($F(1,29)= 4.95$; $p= 0.033$) and left ($F(1,29)= 4.46$; $p= 0.042$) sensorimotor cortex during non-action compared to action verb processing (see figure 3). The differences between AWS and FS were not strong enough to be significant for the sensor-space analysis.

Table II: Significant results of the source reconstruction for both time windows.
Reported are the coordinates of local maxima in MNI space which are part of larger clusters as well as the number of voxels per cluster (2 mm x 2 mm x 2 mm). A description of the region that contains these coordinates (based on both macroscopical parcellation and BA labelling) is added. The last column shows which verb type or group evoked most energy.

Time	***Macroscopic***		***Coordinates (MNI)***			***P***	***Extent***	***Highest***
interval	***anatomical name***	***BA***	***x***	***y***	***z***	***value***	***(voxel)***	***activation***
155 – 174 ms: action vs non-action verbs for the FS (Vanhoutte et al., 2015b)								
	R precentral gyrus	BA 6	18	-24	68	0.026	158	Action
	L precentral gyrus	BA 4	-14	-28	71	0.027	110	Action
155 – 174 ms: FS vs AWS for the non-action verbs								
	R precentral gyrus	BA 6	18	-24	68	0.019	189	AWS
	L precentral gyrus	BA 4	-14	-26	70	0.030	65	AWS
219 – 238 ms: sensor-space analysis for the FS (Vanhoutte et al., 2015b)								
	R middle frontal gyrus	BA 9	32	28	42	0.011	164	Action
	L middle frontal gyrus	BA 9	-26	26	35	0.011	222	Action
313 – 352 ms: sensor-space analysis for the AWS								
	R precentral gyrus	BA 6	22	-16	72	0.033	302	Non-action
	L precentral gyrus	BA 6	-10	-8	72	0.042	53	Non-action

Figure 4 shows the GFP and the map of current estimates of the AWS. The earliest prominent peak activity over pre- and post-central areas occurred at 166 ms for the action verbs and at 168 ms for the non-action verbs. As these situated close around 165 ms, the peak observed in the FS, it was decided to evaluate the same time window to enable a comparison between AWS and FS by use of a full factorial general linear model.

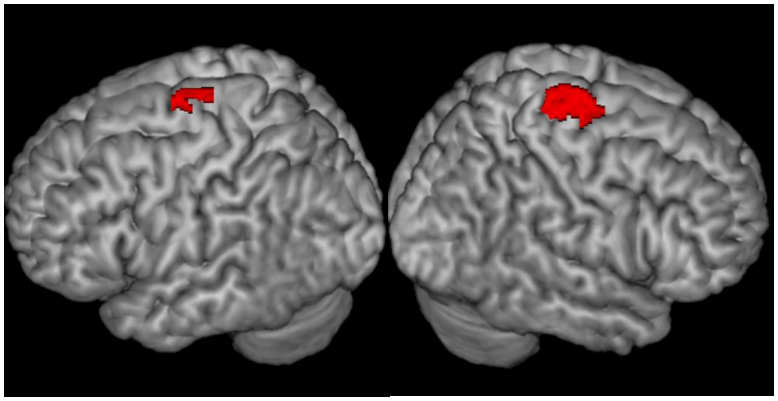

Figure 3: Result of the sensor-space analysis of the AWS.
Source reconstruction for the statistical significant difference between action and non-action verbs in the time window 313 - 352 ms. An activation focus located in bilateral sensorimotor cortex can clearly be identified.

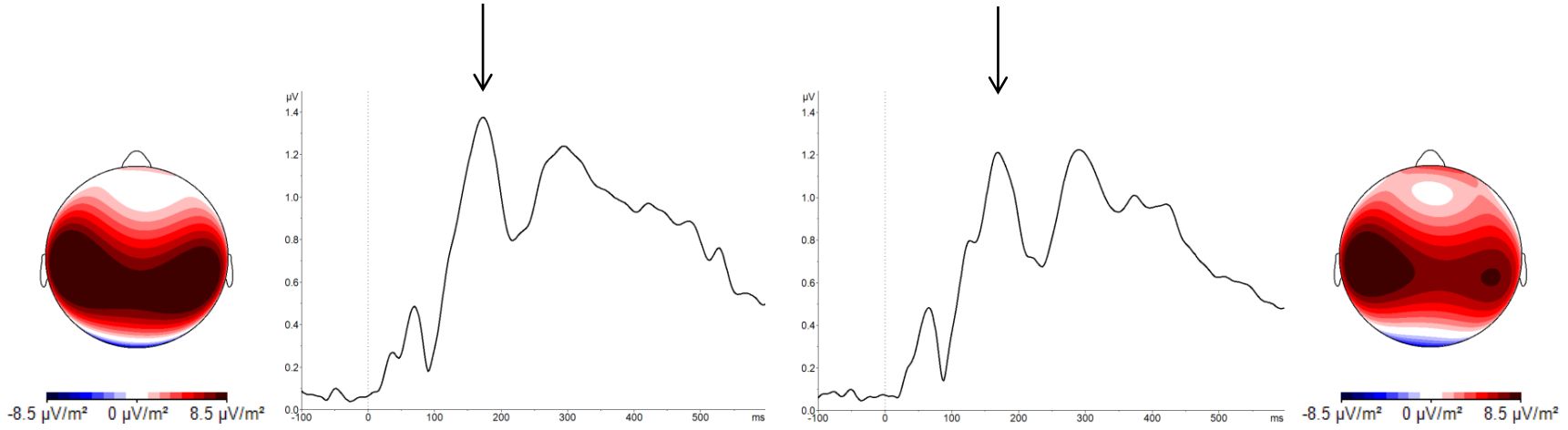

Figure 4: GFP calculated over all (pre)frontal and parietal sensors for all AWS.
Non-action verbs are shown on the left, action verbs on the right. The current estimates maps at 168 ms for the non-action verbs and 166 ms for the action verbs are presented as well. This is the earliest time point with clear activity over pre- and post-central gyrus.

From 155 to 174 ms, source reconstruction of the grand averages revealed for both verbs widespread activation in perisylvian regions, including superior temporal cortex and inferior frontal gyrus, supramarginal gyrus, pre- and postcentral gyrus. Also prominent activity was seen in occipital, inferior temporal and fusiform gyrus (see figure 5). Both left and right hemisphere showed similar patterns of activity. Statistical analysis only focused on the sensorimotor cortex (pre- and postcentral gyrus). No significant difference was found between both verbs for the AWS and between both groups for the action verbs. A significant result did appear when comparing non-action verb processing between both groups. AWS showed significantly more activity than FS in right (p= 0.019) and left (p= 0.030) sensorimotor cortex.

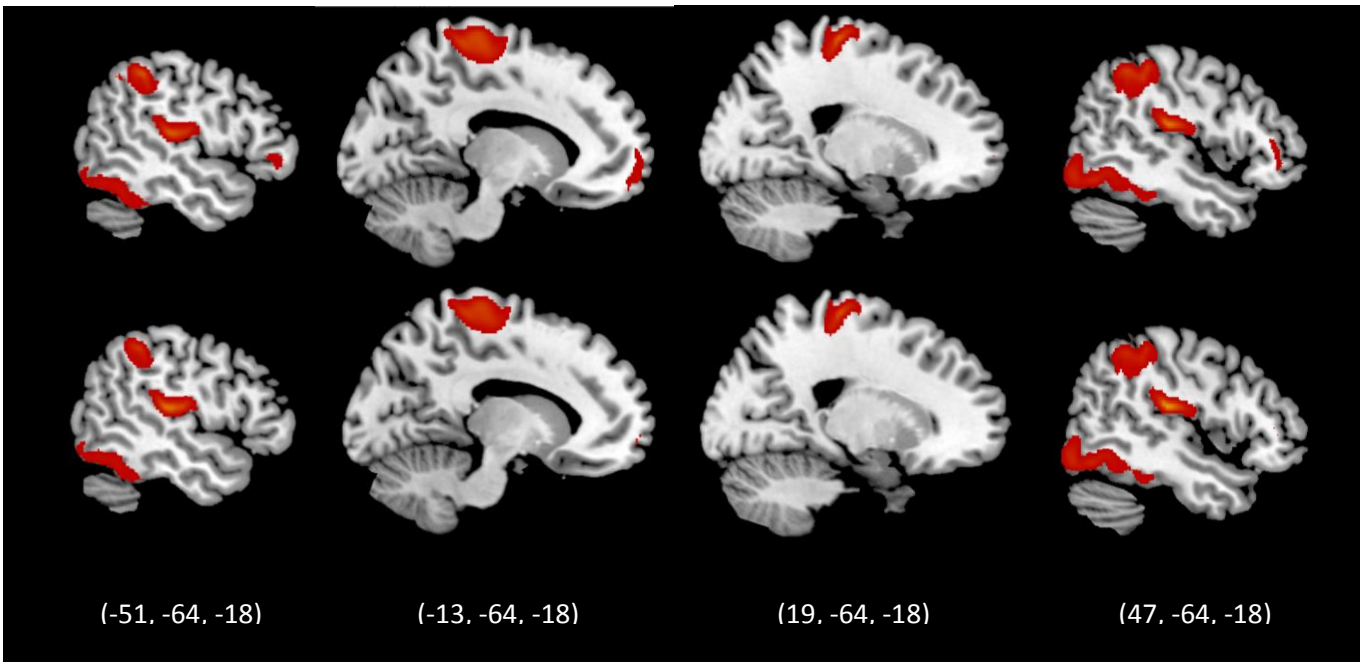

Figure 5: Source reconstruction of the grand average ERP's of the AWS from 155 to 174 ms.
Four sagittal slices are shown for non-action verbs (top diagram) and action verbs (bottom diagram). The most prominent activation clusters (> 2 standard deviations calculated over the whole volume of reconstructed activity) are depicted in red. The two slices on the left are located in the left hemisphere, the two slices on the right are located in the right hemisphere. Corresponding MNI coordinates are shown underneath the pictures (x, y, z).

4. Discussion

The present study aimed at evaluating the time points of motor related activity in a group of AWS during a perception task that triggered hand motor representations of the brain. Silent reading of hand action verbs was compared to silent reading of abstract, non-action verbs. A silent reading task was chosen to exclude any interference from movement preparation and execution or from aberrant auditory processing. The results of the AWS were compared to the previously obtained results from a group of healthy FS (Vanhoutte et al., 2015b).

The ERP waveforms of the AWS did not differ from those found previously in FS. Source reconstruction revealed that the earliest most prominent peak activity over pre- and post-central areas was identified at 166 and 168 ms for action and non-action verbs respectively, which is very close to the 165 ms observed in the FS group. The sensor-space analysis found a maximal motor related difference between action and non-action verbs at 332 ms.

4.1. Temporal coordination of motor related activation

The present study confirms that the neural timing of motor activations in stuttering can be considerably altered, even during a visual word recognition task. Although the earliest activity over pre-and post-central areas was found around the same time, the sensor-space analysis revealed that the maximal motor related difference between both verb types was delayed with about 100 ms. While FS showed a divergence at 228 ms in bilateral DLPFC, AWS displayed a distinction around 332 ms in bilateral sensorimotor cortex.

In stuttering, neural timing alterations in speech motor systems have been observed by Salmelin et al., (2000) and Biermann-Ruben et al., (2005) during a single word and sentence production task respectively. The present findings show that these timing alterations do not seem to require overt speech production, which was also observed by Liotti et al., (2010) during an auditory perception task. An early motor hyperactivation together with a late auditory hypoactivation was suggested to reflect a disturbed interplay between auditory and motor areas. The present study adds that temporal changes in neural motor control during speech perception can also present without dysfunctions in auditory processing and/or auditory-motor integration. Overall, neurological research in stuttering might focus more on timing aspects of motor control as even simple tasks evoke considerable alterations.

4.2. Reversed sensorimotor recruitment

The present results contribute to a growing amount of evidence for motor abnormalities in stuttering (Brown et al., 2005). A reversed motor related activation was observed in AWS from 313 to 352 ms. Non-action verbs evoked a significantly higher activation in bilateral sensorimotor cortex. In FS, larger activations occurred during action verb processing in bilateral sensorimotor cortex from 155 to 174 ms and in bilateral DLPFC from 219 to 238 ms. The decreased sensorimotor recruitment during hand action compared to non-action verb processing is suggested to be linked with the impaired hand motor control in stuttering (Busan et al., 2011).

Motor impairments in stuttering have been found to extend to non-speech oral movements (Chang et al., 2009) and finger movements. Not only behavioural deficits have been described like prolonged initiation, execution and reaction times for finger and manual tasks (Bishop et al., 1991; Smits-

Bandstra et al., 2006; Webster, 1997; Webster and Ryan, 1991), also neural dysfunctions have been reported like an imbalanced lateralization during finger tapping (Morgan et al., 2008; Neef et al., 2011). Of particular interest for the present findings is the decreased excitability of hand motor representations. AWS showed reduced motor evoked potentials from hand muscles following transcranial magnetic stimulation of the hand motor cortex (Busan et al., 2011). Because the current action verbs described hand and/or arm movements, they sparked the upper limb representation in the motor cortex (Hauk et al., 2004; Vanhoutte et al., 2015b). Due to the decreased excitability of hand motor regions, sensorimotor activation of the hand representation during action verb processing will be hampered. Consequently, action verbs will not be able to evoke stronger sensorimotor activation than non-action verbs in stuttering.

The mutual comparison of sensorimotor cortex activation in FS and AWS in the early time window 155 – 174 ms revealed another interesting finding. No difference was found between both groups during action verb processing. Non-action verbs, on the other hand, evoked increased sensorimotor activity in AWS compared to FS. At first sight, this observation seems to contradict the previous hypothesis of decreased hand motor activation. However, another motor activation system might have occurred and resulted in a motor increase during non-action verb processing.

Motor cortex is generally found to be hyperactive in stuttering during a variety of tasks (Brown et al., 2005), even during silent reading of nouns not specifically related to motor semantic features (De Nil et al., 2000, 2003). This motor hyperactivation can already present within the first 100 ms after stimulus presentation (Liotti et al., 2010). The increase in sensorimotor cortex activation during non-action verb processing at 155 to 174 ms is suggested to be a reflection of the general motor hyperactivation typically seen in stuttering, independent of stimulus material or overt speech requirements. The hand action verbs are hypothesized not to follow this general motor increase as they relied specifically on the impaired hand motor region which is characterized by a decreased excitability (Busan et al., 2011). It is hypothesized that these two different activation patterns are at the origin of the reversed sensorimotor activation.

4.3. Linguistic processing

It is an ongoing debate whether stuttering is a language or a motor disorder or a combination of both (Kent, 2000). The present study was not designed to make a sound conclusion on this matter, but suggests that motor deficits may present without neurophysiological alterations in linguistic processing.

AWS did not differ from FS for any peak, neither for amplitude nor for latency. While N1 is linked with visual word form processing (Brem et al., 2006; Salmelin, 2007; Tarkiainen et al., 1999), the exact function of the P2 is uncertain. It has been suggested to be related to phonological and early

lexico-semantic and -syntactic processes (Dambacher et al., 2006; Palazova et al., 2001; Takashima et al., 2001; Zhang et al., 2009). The N400 reflects integration of different kinds of information in large scale networks (Hauk et al., 2012; Kutas & Federmeier, 2000). AWS even display the normal N400 concreteness effect similar to the FS: action verbs evoked a significantly larger N400 than non-action verbs which is compatible with concrete words evoking larger N400 amplitudes than abstract words (e.g. Gullick et al., 2013). The larger N400 for action verbs would be related to a larger amount of neural correlates that require more semantic integration (Xiao et al., 2012). In sum, AWS and FS seem to perform similarly on visual and linguistic processing during the present task.

Previous linguistic EEG studies evaluating reading in stuttering did not report abnormalities in N1 and P2 either (Weber-Fox, 2001; Cuadrado & Weber-Fox, 2003; Weber-Fox et al., 2004, 2008). The N400 on the other hand is frequently reported to be altered. Large methodological differences can explain the contradiction with the present N400 finding. Previous studies documenting abnormalities in N400 used primed picture naming or picture-word primed (Maxfield et al., 2010, 2011, 2014) and sentence processing tasks (Weber-Fox, 2001; Weber-Fox & Hampton, 2008; Weber-Fox et al., 2013). The present study encompassed single word reading which is considerably less complex and demanding. Indeed, when evaluating rhyming based on word pairs, no amplitude abnormalities occurred in children who stutter, neither in the N400 evoked by the prime nor the one evoked by the target (Weber-Fox et al., 2008). Overall, it seems that neurophysiological alterations in stuttering during language processing only appear during more complex and demanding tasks (Weber-Fox et al., 2004).

4.4. Bilateral findings

The significantly lower sensorimotor activity during action verb processing was found bilaterally which is somewhat unexpected as decreased hand motor excitability was only observed over the left hemisphere (Busan et al., 2011). The exact origin of the bilateral result cannot be determined, though several observations might have contributed to this finding. From a language perspective, hand action verbs have frequently been reported to evoke bilateral motor activations (Hauk & Pulvermüller, 2004b; Raposo et al., 2009; Rüschemeyer et al., 2007; Tettamanti et al., 2005). Also abstract verb processing has been shown to rely on both hemispheres (Rodriguez-Ferreiro et al., 2010). From a stuttering perspective, motor hyperactivation is most frequently described over right hemisphere (Brown et al., 2005) which may cause more pronounced differences between action and non-action verbs in this hemisphere. Secondly, left hemisphere is, by most researchers, posited to contain the primary structural anomaly for stuttering which would be situated in the neighbourhood of the left sensorimotor cortex (Chang et al., 2008; Connally et al., 2014; Cykowski et al., 2010;

Sommer et al., 2002; Watkins et al., 2008). This structural anomaly may, however, vary largely among individuals which may evoke less consistency in left sensorimotor activity.

4.5. Additional considerations

The strength of EEG research concerns its excellent temporal resolution. The major aim of this study was to evaluate temporal coordination of motor related activations. As motor involvement in the present task is not linked to a certain ERP (e.g. Pulvermüller et al., 2001, 2005; Vanhoutte et al., 2015b), source reconstruction was performed. Spatial resolution of EEG research is, however, limited and therefore, its results should be interpreted with caution. To meet this limited spatial resolution, no fine grained analyses nor interpretations were performed (e.g. making a distinction between premotor and motor cortex) though this might have provided valuable information.

5. Conclusion

Neural timing of motor activations is altered in stuttering, even during a silent reading task. Although the earliest activity over pre- and post-central areas was found around the same time (165 ms), the maximal motor related difference between both verb types was delayed with about 100 ms (at 228 ms for FS in DLPFC, at 332 ms for AWS in sensorimotor cortex). This difference even showed a reversed activation pattern: non-action verbs showed more sensorimotor activation than hand action verbs. The increased activity during non-action verb processing is suggested to reflect a general motor hyperactivation typically seen in stuttering. The hand action verbs are hypothesized not to follow this general motor increase as they specifically rely on the hand motor region which is suggested to show a decreased excitability. Overall, neural motor abnormalities in stuttering are confirmed not to require (speech) movement execution.

Chapter 8

CNV amplitude as a neural correlate for stuttering frequency: a case report of acquired stuttering

Vanhoutte Sarah, Van Borsel John, Cosyns Marjan, Batens Katja, van Mierlo Pieter

Hemelsoet Dimitri, Van Roost Dirk, Corthals Paul, De Letter Miet, Santens Patrick

Neuropsychologia (64), 349-59.

Abstract

A neural hallmark of developmental stuttering is abnormal articulatory programming. One of the neurophysiological substrates of articulatory preparation is the contingent negative variation (CNV). Unfortunately, CNV tasks are rarely performed in persons who stutter and mainly focus on the effect of task variation rather than on interindividual variation in stutter related variables. However, variations in motor programming seem to be related to variation in stuttering frequency. The current study presents a case report of acquired stuttering following stroke and stroke related surgery in the left superior temporal gyrus. A speech related CNV task was administered at four points in time with differences in stuttering severity and frequency.

Unexpectedly, CNV amplitudes at electrode sites approximating bilateral motor and left inferior frontal gyrus appeared to be *inversely* proportional to stuttering frequency. The higher the stuttering frequency, the lower the activity for articulatory preparation. Thus, the amount of disturbance in motor programming seems to determine stuttering frequency. At right frontal electrodes, a relative increase in CNV amplitude was seen at the test session with most severe stuttering. Right frontal overactivation is cautiously suggested to be a compensation strategy. In conclusion, late CNV amplitude elicited by a relatively simple speech task seems to be able to provide an objective, neural correlate of stuttering frequency. The present case report supports the hypothesis that motor preparation has an important role in stuttering.

Keywords

contingent negative variation, stuttering, stuttering severity, %SS, motor preparation

1. Introduction

Stuttering is a speech disorder primarily characterized by the occurrence of speech blocks, prolongations and/or repetitions of sound or syllables. When the disorder begins in early childhood, it is called developmental stuttering (Bloodstein & Ratner, 2008). However, an acquired form of stuttering following brain damage exists as well. This form is referred to as neurogenic stuttering and typically has its onset during adulthood (Van Borsel, 1997; Duffy, 2013). Neurogenic stuttering has been associated with a variety of lesions that can be located in all cortical lobes of both hemispheres as well as in the basal ganglia, thalamus, cerebellum, corpus callosum and brain stem (for a review, see Van Borsel, 1997; De Nil et al., 2009). Many of these areas are also assumed to be involved in developmental stuttering (e.g. Chang et al., 2009; Lu et al., 2010a; Watkins et al., 2011; Xuan et al., 2012). Although originally thought to be two different entities, it now seems that both types of stuttering may share common neural characteristics (Theys et al., 2012).

A neural hallmark of developmental stuttering is abnormal motor programming. Several studies found anatomical and functional disturbances in left inferior frontal gyrus (IFG), the core cortical region of motor preparation, and its connections. Besides structural anomalies in grey and white matter (Sommer et al., 2002; Chang et al., 2008, 2011; Watkins et al., 2008; Kell et al., 2009; Cykowski et al., 2010), uni- and bilateral hypo- and hyperactivations have been described in both silent reading and overt speech production (Fox et al., 1996; De Nil et al., 2000, 2003; Watkins et al., 2008). The most recurrent finding is an anomalous engagement of the right frontal operculum (RFO), the homologue of Broca's area (for a meta-analysis, see Brown et al., 2005). Increased activity in left IFG has also been observed during rest (Xuan et al., 2012). Magneto-encephalography revealed that adults who stutter (AWS) first activate left motor cortex and secondly left IFG during overt reading. Thus, AWS seem to initiate motor programmes before preparing the articulatory code (Salmelin et al., 2000).

One of the electrophysiological substrates of motor preparation is the contingent negative variation (CNV) .The CNV is an event-related, slow negative potential that occurs between two defined stimuli. The first stimulus is the warning stimulus (S1) which announces the imperative stimulus (S2) which on his turn requires a response (Walter et al., 1964; Rohrbaugh & Gaillard, 1983; McCallum, 1988; Regan, 1989; Golob et al., 2005). This response is typically a motor response though cognitive tasks have been reported as well (e.g. Cui et al., 2000; Bares et al., 2007). If the interval between the onset of S1 and S2 is ≥ 2 seconds, two CNVs can be distinguished within this interstimulus interval. The first one, the initial CNV, is related to orientation and is induced by the warning stimulus. It has its greatest amplitude at frontal sites within the first second following S1. The second one, the late CNV, occurs before S2 and has a wide cortical distribution with a maximum amplitude at central electrodes (Walter et al., 1964; Loveless & Sanford, 1974; Rohrbaugh & Gaillard, 1983; McCallum, 1988; Regan,

1989). The late CNV is reported to have multiple cortical and subcortical generators: prefrontal, premotor, primary motor, anterior cingulate, somatosensory and parietal regions as well as the basal ganglia and thalamus. Hence, the late CNV is generally accepted to measure the neuronal activity within the basal ganglia-thalamo-cortical (BGTC) loop (Lamarche et al., 1995; Hamano et al., 1997; Gomez et al., 2003; Bares et al., 2007; Fan et al., 2007). This late CNV is suggested to represent primarily motor preparation, and, additionally, sensory anticipation for S2 (Bender et al., 2004; Bares et al., 2007).

CNV research mostly implies a motor response from the limbs. Only a few speech related CNV studies have been performed (e.g. Michalewski & Weinberg, 1977; Mock et al., 2011) and they rarely concerned stuttering. Pinsky & McAdam (1980) found no significant difference in speech CNV amplitude between 5 AWS and 5 control participants. Prescott and Andrews (1984), and Prescott (1988) evaluated the influence of the complexity of the speech response on the CNV amplitude in AWS. In their first study, no significant results were found (Prescott & Andrews, 1984). In the second study, AWS displayed larger CNV amplitudes than fluent speakers for familiar words, which are highly practiced speech responses and therefore very likely to be completely pre-programmed, suggesting that AWS have difficulties establishing efficient motor programs (Prescott, 1988). While these 2 studies mainly focused on the effect of task complexity on motor preparation, the effect of individual variation in stuttering severity has not been explored thus far. Nonetheless, Zimmerman & Knott (1974) observed large interindividual variations among stuttering participants in CNV amplitude and morphology. Several stuttering frequency and severity measures are repeatedly reported to correlate positively with cortical regions (Braun et al., 1997; Fox et al., 2000; Chang et al., 2009; Kell et al., 2009; Ingham et al., 2012) and subcortical brain structures like thalamus and basal ganglia (Braun et al., 1997; Giraud et al., 2008; Kell et al., 2009, Ingham et al., 2012) known to be involved in motor preparation. As on one hand, these regions are part of the BGTC – loop and on the other hand, the late CNV is known to measure the activity in this loop (Fan et al., 2007), a positive association between CNV amplitude and stuttering frequency/severity may be expected. More specifically, the amplitude of the late CNV during a speech production task is hypothesized to increase with increasing stuttering severity/frequency.

The current study presents a case of acquired stuttering following stroke in left superior temporal gyrus (STG) and stroke related surgery. A speech related CNV task was administered by use of electro-encephalography (EEG) at four points in time with differences in stuttering frequency. Due to its excellent temporal resolution, EEG allows one to look at a particular process with millisecond precision. Due to its limited spatial resolution however, EEG data can only provide activation information of broad neurological areas, not of specific brain regions.

2. Method

2.1. Participant

2.1.1. General information

MH is a 28-year-old right-handed, highly educated woman and native speaker of Dutch. At the time she suffered a stroke, she was working as a psychologist. There was no history of hearing complaints, psychiatric disorders, dyslexia or other speech-language problems prior to her neurological event. In addition, there was no family history of recovered or persistent developmental stuttering or cluttering. MH has a corrected-to-normal vision and took no medication apart from contraception. She gave her written informed consent to participate in this study, in accordance with the declaration of Helsinki. The study was approved by the local ethics committee.

2.1.2. Medical history

At birth, MH suffered from sepsis for which she spent several weeks in an incubator. Her psychomotor development, however, was normal. In 2010, after 7 years of complaints of fatigue and a regular occurrence of headache, a tentative diagnose of narcolepsy was made based on a polysomnography with a Multiple Sleep Latency Test. No cataplexy, sleep paralysis or hypnagogic hallucinations occurred. A brain MRI was normal. Methylphenidate, and subsequently modafenil were prescribed, however without any adequate effect. At the time of the stroke, fatigue had diminished and MH no longer took these medications.

2.1.3. Case report

Over a period of 2.5 months, MH sustained 5 hemorrhagic strokes from a cavernoma in the left temporal area. They were characterized by linguistic disturbances, especially auditory comprehension problems, that took on average 60 minutes after which MH recovered completely. No other motor or cognitive disturbances were reported. Stuttering symptoms started to appear a few days after the third stroke. A detailed time line of the neurologic events, hospitalizations and neurophysiologic evaluations can be found in figure 1.

After this third stroke, MH was admitted to the hospital for the first time. On admission, clinical neurologic assessment was normal. An urgent brain MRI revealed a subacute intraparenchymatic haematoma in the left STG with moderate perilesional oedema suggestive for a venous cavernoma (figure 2A). Conventional angiography showed no abnormalities. Because the linguistic symptoms appeared intermittently, a possible epileptic nature was suspected. Therefore, levetiracetam, 2 x 500 mg/day, was started. A few days after the third stroke, stuttering started to emerge. Since no increase in bleeding was seen on a brain Computerized Tomography (CT), an increase in oedema was suggested to be the origin of stuttering onset.

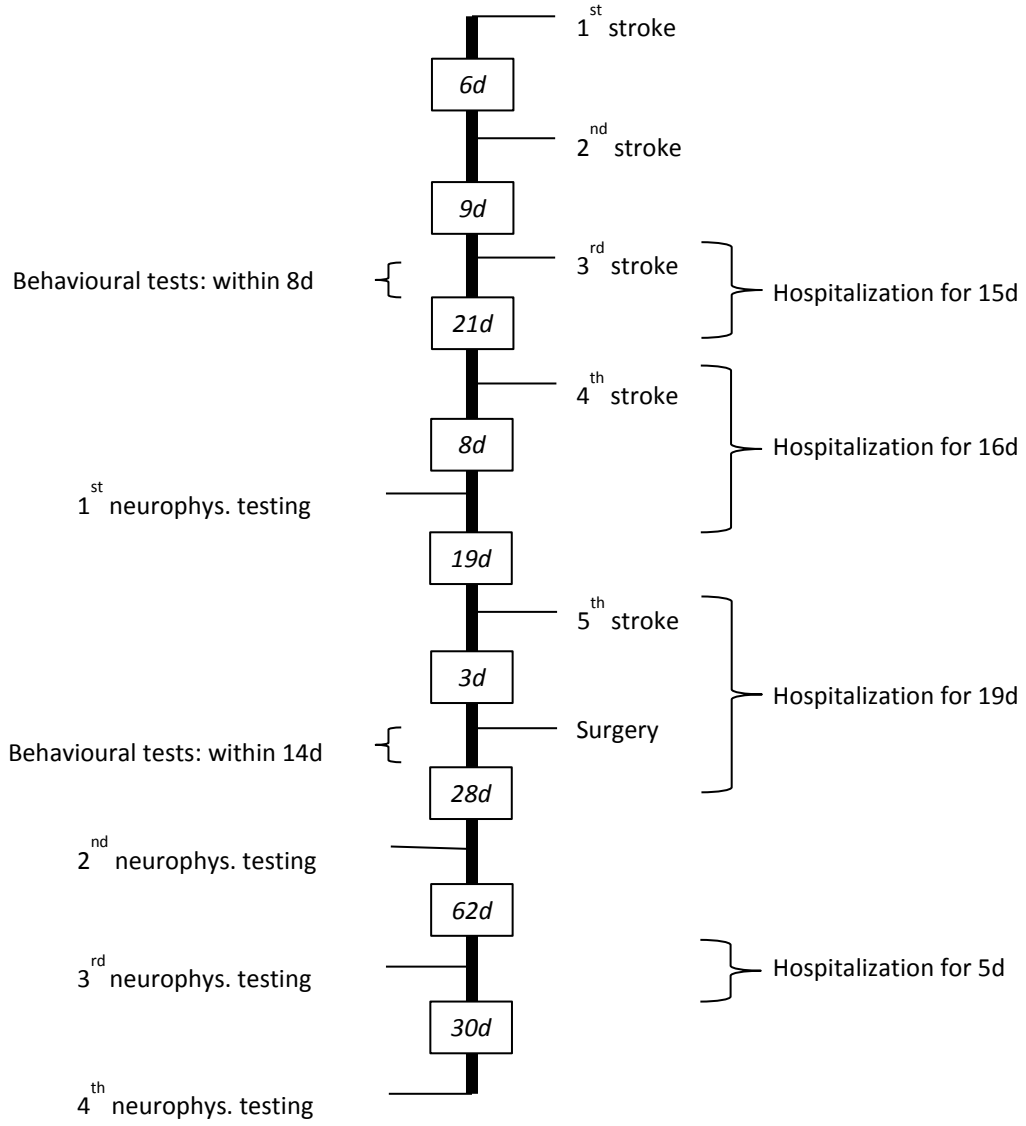

Figure 1: A detailed time scale of all events. Durations are expressed in number of days (d).

Behavioural assessment revealed no linguistic problems. MH obtained the maximum score on both the Token Test of the Aachen Aphasia Test (AAT - Dutch edition; Graetz et al., 1991) and the writing-on-dictate subtest (test 42) of the Psycholinguistic Assessment of Language Processing in Aphasia (PALPA; Kay et al., 1992) – Dutch edition (Bastiaanse et al., 1995).

After a fourth episode of aphasia, MH was re-admitted. The stuttering now seemed to be worse. Clinical neurological examination was normal. CT revealed a slight increase of the intracerebral bleeding in the left temporal area. An additional Fluorine-18-Fluorodeoxyglucose Positron Emission Tomography (FDG-PET) scan showed hypoperfusion near the left STG. About 10 days later, another episode of phatic problems occurred.

Due to the rapid recurrence of the events, a resection of the lesion was performed. Histopathology revealed an arteriovenous malformation (AVM). Initially after surgery, very discrete linguistic

problems were noted which normalized rapidly without substantial logopaedic support. A few days after surgery the Token Test of the AAT was re-administered. Due to the localization of the lesion in the left STG, a more comprehensive evaluation of phonological processing skills was performed as well. These phonological processes include detection, identification and discrimination of spoken phonemes and the recognition of a spoken word as being part of the mental lexicon (McClelland & Elman, 1986; Poeppel et al., 2008). This can be evaluated by subtests 1, 2 and 5 of the PALPA (Kay et al., 1992; Bastiaanse et al., 1995). Phoneme discrimination was assessed by having MH judge whether aurally presented minimal pairs of pseudowords (subtest 1) and real words (subtest 2) were similar or not. Lexical decision was measured by subtest 5 in which 80 real and 80 pseudowords were presented aurally. On all these tests, MH obtained the maximum score. Nevertheless, mild stuttering persisted. Both selective angiography and MRI showed a favourable post-surgery image with no arguments for a residual AVM (figure 2B).

About 13 weeks after surgery, MH was re-admitted to the hospital due to a sudden increase in stuttering severity. Clinical neurological examination was normal. Both angiography and MRI were unchanged. No venous anomaly, arteriovenous fistula or AVM could be seen and MH was dismissed. Five months after surgery, MH resumed work on a part-time basis. No speech therapy was initiated for her stuttering. Anti-epileptic treatment was ultimately stopped.

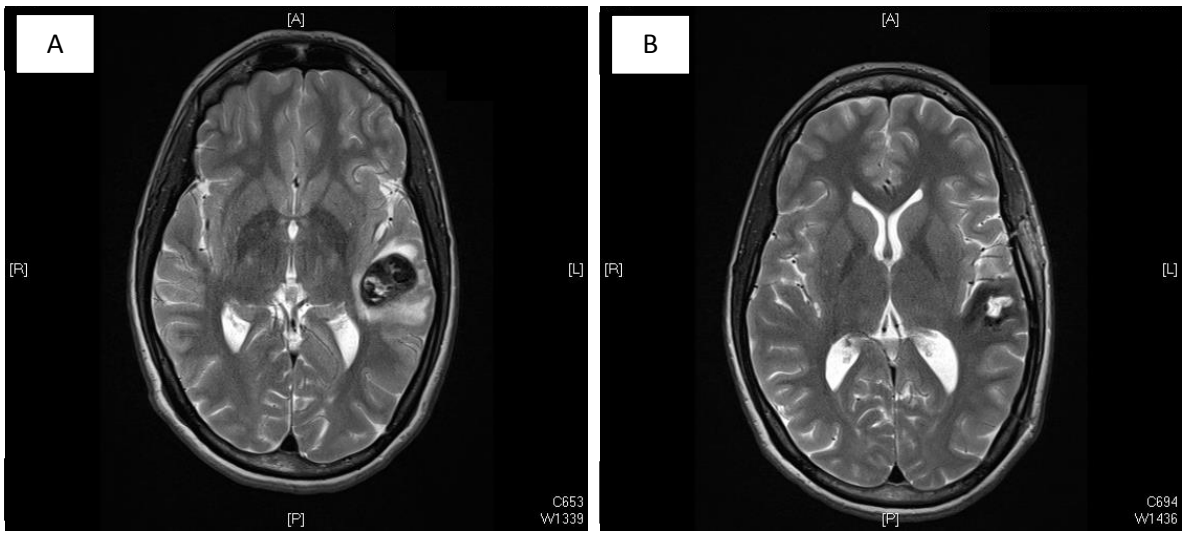

Figure 2: MRI scan, T2-axial, after the third stroke (A) and 2 months after surgery (B).

2.2. Procedure

The neurophysiological testing was executed once pre-surgery and 3 times post-surgery: (1) after one month, (2) after three months, (3) after four months. Each evaluation followed the same procedure. First, speech and reading samples were collected. Secondly, the CNV paradigm was performed.

Finally, three additional phonological tasks were administered that were presented in a randomized order over test sessions. The CNV task was always performed before the phonological tasks to limit the influence of fatigue. At the 4th testing, the phonological tasks were not administered. There were no differences between test sessions concerning medication status as at all test moments, only levetiracetam (2 x 500 mg/day) was taken.

2.2.1. Speech samples

On each test moment, a conversational and reading speech sample was collected. MH read the Dutch translation of the text 'The north wind and the sun' (International Phonetic Association, 1974). During the conversational speech sample MH engaged a conversation with the investigator about work/family/hobby. Due to a sudden increase in stuttering severity, a more extensive speech evaluation was done at the 3rd testing. Automatic speech (counting, reciting the days of the week and the months of the year) and repetition of words and sentences with increasing length was included as well. Speech samples were videotaped using a Canon ACV HD (1920 x 1080) camera and audiotaped in PRAAT, a free software program for acoustical analysis (Boersma & Weenink, Phonetic Sciences, University of Amsterdam, Amsterdam, The Netherlands) using a Samsung CU01 microphone placed 50 cm in front of the participant.

Speech samples were judged for stuttering severity by means of the Stuttering Severity Instrument, fourth edition (SSI-4; Riley, 2008) and percent stuttered syllables (%SS) was calculated following the principles of the Stuttering Measurement System (Ingham & Ingham, 2011). Part-word (sound/syllable) repetitions, prolongations, blocks, broken words and tense pauses (American Speech-Language-Hearing Association, 1999; Yaruss, 1997) were counted as stuttered syllables. It is an ongoing debate whether or not to count monosyllabic word repetitions as stutters (Einarsdottir & Ingham, 2005). In this study, repetitions of monosyllabic words were considered as stuttered dysfluencies when they were repeated at a high rate (Bezemer et al., 2010; Guitar, 2006), with apparent undue stress, tension or struggle (American Speech-Language-Hearing Association, 1999; Van Zaalen & Winkelman, 2009) or when the number of repetition units was 3 or more (Boey et al., 2009; Gregory, 1993). Because stuttering severity can vary considerably over the course of a conversation, long speech samples are recommended to obtain a reliable representation of the stuttering pattern (Sawyer & Yairi, 2006). This holds especially true for a single case study. Since the shortest conversation sample consisted of 735 syllables, the first 735 syllables of each sample were evaluated.

All samples were scored independently by two speech language pathologists (SLP) specialized in stuttering. One of the raters was blind to the sequence of the test sessions. Inter-rater reliability was

assessed by calculating the intraclass correlation coefficient (ICC). All ICC's were high which ensured good to even excellent agreement. Any points of disagreement were discussed to reach consensus.
Stuttering severity was also perceptually judged by three other SLPs specialized in stuttering using the scale from the Camperdown Program (O'Brian et al., 2004; Karimi et al., 2013). The latter is a nine point scale in which 1 = no stuttering, 2 = extremely mild stuttering, and 9 = extremely severe stuttering. Conversation and reading samples were judged separately. Samples were presented in a randomized order and scored independently by the judges. The severity of each sample was then determined by calculating the mean of the severity scores assigned (table I).

2.2.2. EEG data acquisition

EEG data were collected with Neuron-Spectrum-5 (4EPM) registration software (Neurosoft, Moscow, Russia). By use of an universal EEG cap (Haube S2), 21 Ag/AgCl electrodes (Fp1, Fpz, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, Oz, O2) were placed on the scalp according to the international 10/20 system. Two more electrodes were placed above the right side of the upper lip and underneath the left side of the lower lip to register the electromyogram (EMG) of the orbicularis oris muscle in a bipolar fashion. An additional electrode on the forehead was used as ground. Neurophysiological data were recorded against a linked ears reference at a sampling rate of 500 Hz (0.01-75Hz band-pass filter). Impedance of each electrode was kept below 5kΩ. During all tasks, MH was encouraged to avoid orofacial movements and to reduce eye-blinks as much as possible. MH's performances were also videotaped using a Canon ACV HD (1920 x 1080) camera.

2.2.3. CNV paradigm

A self-composed picture naming task was administered in which S1 consisted of a picture that was shown for 1 second. The S2, in the form of a short, black line, appeared 2 seconds after S1 onset (the foreperiod duration was 2 seconds) indicating that MH should name the picture as quickly as possible. S2, shown for 2 seconds, was followed by a black screen for another 2 seconds. If MH continued to stutter on a word once this black screen appeared, she was instructed to stop speaking in order not to contaminate the next trial with muscular artefacts (for a diagram of the CNV task, see figure 3A). One hundred and ten black and white pictures were shown on a white background in the middle of a computer screen that was placed one meter in front of MH. She was instructed to name only one word or to say 'pass' if she didn't know the noun.
The pictures were selected from a picture naming norms database, provided by the Department of Experimental Psychology from the Ghent University, Belgium (Severens et al., 2005). For further analysis, speech onset had to be determined. Articulatory movements were shown to precede vocalization during a Bereitschaftspotential paradigm. Depending on the initial phoneme, the lips or

the tongue were the first source (McArdle et al., 2009). Lip movements are easier to detect than tongue movements with EMG. Therefore, pictures were chosen that referred to a noun that had a bilabial (/m/, /w/, /b/, /p/) or labiodental (/f/, /v/) initial phoneme. For the 4 sessions, MH correctly identified 106, 107, 106, and 107 pictures respectively. Some responses were additionally excluded from further analyses because (1) the word was produced before S2 was shown, (2) the produced word did not have a labiodental or bilabial initial phoneme, (3) MH swallowed or made an inappropriate lip movement within 1500 ms preceding S2 which was judged based on the videotape recordings and visual inspection of the EMG signal. Stuttered responses would have been analysed separately. However, no stutters occurred. This was judged on-line by the first SLP and off-line based on the videotape recordings by the second SLP. In this way, the following number of trials were preserved for further off-line EEG analyses: 100, 104, 101, 102 for the 4 sessions respectively.

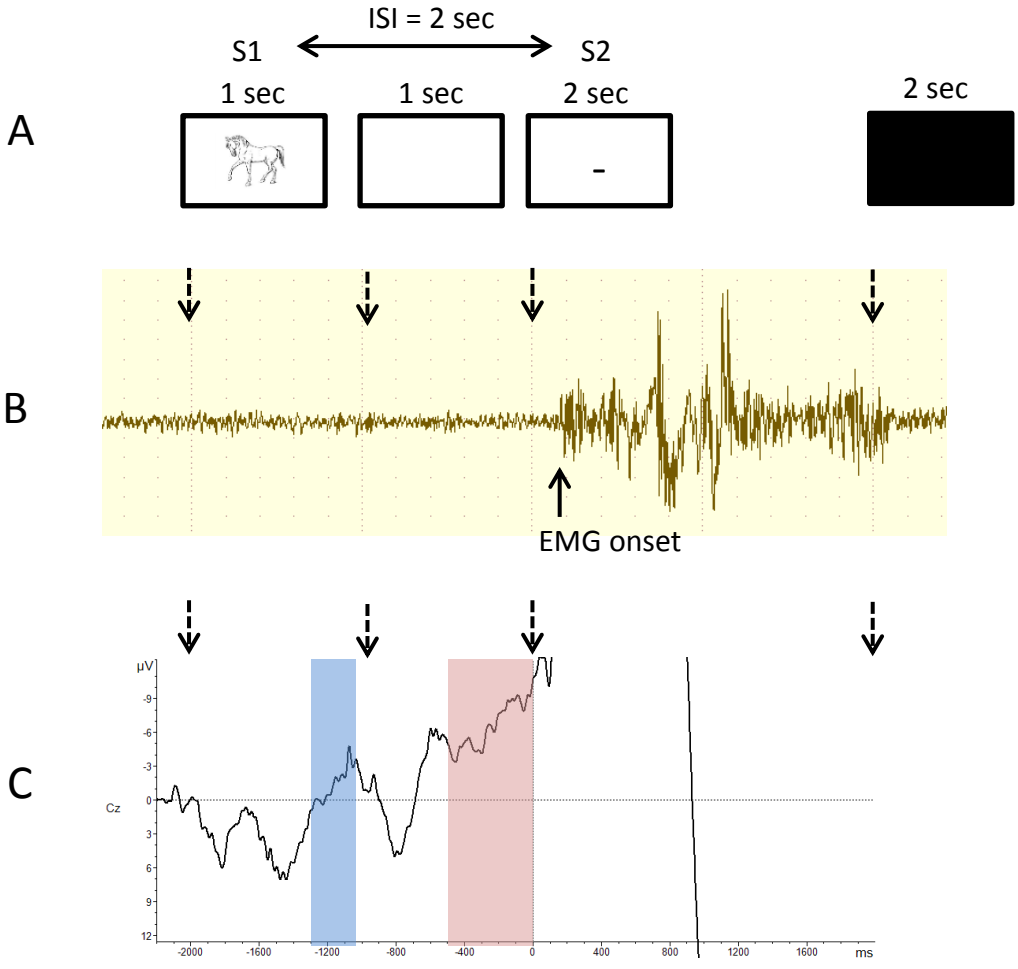

Figure 3: **(A)** Diagram of the picture naming CNV task. The warning stimulus (S1) consists of a picture, the imperative stimulus (S2) of a short, black line that prompts the participant to name the picture. Interstimulus interval (ISI) is 2 s. **(B)** EMG signal of the orbicularis oris muscle of one response. The dotted arrows represent the onset of the corresponding image in (A). The full arrow represents the onset of the EMG signal. **(C)** S-locked average at Cz at the first test session. Latency (x-axis) is represented in milliseconds (ms) and amplitude (y-axis) in microvolts (µV). Negative is plotted upwards. Baseline is the first 200 ms op the epoch i.e. 200 ms before S1 onset. The 0 ms point is S2 onset. While the blue bar indicates the early CNV, the pink bar highlights the late CNV. Again, dotted arrows represent the onset of the corresponding image in (A).

These off-line analyses were performed using BrainVision Analyzer 2 (Brain Products, Munich, Germany). After additional filtering (0.01-30 Hz band-pass filter, Notch filter 50 Hz), eye artefacts were removed by Independent Component Analysis (Mennes et al., 2010). Two components (eye blinks; left-right eye movements) were excluded based on inspection of the components' spatial distribution. It is recommended to analyse language related brain activities not only time-locked to stimulus onset, but also time-locked to response onset. Activities linked to response execution emerge time-locked to the response and might consequently be reduced in analyses time-locked to stimulus presentation (Riès et al., 2013). Therefore, data were analysed with respect to S2 and lip movement onset, from here on referred to as stimulus and response locked respectively. For the stimulus locked analyses, the continuous EEG data were segmented into epochs of 4200 ms, starting 2200 ms prior to S2, and baseline corrected to the first 200 ms of the epochs (Luck, 2005), which is the 200 ms time window before S1 onset. For the response locked analyses, lip movement onset was visually determined based on the EMG data. Therefore, the EMG data were separately band-pass filtered from 15 Hz to 100 Hz to reduce the contamination by motion artefacts and non-myogenic potentials (Van Boxtel, 2001). The continuous EEG data were also segmented into epochs of 4200 ms, starting 2300 ms prior to lip EMG onset, and baseline corrected to the first 200 ms of the epochs (Luck, 2005). The starting point of the segmentation was somewhat different than in the stimulus locked analyses due to a reaction time delay. Reaction time was determined as the time between S2 and lip EMG onset (see figure 3B). For the 4 test sessions respectively, 55 %, 65 %, 40 % and 40 % of the responses had an EMG onset occurring after S2. If the segmented epochs started 2200 ms before lip movement onset and were baseline corrected to the first 200 ms, on average 50 % of the responses would have a baseline that contained a part of the visual evoked potentials elicited by S1. This would have added a serious amount of noise to the data since baselines should be as neutral as possible and are not allowed to contain any kind of potentials. Therefore, the starting point of the segmentation was put 100 ms earlier and thus started 2300 ms prior to lip movement onset. For all test sessions, more than 90 % of all responses had a reaction time ≤ 100 ms. All trials containing artefacts were manually excluded (Cui et al., 2000; Bares et al., 2007; Mock et al., 2011). By averaging over corresponding epochs, the CNV potential could be computed for each test moment. For the 4 test sessions, the average was based on 92, 96, 91, 99 and 89, 95, 91, 102 trials for the stimulus locked and response locked analyses respectively.

For both stimulus and response locked analysis, mean amplitude was calculated from -500 – 0 ms since this time window contained the maximal variation of the CNV potential. This was done for all frontal (F7, F3, Fz, F4, F8), central (C3, Cz, C4) and 2 temporal (T3, T4) electrodes. The latter electrodes were situated above the lesion site and its contralateral homologue. The other electrodes were located near regions important for speech preparation and execution.

2.2.4. Phonological assessment

As mentioned above, a detailed analysis of phonological processing skills was recommended due to the localisation of the lesion. As behavioural tests may not be sensitive enough to detect very mild language problems, an additional neurophysiological examination was performed at the first three test sessions. Tests for this additional examination were selected from Aerts et al., (2013) in which a detailed description of the tasks and their analyses can be found. All tasks were auditory oddball paradigms in which the proportion of standard vs deviant stimuli was 4/1. Phoneme discrimination was evaluated in an attended (P300) and unattended (Mismatch Negativity - MMN) condition. Both contained [bə] as standard and [gə] as deviant phonemes. An unattended MMN paradigm was used as word recognition task in which real words were presented as standard and pseudowords as deviant stimuli. During the P300, MH had to press a button when hearing the deviant stimulus. During the MMN tasks, MH was instructed to ignore the stimuli and focus on a silent movie. All stimuli were presented binaurally with Apple Inc. earphones, placed directly into the external ear, at a comfortable listening level of ca. 70 dB.

Peak latency and amplitude were measured at Fz/Cz for the MMN wave and at Pz for the P300 wave in the unattended and attended phoneme discrimination task respectively. In the word recognition task, both real and pseudowords evoked the successive peaks N100, P200 and N400. Peak latency and amplitude were calculated at F3/Fz/F4 for P200 and at Cz for N100 and N400. These values were compared to the norms obtained in Aerts et al. (2013). Although scores that fall within 2 SD from the mean are usually considered normal, scores falling between 1.5 and 2 SD are already borderline (Lezak et al., 2004). Therefore, only latency and amplitude values falling within 1.5 SD from the averages obtained in Aerts et al., (2013) were considered normal.

3. Results

3.1. Speech samples

In table I, an overview of all stuttering related scores is given. According to the SSI-4 (Riley, 2008), no stuttering could be diagnosed pre-surgery and a very mild stuttering severity occurred post-surgery. Although all post-surgery test sessions showed only minor differences in total score, a large variation in %SS during conversation occurred. Based on these scores, MH stuttered moderately during the 3rd and mildly during the other test sessions (Onslow, 2000). Conversely, no such variation was observed in non-propositional speech. MH rarely stuttered during reading. Moreover, no stutters were noted during automatic speech and during repetition of words and sentences at the 3rd testing.

Table I: Results of the SSI-4 (Riley, 2008) obtained after consensus. The corresponding ICC's and the mean severity rating score obtained from 3 clinicians.

	Stuttering Severity Instrument (SSI-4)									**Severity Rating**	
	Reading		Conversation		Duration		Physical	Total	Severity	Reading	Conversation
	%SS	Score	%SS	Score	Average	Score	concomitants	score	label		
Pre-surgery	0	0	.8	2	0.6	4	1	7	Not stuttering	1.0	1.7
1 month post-surgery	.5	2	1.4	2	1.2	6	2	12	Very mild	2.0	2.3
3 months post-surgery	0	0	7.3	6	1.2	6	4	16	Very mild	1.0	5.0
4 months post-surgery	0	0	2.5	3	0.6	4	6	13	Very mild	1.0	2.5
ICC	80		98		82		88	90			

The severity rating by the three judges mirrored the results of %SS for both reading and conversation. Only at the 2nd test session, extremely mild stuttering was perceived during reading. For conversation, test sessions 1, 2, and 4 gave comparable results, while test session 3 obtained a much higher score.

MH exclusively stuttered at word initial phonemes. No stutters involving entire syllables or longer linguistic units were noted. These stutters were mainly blocks and prolongations. Additionally, a slight increase in amount of physical concomitants seemed to appear over time. MH sometimes nodded her head and frowned. At the 4th evaluation, a glottal fry could be heard occasionally. All these behaviours were transient and mostly scored as 'barely noticeable for a casual observer'.

Concerning avoidance and escape related secondary behaviours, MH was observed to look for synonyms and to break off sentences when a stutter appeared or was anticipated. Remarkably, she was scarcely aware of this behaviour herself. When asked whether she applied some tricks to avoid/escape a stutter, she mentioned not to do so. MH also underestimated her stuttering severity. Both at the 2nd and 4th testing, she said that the stuttering was almost gone, while both speech therapists could clearly distinguish several stutters during the conversation. Overall, MH was concerned about her stuttering, especially in the beginning, when the stuttering appeared, and at the 3rd testing, when the sudden increase had occurred.

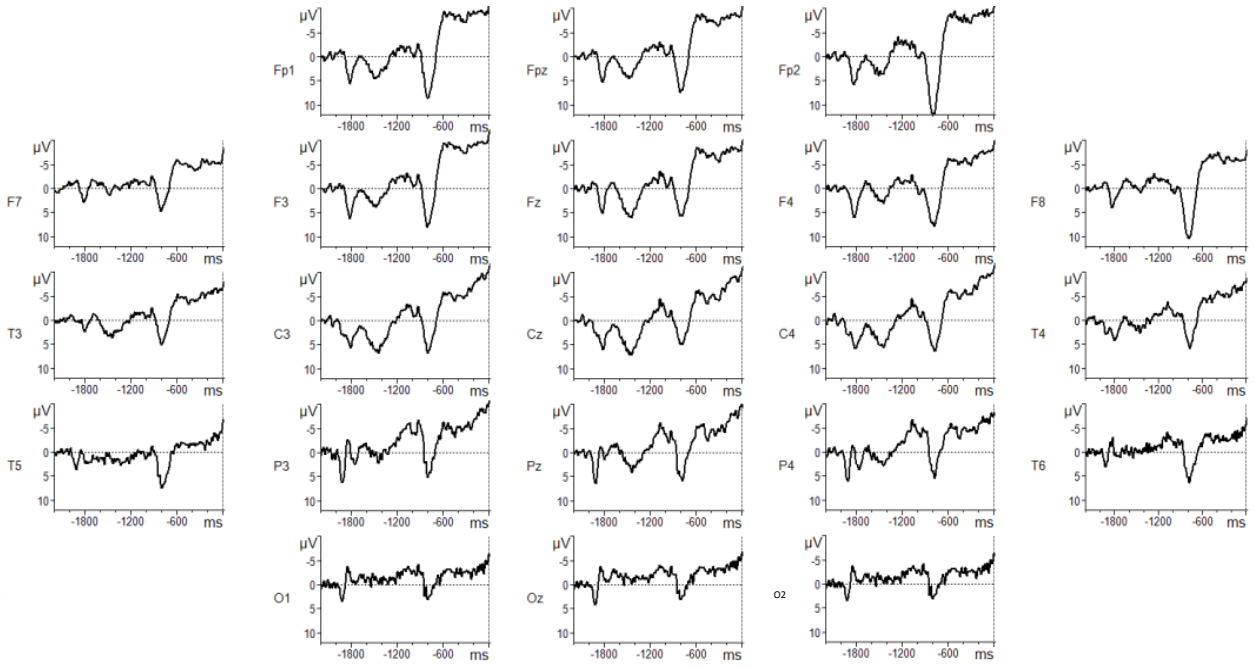

Figure 4: Stimulus locked analysis of all electrodes at the first test session. Latency (x-axis) is represented in milliseconds (ms) and amplitude (y-axis) in microvolts (μV). Negative is plotted upwards. Baseline is the first 200 ms op the epoch i.e. 200 ms before S1 onset. The 0 ms point is S2 onset.

3.2. CNV paradigm

A typical CNV wave was evoked, as can be seen in figure 3C and 4. After visual and linguistic processing of the pictures (S1), a clear increase in negativity occurred between 700 and 1000 ms following S1. This early CNV (blue bar in figure 3C) was seen over (pre)frontal, central and parietal electrodes. At 1000 ms, the early CNV was interrupted by a new phase of visual processing because at this point in time the picture disappeared from the screen. Shortly hereafter, a steep increase in negativity could be observed, peaking around the presentation of S2. This negativity is the late CNV (pink bar in figure 3C) and has a wide scalp distribution. The largest CNV was elicited in the pre-surgery test session (figure 5, 6 and 7).

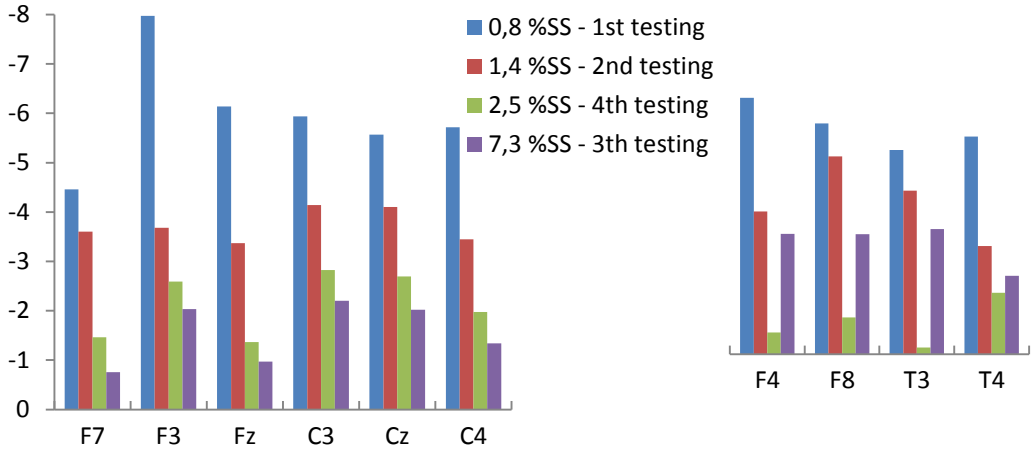

Figure 5: Mean CNV amplitude (µV) of the response locked analysis at all test sessions. Bars are coloured corresponding to % SS during conversation.

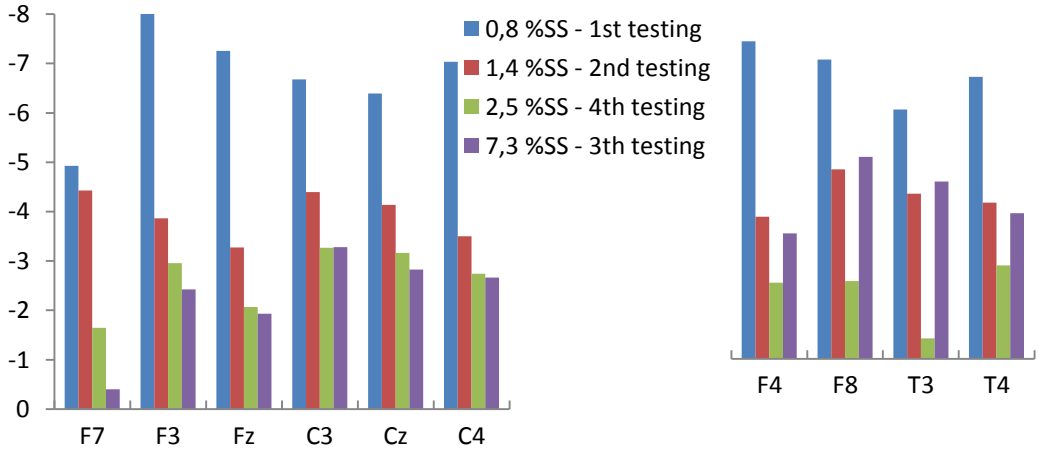

Figure 6: Mean CNV amplitude (µV) of the stimulus locked analysis at all test sessions. Bars are coloured corresponding to % SS during conversation.

Two different activity patterns could be discerned which were most clearly seen in the EMG averaged data. Over bilateral and midline central (C3, Cz, C4) and left and midline frontal (F7, F3, Fz) electrodes, CNV amplitude was inversely proportional to stuttering severity and frequency during conversation. The more MH stuttered, the lower the CNV amplitude became. The remaining 4

electrodes (T3, F4, F8, T4) showed a different pattern that was particularly observed in the response locked analyses. While the CNV amplitude at the 1st, 2nd and 4th test session did show the inverse association with stuttering symptoms, the CNV amplitude measured at the 3rd testing did not. Although MH stuttered more at the 3rd than at the 4th testing, the CNV was much higher at the 3rd test session. Note that at the 1st, 2nd and 4th testing, 3 out of these 4 electrodes were amongst the lowest amplitudes of all electrodes. However at the 3rd testing, T3, F4 and F8 had the highest amplitude of all (see figure 5). Thus, the CNV at these electrodes showed a *relative increase* compared to the CNV over central and left frontal areas.

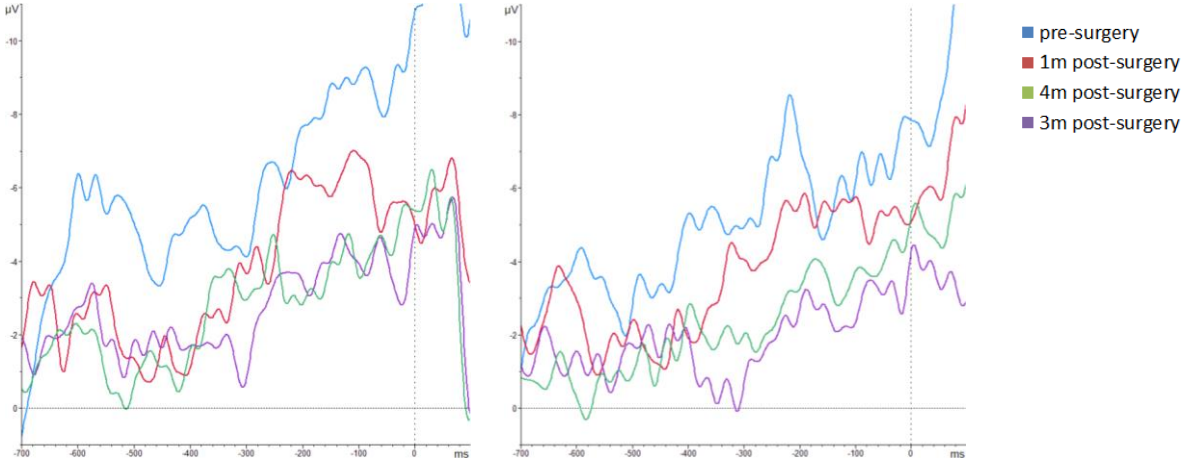

Figure 7: The late CNV at Cz for all test sessions, the 700 ms preceding the averaging point are shown. On the left, the stimulus locked analysis is displayed in which 0 ms represents S2 onset. On the right, response locked analysis is depicted in which 0 ms represents EMG onset. Negative is plotted upwards.

3.3. Phonological evaluation

All neurophysiological tests evoked clear event-related potentials in which all peaks could be distinguished (see figure 8). Only minor deviations were seen in latency measures (table II). These deviations solely encompassed faster latencies than average. All amplitude measures were within normal limits (table III).

Table II: Latency (ms) values of the neurophysiological assessment of auditory phonological processing.

	Phoneme discrimination		**Auditory word recognition**					
			Words			Pseudowords		
	MMN	P300	N100	P200	N400	N100	P200	N400
Pre-surgery	***115***	426	98	205	406	98	159	530
1 month post-surgery	***120***	372	***68***	168	416	72	153	***392***
3 months post-surgery	140	378	***70***	181	544	90	155	464
Norms: M (SD)	171 (28.17)	409 (37.71)	92 (8.41)	182 (18.74)	494 (60.02)	94 (16.39)	170 (19.40)	507 (54.51)

Latencies not falling within M ± 1.5 SD are italicized and displayed in bold. Norm scores are obtained from Aerts et al., (2013).

Table III: Amplitude (µV) values of the neurophysiological assessment of auditory phonological processing.

	Phoneme discrimination		**Auditory word recognition**					
			Words			Pseudowords		
	MMN	P300	N100	P200	N400	N100	P200	N400
Pre-surgery	-1.37	21.41	-2.15	3.17	-2.05	-1.70	4.67	-3.18
1 month post-surgery	-5.32	16.56	-2.55	3.27	-2.14	-2.06	5.15	-5.33
3 months post-surgery	-1.86	20.67	-1.55	1.44	-2.47	0.55	4.06	-4.40
Norms: M (SD)	-4.48 (2.12)	13.03 (5.65)	-2.03 (1.12)	1.67 (1.34)	-3.26 (1.26)	-3.16 (2.35)	3.52 (3.26)	-4.37 (2.83)

All amplitudes fall within M ± 1.5 SD. Norm scores are obtained from Aerts et al., (2013).

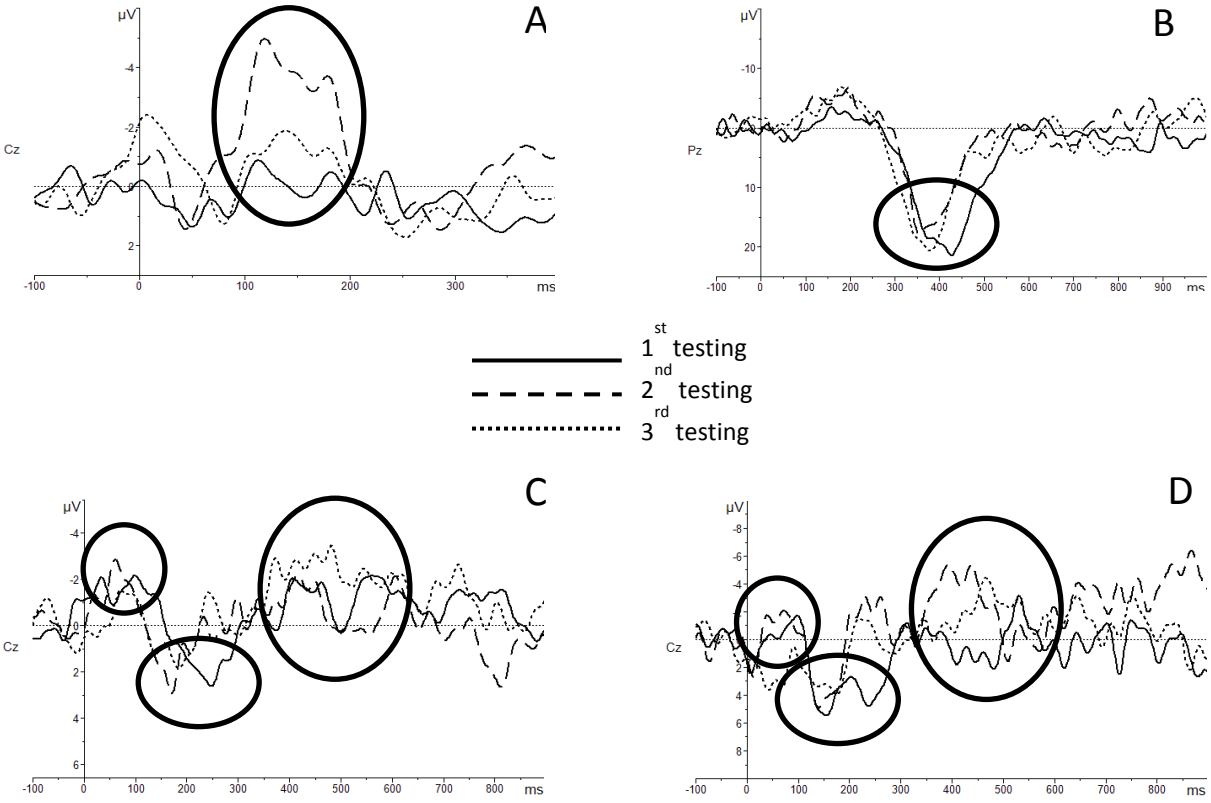

Figure 8: Event-related potentials (ERP) evoked by the phonological tasks. Latency (x-axis) is represented in milliseconds (ms) and amplitude (y-axis) in microvolts (µV). Negative is plotted upwards. Top: the unattended and attended phoneme discrimination tasks: (A) MMN at Cz (B) P300 at Pz. Bottom: the word recognition tasks evoked the successive peaks N100, P200 and N400: (C) real words at Cz (D) pseudowords at Cz.

4. Discussion

In the present case report, motor preparation was evaluated by a CNV task at several points in time with differences in stuttering severity and frequency. A typical CNV wave was evoked by a picture naming task. Besides an early CNV at 700 – 1000 ms following S1, S2 was preceded by a second and larger negativity over (pre)frontal, central, parietal and temporal areas. The latter wave is the late CNV related to motor preparation.

4.1. CNV amplitude related to motor preparation

Surprisingly, a reversed association appeared between late CNV amplitude and stuttering frequency during conversation. The higher the stuttering frequency, the smaller the CNV amplitude. This observation was mainly seen in the response locked analysis which highlights the importance of taking reaction time into account in stuttering (Smits-Bandstra & Gracco, 2013). As outlined in the method section, activities linked to response execution might be reduced in the stimulus locked analysis (Riès et al., 2013). The reduction in CNV amplitude was observed over bilateral and midline central, and over left and midline frontal electrodes. These electrode sites approximate bilateral (pre)motor, somatosensory areas and left IFG, which are known to be responsible for motor preparation and execution (Price, 2012). The present study suggests that articulatory preparation has an important role in stuttering. The amount of reduction in motor programming activation seems to be related to the amount of stutters that will occur during conversation.

This observation is opposite to the hypothesis put forward in the introduction. Neuroimaging research mostly described positive correlations between stuttering severity/frequency and several (sub)cortical brain structures that are part of the BGTC – loop (Braun et al., 1997; Fox et al., 2000; Giraud et al., 2008; Chang et al., 2009; Kell et al., 2009; Ingham et al., 2012). As the amount of activity in this loop is known to be positively associated with the CNV amplitude (Fan et al., 2007), an increased amplitude was expected. Indeed, some older CNV reports in stuttering found an enlarged CNV amplitude (Prescott & Andrews, 1984; Prescott, 1988). However, all these studies concern developmental stuttering. Although neurogenic and developmental stuttering are suggested to share common neural substrates (Theys et al., 2012), its translation in CNV amplitude seems to be different. The neural network involved in fluent (and stuttered) speech is suggested to be differently interrupted causing the opposite observation in MH.

According to the DIVA (Directions into Velocities of Articulators) speech model, two systems are necessary for fluent speech: a feedforward and a feedback system. Motor preparation is provided by the so called Speech Sound Map situated in the caudoventral portion of the precentral gyrus. The initiation and sequencing of different speech motor programs would depend on activity in the basal ganglia and the thalamus. Both are part of the feedforward system (Guenther, 2006). As the CNV is related to motor programming and the BGTC – loop, this potential would reflect activity in the feedforward loop. Left STG on the other hand, MH's lesion site, is situated in the feedback system. In this system, the expected and the actual sensory speech output are compared and corrected if necessary (Guenther, 2006; Golfinopoulus et al., 2010). Thus, MH's lesion site is primarily affecting another subsystem than the one that is measured by the CNV.

Moreover, developmental stuttering is suggested to have its primary lesion in the proximity of the left IFG (Sommer et al., 2002; Chang et al., 2008, 2011; Watkins et al., 2008; Kell et al., 2009;

Cykowski et al., 2010) which is located in the feedforward system. This lesion will affect auditory-motor integration and provide aberrant input to the basal ganglia (Giraud et al., 2008). In the presented case, auditory-motor integration will probably be disturbed as well due to the lesion in the feedback system. However, since MH's lesion is located in a different region, and in a different subsystem of the DIVA model, than the hypothesized lesion in developmental stuttering, its impact on auditory-motor integration, on left IFG activation and consequently on the BGTC – loop, might be different as well. Hence, the direction of the CNV amplitude alteration might be reversed.

Since the present study concerns a case description, no correlation analysis could be performed. Future group studies may clarify whether a correlation between CNV amplitude and stuttering severity/frequency exists. Moreover, these group studies are recommended to involve developmental stuttering to see whether the hypothesized positive correlation based on the literature and the alternative explanation for the reversed association found in MH hold true. But taken together, late CNV amplitude elicited by a relatively simple speech task is hypothesized to provide an objective, neural correlate of stuttering frequency.

Note that MH did not stutter during the CNV task. Isolated word production usually evokes no or very little stuttering (Brown, 1938; Adams et al., 1973) probably because it requires relatively little effort by the neural speech motor system (Bloodstein & Ratner, 2008). So, even without stuttered speech during task performance, a substantial motor programming dysfunction may be present. This observation suggests that when a limited load is put on the speech motor system, motor programming disturbances are either not enough to evoke stuttering or are surmountable by compensation strategies.

In the response-locked analysis, the reversed activity pattern was not observed over F4, F8, T3 and T4. Late CNV was larger at the 3rd compared to the 4th test session though %SS was larger at the 3rd session. At the 1st, 2nd and 4th testing, 3 out of these 4 electrodes were amongst the lowest amplitudes of all electrodes. Conversely, 3 out of these 4 (T3, F4 and F8) had the highest amplitude of all at the 3rd evaluation. Thus, at the 3rd evaluation, the CNV over these electrodes showed a relative increase compared to the CNV over central and left frontal sites. It is tempting to suggest that this relative increase at the right-sided electrodes is related to compensation strategies and at T3 to the cause of the stuttering worsening since T3 is closely located to left STG, MH's lesion site. This suggestion concurs with a traditional divergence made between left and right hemisphere in developmental stuttering. While left hemisphere observations would reflect the primary deficit (Sommer et al., 2002; Chang et al., 2008; 2011), right-sided activations would result from compensatory processes. Especially right frontal regions are reported in this respect (Braun et al., 1997; Preibisch et al., 2003). RFO is the most frequently reported brain region to show anomalous right activation in AWS (for a meta-analysis, see Brown et al., 2005). Its overactivation is suggested to

compensate for a deficient signal transmission in left hemisphere areas for motor preparation and execution (Sommer et al., 2002; Watkins et al., 2008; Chang et al., 2011). As F4, F8 and T3 are closely located to RFO and to the contralateral homologue of MH's lesion site, these electrodes might have registered this overactivation. However, to substantiate this hypothesis, source reconstruction techniques should be applied on the data. Unfortunately, source localization would not provide reliable results because the present manuscript concerns a case report in whom data is collected with 21 electrodes. In sum, right sided increases in CNV amplitude observed at the 3rd evaluation are cautiously suggested to reflect an attempt to deal with the increase in stuttering frequency.

4.2. Stuttering frequency, not severity

CNV amplitude was inversely proportional to both %SS during conversation and overall stuttering severity as measured by the SSI-4 (Riley, 2008). However, the latter measure only showed small differences between the post-surgery evaluations. Such small differences are very unlikely to have caused such obvious changes in neural activity. Therefore, the pattern in CNV amplitude observed over central and left frontal sites is assumed to be related to stuttering frequency during conversation rather than to overall stuttering severity. Similarly, previous studies documenting associations between neural findings and stutter related variables, mostly found this correlation with a measure related to stuttering frequency (Braun et al., 1997; Fox et al., 2000; Giraud et al., 2008; Chang et al., 2009; Kell et al., 2009; Ingham et al., 2012). The severity measure differs from stuttering frequency in that it also includes 'physical concomitants'. These secondary behaviours do not belong to the primary speech characteristics of stuttering. They are, at least partly, not the result of neural disturbances, but rather of the coping behaviour by the speaker to his/her stuttering.

Also the severity ratings provided by the judges, mirror the %SS scores. This is in line with previous research reporting high correlations between %SS and clinician severity rating (O'Brian et al., 2004; Karimi et al., 2013).

4.3. Other influencing factors

Although influences from attention deficits cannot be excluded, the present data provide several arguments for stuttering frequency to be the main contributor to CNV changes. First, the reversed association pattern is observed over brain regions that are well-known to be primarily involved in motor functions (Price, 2012). Secondly, the relative increase during the 3rd testing at right frontal areas is a typical compensation strategy for motor difficulties in stuttering (Preibisch et al., 2003). Also a retest-effect is very unlikely to have occurred since CNV amplitude measures are shown to be reliable and stable (Kropp et al., 2000). Moreover, if there had been a retest-effect, all electrodes should have shown a similar, decreasing pattern over consecutive test sessions. Finally, although

MH's neurological condition was not entirely stable over time (after the pre-surgery test session, MH experienced one more stroke and the surgery in itself), no variance in neurological condition between all three post-surgery sessions was present. Therefore, its influence on the present results will be limited to even absent.

As the left STG is a key area for auditory and phonological processing (Salmelin, 2007; Vigneau et al., 2006), problems in these domains may arise. However, MH was flawless at behavioural auditory language testing. Also neurophysiological examination revealed no particular auditory or language deficits. The only observed deviations concerned faster latencies than average which can but be seen as an alteration, not as a dysfunction. Even more so because the amplitudes of all peaks were within normal limits at all test sessions. Early sensory-perceptual processes and intermediate stages of auditory feature analyses are reflected by N100 and P200 (Cooper et al., 2006; Näätänen et al., 2011). The latter peak also reflects some phonological processing (Zhang et al., 2009). In addition, phonological processing was specifically evaluated by the MMN and P300 task which required phoneme discrimination (Aerts et al., 2013). As all these peaks were within normal limits, MH can be concluded to have normal auditory and phonological processing skills. Finally, following the N400 results, also lexical processing (pseudo word processing) and semantic integration (real word processing) seems to be unaffected (Kutas & Federmeier, 2000; Giaquinto et al., 2007; Hauk et al., 2012). In conclusion, both behavioural and neurophysiological evaluation revealed no remarkable deficits in auditory, phonological and lexico-semantic processing that might have had a modifying role in CNV amplitude variation.

4.4. Acquired stuttering

Stuttering following brain damage may not always be 'neurogenic' stuttering. Psychogenic stuttering has been described as well and the differential diagnosis may be complex. One of the reasons is that literature on their stuttering pattern is characterized by conflicting observations. Even attempts to find correspondences between patients with a similar neurogenic aetiology resulted in contradictory results (Theys et al., 2008; De Nil et al., 2009). The present case report is no exception to this. MH stutters were mainly blocks and prolongations which contradicts the general finding that repetitions are the predominant speech characteristic in most neurogenic and psychogenic stuttering patients (Theys et al., 2008; Van Borsel, 2011). For the following reasons however, MH was concluded to suffer neurogenic and not psychogenic stuttering. Psychogenic stuttering patients (1) typically stutter during all speech modalities, (2) are generally found to be indifferent to their stuttering, and (3) often had earlier psychosomatic disorders (Van Borsel, 2011). In contrast, MH only stutters during conversation, is clearly concerned about her stuttering and had no previous psychogenic related complaints. Moreover, stuttering onset is clearly linked with a neurological event. Although

stuttering occurred a few days after a stroke with no observed increase in bleeding, neurologists suggested the increase in oedema would be the cause of the somewhat delayed appearance. Finally, MH's lesion site, left STG, is well known to have a crucial role in fluent and stuttered speech (Brown et al., 2005; Guenther, 2006).

As mentioned before, no uniformity can be found among neurogenic stuttering patients. This accounts for MH as well. Neurogenic AWS would be more likely to stutter during non-propositional speech than developmental AWS (Helm-Estabrooks et al., 1986; Helm-Estabrooks, 1993). However, MH rarely stuttered during reading, automatic speech and repetition. Her %SS during conversation on the other hand, increased to a moderate level. This opposite pattern has been described in some cases with neurogenic stuttering following stroke, traumatic brain injury and brain surgery (Theys et al., 2008). It is even a recurrent finding after thalamic stroke (Abe et al., 1993; Van Borsel et al., 2003b).

Overall, MH's stutters occurred exclusively in word initial position, which is a typical finding in both developmental and neurogenic stuttering (Bloodstein & Ratner, 2008; De Nil et al., 2009). MH' stuttering solely involved sounds, not syllables or longer units. MH displayed only limited secondary behaviours that were less elaborate and more transient than typically seen in developmental stuttering. Indeed, secondary behaviour is suggested to occur less frequently or to be absent in neurogenic AWS because they stutter mostly for a relatively short period of time. Full blown secondary behaviour may, as is the case in developmental stuttering, appear after stuttering for a significant period of time (De Nil et al., 2009). Note that in line with this suggestion, a slight increasing trend in physical concomitants was observed in MH, despite a decrease in stuttering frequency at the 4th session.

One last form of acquired stuttering is pharmacogenic stuttering. Several medicines have been described to influence fluency/stuttering (for a review, see Brady, 1998; and Boyd et al., 2011). At all test sessions, MH only took levetiracetam, an antiepileptic medicine. Several antiepileptic drugs have been found to affect fluency (Sechi et al., 1997; Brady, 1998; Mula et al., 2003). However, levetiracetam is consistently described to reduce stuttering (Canevini et al., 2002; Sechi et al., 2006). Therefore, MH's stuttering is very unlikely to have a pharmacogenic origin.

5. Conclusion

For the case described, CNV amplitude is shown to be inversely related to stuttering frequency during conversation. The larger the stuttering frequency, the smaller the CNV amplitude. Thus, the amount of disturbance in articulatory preparation seems to be related to the amount of stutters that will occur during conversation. During task performance, no stuttering appeared. This observation suggests that when only a limited load is put on the speech motor system, motor programming

disturbances are either not enough to evoke stuttering or are surmountable by compensation strategies. At the test session with most severe stuttering, such a cortical compensatory mechanisms was cautiously suggested to be triggered at right frontal electrodes.

Overall, motor preparation is suggested to have an important role in stuttering. Late CNV amplitude elicited by a relatively simple speech task seems to be able to provide an objective, neural correlate of stuttering frequency.

Chapter 9

Increased motor preparation activity during fluent single word production in developmental stuttering:

a correlate for stuttering frequency and severity

Vanhoutte Sarah, Santens Patrick, Cosyns Marjan, van Mierlo Pieter, Batens Katja

Corthals Paul, De Letter Miet, Van Borsel John

Neuropsychologia (75), 1-10

Abstract

Abnormal speech motor preparation is suggested to be a neural characteristic of stuttering. One of the neurophysiological substrates of motor preparation is the contingent negative variation (CNV). The CNV is an event-related, slow negative potential that occurs between two defined stimuli. Unfortunately, CNV tasks are rarely studied in developmental stuttering (DS). Therefore, the present study aimed to evaluate motor preparation in DS by use of a CNV task. Twenty five adults who stutter (AWS) and 35 fluent speakers (FS) were included. They performed a picture naming task while an electro-encephalogram was recorded. The slope of the CNV was evaluated at frontal, central and parietal electrode sites. In addition, a correlation analysis was performed with stuttering severity and frequency measures.

There was a marked increase in CNV slope in AWS as compared to FS. This increase was observed over the entire scalp with respect to stimulus onset, and only over the right hemisphere with respect to lip movement onset. Moreover, strong positive correlations were found between CNV slope and stuttering frequency and severity. As the CNV is known to reflect the activity in the basal ganglia-thalamo-cortical – network, the present findings confirm an increased activation of this loop during speech motor preparation in stuttering. The more a person stutters, the more neurons of this cortical-subcortical network seem to be activated. Because this increased CNV slope was observed during fluent single word production, it is discussed whether or not this observation refers to a successful compensation strategy.

Keywords

contingent negative variation, stuttering severity, stuttering frequency, compensation, motor preparation, basal ganglia, dopamine

1. Introduction

Stuttering is a speech disorder primarily characterized by the occurrence of speech blocks, prolongations and/or repetitions of sound or syllables. These may be accompanied by accessory (secondary) behaviours, i.e. behaviours used to escape and/or avoid these speech events (American Speech-Language-Hearing Association, 1999). When the disorder begins in early childhood, it is called developmental stuttering (DS) (Bloodstein &Ratner, 2008; Van Borsel, 2014). One of the neural characteristics of DS is abnormal motor preparation. Motor preparation contains all processing stages in which a phonological word is transferred into concrete, context-specific articulatory motor commands. According to some theories, it includes phonological word encoding as well (Peters et al., 2000; Indefrey & Levelt, 2004; Indefrey, 2011).

Apart from theoretical arguments for speech motor planning deficits in stuttering (Venkatagiri, 2004; Peters et al., 2000; Packman et al., 1996), neurological evidence is available. The most important cortical structure for motor preparation is the premotor cortex (PMC) with a distinct role of its ventral part (vPMC) for speech (Golfinopoulus et al., 2010). Adjacent to and partly overlapping with vPMC is the inferior frontal gyrus (IFG) which includes in the left hemisphere, the well-known Broca's area (Brodmann area 44, 45). Several studies on stuttering reported both anatomical and functional disturbances in this region. Uni- and bilateral hypo- and hyperactivations have been described at rest, in silent reading and overt speech production (Fox et al., 1996; De Nil et al., 2000, 2003; Watkins et al., 2008; Xuan et al., 2012). The most recurrent finding is an anomalous right laterality in activity of the frontal operculum, the homologue of Broca's area (for a meta-analysis, see Brown et al., 2005). Three magneto-encephalography studies revealed some interesting findings as well. Walla et al., (2004) observed in adults who stutter (AWS) a decreased preparatory activity in or close to bilateral motor cortex preceding overt word reading. Sowman et al., (2012) showed large differences in inferior frontal areas between fluent and stuttered speech. In this case report, blocks, as compared to fluent utterances, were associated with decreased activation in left and increased activation in right IFG extending into orbitofrontal areas. Finally, Salmelin et al., (2000) found an advanced activation of left motor cortex and a delayed activation of left IFG during overt reading. AWS were suggested to initiate motor programmes before preparing the articulatory code. This timing deficit has been linked with decreased white matter density in tracts connecting Broca's area and left motor cortex (Sommer et al., 2002; Chang et al., 2011).

When considering motor preparation, subcortical influences must be taken into account as well. The GODIVA (Gradient Order Directions Into Velocities of Articulators) model, an extension of the DIVA model (Guenther, 2006) provides an explanation on how speech movements are selected, sequenced and initiated (Bohland et al., 2010). This model highlights the crucial role of the thalamus and basal ganglia in motor preparation. These subcortical structures form a reciprocal loop with vPMC: the

basal ganglia-thalamo-cortical (BGTC) loop. An alteration of activation in this loop has repeatedly been shown in AWS. Moreover, these altered activations seem to correlate positively with stuttering frequency and severity (Braun et al., 1997; Fox et al., 2000; Giraud et al., 2008; Chang et al., 2009; Kell et al., 2009; Ingham et al., 2012).

One of the electrophysiological substrates of motor preparation is the contingent negative variation (CNV).The CNV is an event-related, slow negative potential that occurs between two defined stimuli. The first stimulus is the warning stimulus (S1) which announces the imperative stimulus (S2) which in its turn requires a response (Walter et al., 1964; Rohrbaugh & Gaillard, 1983; McCallum, 1988; Regan, 1989; Golob et al., 2005). This response is typically a motor response, though cognitive tasks have been reported as well (e.g. Cui et al., 2000; Bares et al., 2007). If the interval between the onset of S1 and S2 is larger than 2 seconds, two CNVs can be distinguished within this interstimulus interval. The first one, the initial CNV, is induced by and related to orientation to the warning stimulus. It has its largest amplitude at frontal sites within the first second following S1. The second one, the late CNV, occurs before S2 and has a wide cortical distribution with a centro-posterior maximum (Walter et al., 1964; Loveless & Sanford, 1974; Rohrbaugh & Gaillard, 1983; McCallum, 1988; Regan, 1989). The late CNV is reported to have multiple cortical and subcortical generators: prefrontal, premotor, primary motor, anterior cingulate, somatosensory and parietal regions as well as the basal ganglia and thalamus. Hence, the late CNV is generally accepted to measure the neuronal activity within the BGTC - loop (Lamarche et al., 1995; Hamano et al., 1997; Gomez et al., 2003; Bares et al., 2007; Fan et al., 2007). This late CNV is suggested to represent primarily motor preparation, and, additionally, sensory anticipation for S2 (Bender et al., 2004; Bares et al., 2007).

CNV research usually requires a motor response from the limbs. Only a few studies required speech or a non-speech oral movement (e.g. Michalewski & Weinberg, 1977; Yoshida & Iizuka, 2005; Mock et al., 2011). Stuttering is even less concerned and only older reports can be found, some of which unfortunately have a poor methodology compared to nowadays' standards (Zimmerman & Knott, 1974; Pinsky & McAdam, 1980). Prescott & Andrews (1984), and Prescott (1988) evaluated the influence of the complexity of the speech response on the CNV amplitude in AWS. In the former study, AWS displayed larger CNV amplitudes than fluent speakers (FS) but not significantly so. In the latter study, a significant increase was found, but only for familiar words. As familiar words are highly practiced speech responses and therefore very likely to be completely pre-programmed, the authors concluded that AWS have difficulties establishing efficient motor programs. This concurs with the suggestion of Venkatagiri (2004) that speech motor planning deficits in stuttering may be restricted to familiar syllable motor plans as opposed to new or unfamiliar utterance plans. The above studies mainly focused on the effect of task complexity on motor preparation, and as such the effect of

individual variation as to stuttering severity remained unexplored. Interestingly, Zimmerman & Knott (1974) observed large inter-individual variations among stuttering participants.

Recently, we explored the effect of stuttering frequency/severity in a case of acquired stuttering following stroke in left superior temporal gyrus (STG) and stroke related surgery (Vanhoutte et al., 2014). A speech related CNV task involving picture naming was administered at four points in time with differences in stuttering frequency. Late CNV amplitude appeared to be inversely proportional to stuttering frequency during conversation, i.e. the larger the stuttering frequency, the smaller the CNV amplitude which was opposite to the postulated hypothesis. As on the one hand, mostly positive correlations have been described between stuttering severity/frequency and the activity in the BGTC - loop (Braun et al., 1997; Fox et al., 2000; Giraud et al., 2008; Chang et al., 2009; Kell et al., 2009; Ingham et al., 2012) and on the other hand, the CNV amplitude is known to represent the amount of activity in this loop (Fan et al., 2007), an increased amplitude with increasing stuttering frequency was expected. However, previous studies all concerned DS. We hypothesized that, in this patient, the neural network involved in fluent (and stuttered) speech was disturbed differently compared to DS, causing the opposite observation. In DS, the hypothesized lesion site is suggested to be in the proximity of the left IFG (Sommer et al., 2002; Chang et al., 2008, 2011; Watkins et al., 2008; Kell et al., 2009; Cykowski et al., 2010), while in our case study the lesion was situated in left STG. Both regions will cause aberrant auditory-motor integration and will have an adverse effect on the cortical input of the BGTC – loop (Giraud et al., 2008). However, the effect may be different because the primary lesion site is different.

Therefore, the present study aimed at evaluating the late CNV as an index of motor preparation activity during overt speech production in AWS with DS. Interactions between structures in the BGTC-loop are complex. In general, however, several structures have repeatedly been reported to show increased activations in stuttering (e.g. Ingham et al., 2012). As such, AWS are hypothesized to show an enlarged CNV amplitude. Secondly, the influence of stuttering severity and frequency was explored by a correlation analysis. In case of a correlation, a positive correlation was expected.

2. Method

2.1. Participants

Originally, 35 AWS with DS and 41 FS were included in the study. Some participants had to be excluded because of (A) technical problems with the microphone (n= 2), (B) abundant EEG artefacts due to speech (n= 3), sweating (n= 3), masseter EMG (n= 1) or secondary behaviour (n= 2), and (C) no (pure) DS (n= 2). Although 28 AWS remained with good quality EEG data, 3 more participants were not included in further analyses because they stuttered on most trials of the experimental task. As is

explained further on, only fluently produced utterances were subjected to further analyses in the present study.

Eventually, EEG data of 25 AWS (M/F ratio: 19/6; mean age: 29.9 years; age range: 18 – 57 years) and 35 FS (M/F ratio: 24/11; mean age: 28.9 years; age range: 18 – 58 years) remained in the sample to be analysed. Both groups did not differ significantly for age and educational background (Mann-Whitney test: p= 0.84 and p= 0.61 respectively). 4 AWS and 3 FS were left-handed (Oldfield, 1971). All AWS had already received one or more stuttering treatments of variable duration and intensity. None of the control participants had a history of stuttering nor had stuttering relatives. Both AWS and FS were monolingual native speakers of Dutch, and reported no history of hearing complaints, dyslexia or other speech-language problems, neurological or psychiatric disorders, and presented with normal or corrected-to-normal vision. None of the participants was on psycho-active drugs. All participants gave their written informed consent in accordance with the declaration of Helsinki. The study was approved by the local ethics committee.

2.2. Speech assessment

In each participant, a conversational speech sample (conversation with the investigator about work/school/hobby/family) and a reading speech sample (reading of the Dutch translation of the text 'The north wind and the sun', International Phonetic Association, 1974) was collected. All samples were videotaped (Canon ACV HD , 1920 x 1080 camera) and also audiotaped in the software program for acoustic analysis Praat (Boersma and Weenink, Phonetic Sciences, University of Amsterdam, Amsterdam, The Netherlands) using a Samsung CU01 microphone placed 50 cm in front of the participant.

Speech samples were judged for stuttering severity by means of the Stuttering Severity Instrument, (SSI-4; Riley, 2008) and percent stuttered syllables (%SS) was calculated following the principles of the Stuttering Measurement System (Ingham & Ingham, 2011). Stuttered syllables included part-word (sound/syllable) repetitions, prolongations, blocks, broken words and tense pauses (American Speech-Language-Hearing Association, 1999; Yaruss, 1997). Repetitions of monosyllabic words were considered as stuttered dysfluencies when they were repeated at a high rate (Bezemer et al., 2010; Guitar, 2006), with apparent undue stress, tension or struggle (American Speech-Language-Hearing Association, 1999; Van Zaalen & Winkelman, 2009) or when the number of repetition units was 3 or more (Boey et al., 2009; Gregory, 1993). Stuttering was diagnosed by a certified speech-language pathologist based on % SS (>3%) and/or the presence of significant speech-related struggle behaviour. Stuttering severity varied considerably between participants. Ten AWS presented with very mild, 7 with mild, 3 with moderate, 3 with severe and 2 with very severe stuttering.

All samples were scored off-line. 25% of samples were re-evaluated by a second evaluator (MC) to assess inter-rater reliability. Both evaluators are speech therapists specialized in stuttering. An intraclass correlation coefficient (ICC) was calculated for overall percentile score on SSI-4 (Riley, 2008), %SS for reading and %SS for conversation. ICC's of 96.3; 99.7 and 99.5 % respectively were obtained, indicating excellent agreement.

2.3. Neurophysiological assessment

2.3.1. EEG data acquisition

EEG data were collected with Neuron-Spectrum-5 (4EPM) registration software (Neurosoft, Moscow, Russia). By use of an universal EEG cap (Haube S2), 21 Ag/AgCl electrodes (Fp1, Fpz, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, Oz, O2) were placed on the scalp according to the international 10/20 system. Two more electrodes were placed above the right side of the upper lip and underneath the left side of the lower lip to register the electromyography (EMG) of the orbicularis oris muscle in a bipolar fashion. Additional reference and ground electrodes were placed on the earlobes and forehead respectively. Neurophysiological data were recorded at a sampling rate of 500 Hz (0.01-75Hz band-pass filter). Impedance of each electrode was kept below 5kΩ. The participants were encouraged to avoid orofacial movements and to reduce eye-blinks as much as possible. Each EEG session was also videotaped using a Canon ACV HD (1920 x 1080) camera.

2.3.2. CNV paradigm

The same picture naming task was used as in the previously described case report (Vanhoutte et al., 2014). The pictures were selected from a picture naming norms database, provided by the Department of Experimental Psychology from the Ghent University, Belgium (Severens et al., 2005). For further analysis, speech onset had to be determined. Articulatory movements were shown to precede vocalization during a Bereitschaftspotential paradigm. Depending on the initial phoneme, the lips or the tongue were the first source (McArdle et al., 2009). Lip movements are easier to detect than tongue movements with EMG. Therefore, pictures were chosen that referred to a noun that had a bilabial (/m/, /w/, /b/, /p/) or labiodental (/f/, /v/) initial phoneme.

The picture was presented as S1 and was shown for 1 second. The S2, in the form of a short, black line, appeared 2 seconds after S1 onset (the foreperiod duration was 2 seconds) indicating that the participant should name the picture as quickly as possible. S2, shown for 2 seconds, was followed by a black screen for another 2 seconds. If the participant continued to stutter on a word once this black screen appeared, he/she was instructed to stop speaking in order not to contaminate the next trial with muscular artefacts (for a diagram of the CNV task, see Figure 1A). One hundred and ten black and white line drawings were shown on a white background in the middle of a computer screen that

was placed one meter in front of the participant. Participants were instructed to name the pictures using only one word or to say 'pass' if they did not know the noun. The percentage of correctly identified pictures was determined and evaluated statistically (see further on).

As the large majority of responses were produced fluently, only fluent utterances were subjected to further electrophysiological analyses. Some responses were additionally excluded if (1) the word was produced before S2 was shown, (2) the produced word did not have a labiodental or bilabial initial phoneme, or (3) the participant swallowed or made an inappropriate lip movement within 1500 ms preceding S2, which was judged based on the videotape recordings and visual inspection of the EMG signal.

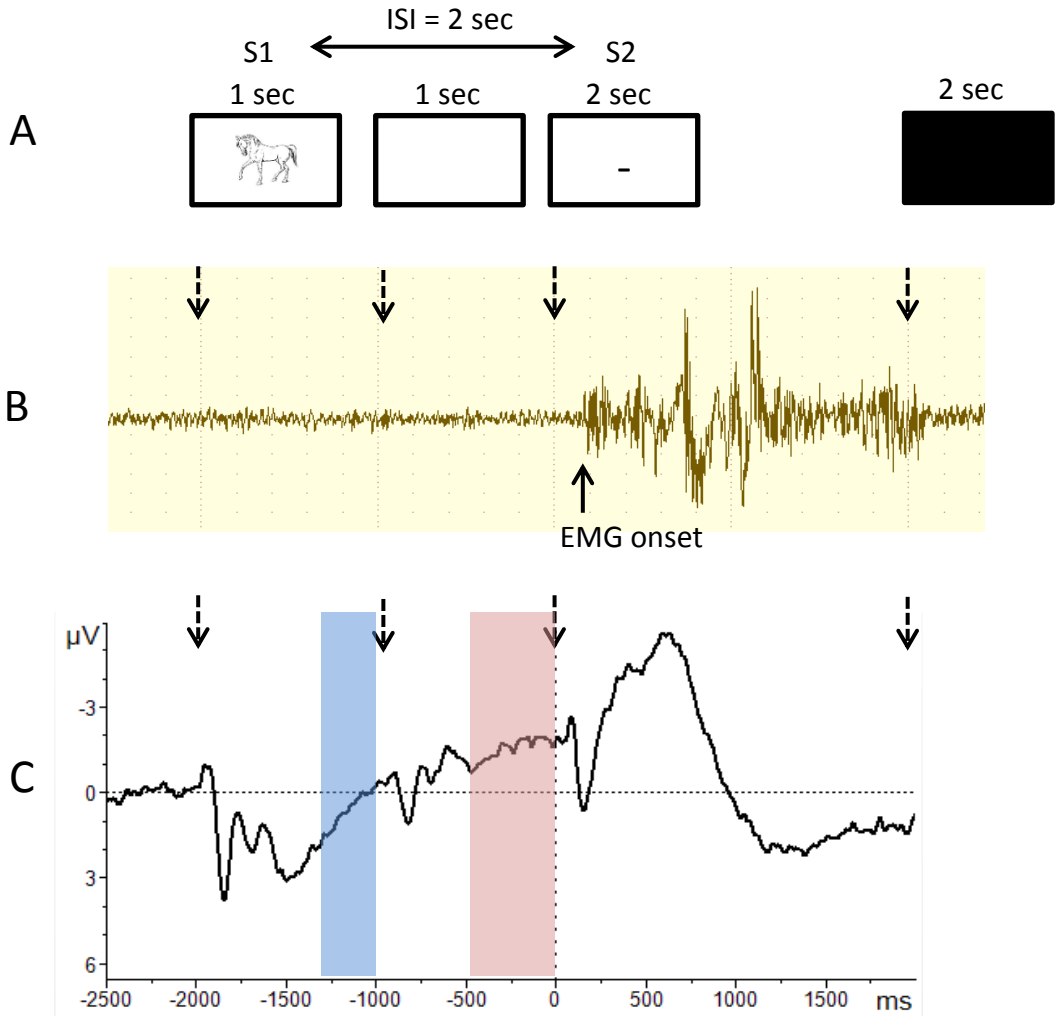

Figure 1: **(A)** Diagram of the picture naming CNV task. The warning stimulus (S1) consists of a picture, the imperative stimulus (S2) of a short, black line that prompts the participant to name the picture as quickly as possible. Interstimulus interval (ISI) is 2 sec. **(B)** EMG signal of the orbicularis oris muscle of one response. The dotted arrows represent the onset of the corresponding image in (A). The full arrow represents the onset of the EMG signal. Reaction time is the time between S2 onset and EMG onset. **(C)** Stimulus locked average at Cz for the FS. Latency (x-axis) is represented in milliseconds (ms) and amplitude (y-axis) in microvolts (µV). Negative is plotted upwards. Baseline is the first 500 ms op the epoch i.e. 500 ms before S1 onset. The 0 ms point is S2 onset. While the blue bar indicates the early CNV, the pink bar highlights the late CNV. Again, dotted arrows represent the onset of the corresponding image in (A).

2.3.3. Off-line EEG analysis

Off-line analysis was performed using BrainVision Analyzer 2 (Brain Products, Munich, Germany). After additional filtering (0.01-30 Hz band-pass filter, Notch filter 50 Hz), eye artefacts were removed by Independent Component Analysis (Mennes et al., 2010). Two components (eye blinks; left-right eye movements) were excluded based on inspection of the components' spatial distribution. It is recommended to analyse speech related brain activities not only time-locked to stimulus onset, but also time-locked to response onset. Activities linked to response execution emerge time-locked to the response and might be slightly reduced in analysis time-locked to stimulus presentation (Riès et al., 2013). This is of particular importance for stuttering as AWS have been described to show slower reaction times (RT) than FS (Smits-Bandstra & Gracco, 2013). Therefore, data were analysed with respect to S2 and lip movement onset, from here on referred to as stimulus and response locked (S- and R-locked), respectively. Lip movement onset was detected by visual inspection of the EMG data of each trial (Van Boxtel et al., 1993; Hasbroucq et al., 1999; Carbonnell et al., 2004). Therefore, the EMG data were separately band-pass filtered from 15 Hz to 100 Hz to reduce the contamination by motion artefacts and non-myogenic potentials (Van Boxtel, 2001). RT was calculated as well. It was determined as the time between S2 and EMG onset for every trial in every participant (Figure 1B).

After segmenting the continuous EEG data into epochs, all trials containing artefacts were manually excluded (Cui et al., 2000; Bares et al., 2007; Mock et al., 2011). By averaging over corresponding epochs, the CNV potential was computed. The average number of trials that were included in the S-locked analysis was 97 for the FS and 77 for the AWS, and for the R-locked analysis: 93 for the FS and 73 for the AWS.

Due to methodological considerations of baseline correction (Luck, 2005), a slope analysis was performed on the CNV which is independent from the baseline (see 2.4. CNV analysis). As such, the baseline correction described in this paragraph is only performed for the construction of the figures (figure 1, 2 and 3) (Carbonnell et al., 2004). The continuous EEG data was segmented into epochs of 4500 ms for the S-locked analysis, starting 2500 ms prior to S2, and into epochs of 4600 ms for the R-locked analysis, starting 2600 ms prior to EMG onset. Baselines were taken from -2500 to -2000 ms for the S-locked and from -2600 to -2100 ms for the R-locked analysis. The baseline correction for the R-locked analysis was different in order to take a RT delay into account. Average RT was 32.8 ms for AWS and 60.9 ms for FS (see 3.1. Behavioural data). If the segmented epochs had been baseline corrected from -2500 to -2000 ms prior to EMG onset, a considerable number of trials would have a baseline that contained a part of the visual evoked potentials elicited by S1. Therefore, baseline correction was performed from -2600 to -2100 ms.

2.4. CNV analysis

The late CNV can be measured by calculating its mean amplitude in the time window that contains its maximal variation. The time window of interest (TOI) for the present study was the 500 ms window preceding S2 and EMG onset. However, after visual inspection of the grand average event-related potential (ERP), mean amplitude appeared rather inappropriate for this data. Instead, a slope measure was preferred. Slope analysis has several advantages over classic mean amplitude analysis (Carbonell et al., 2004). First, they are independent of the baseline. This was of particular interest for the R-locked analysis. As RT varies largely between and even within participants, a fixed baseline correction would be performed on a different part of the EEG epoch for every trial and participant. Therefore, an ERP analysis that is independent from this varying variable is advisable. Secondly, slope measurements are independent of the amplitude level preceding the TOI. If both groups already differ in their amplitude at -500 ms, it is impossible to interpret differences in the late CNV.

To overcome these difficulties, a slope measure was developed. Although peak-to-peak amplitude may be very useful for this purpose, mean amplitude is generally preferred above peak amplitude (Handy, 2005). Therefore, a mean-to-mean amplitude was used in which the absolute value of the difference between the mean amplitude of the first (-500 to -400 ms) and the last (-100 to 0 ms) 100 ms of the TOI was computed (Luck, 2005). This was done for frontal (F3, Fz, F4), central (C3, Cz, C4) and parietal (P3, Pz, P4) electrodes as the late CNV was most pronounced at these sites.

2.5. Statistical analysis

Statistical analysis was performed in IBM SPSS Statistics 22.0 for both S- and R-locked analysis separately. A linear mixed model approach was applied to take the repeated measures design into account. Region (frontal, central, parietal) and Hemisphere (left, midline, right) were inserted as repeated variable. Both were also inserted as factor, together with Group (FS, AWS) as a third factor. Additionally, a correlation analysis was performed between the CNV slope and the following stuttering measures: stuttering frequency during reading, during conversation and stuttering severity. For the latter variable, the overall percentile score of the SSI-4 was calculated. Two behavioural measures were statistically compared between AWS and FS: (1) the accuracy in picture naming was evaluated by a Mann-Whitney U test, and (2) RT was assessed by a linear mixed model approach with Group (FS, AWS) as factor and Picture as repeated variable. Significance values were set at $\alpha \leq 0.05$ for main and interaction effects. All further pairwise comparisons were Bonferroni corrected.

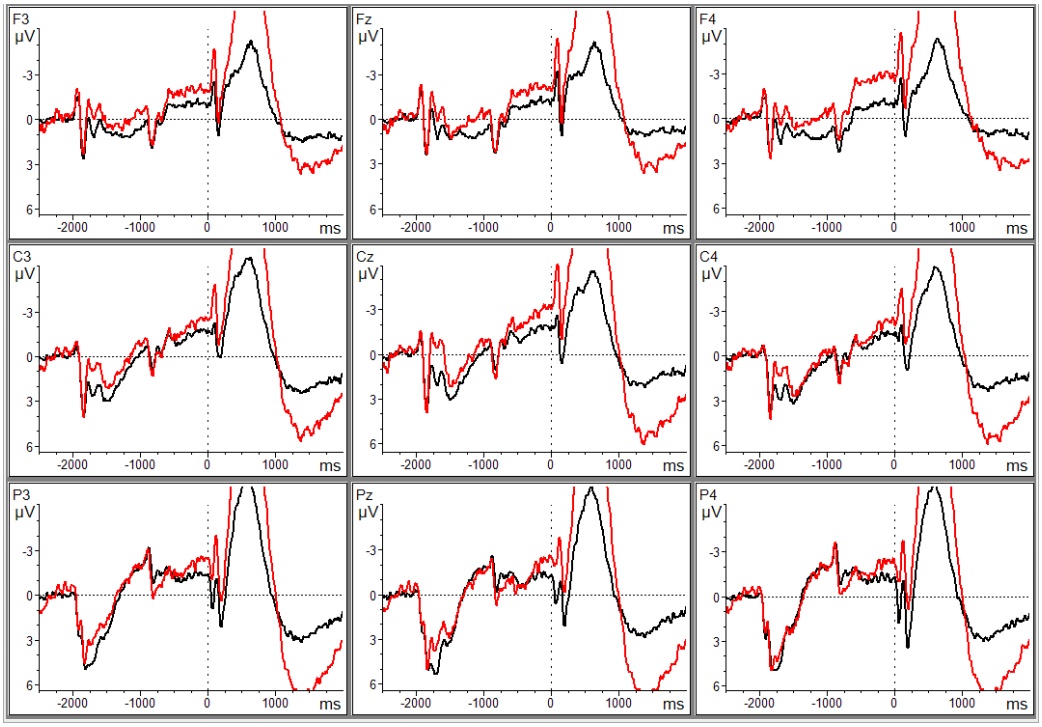

Figure 2: S-locked analysis for all electrodes of interest. Latency (x-axis) is represented in milliseconds (ms) and amplitude (y-axis) in microvolts (µV). Negative is plotted upwards. Baseline correction, which is only performed for the construction of the figures, is the first 500 ms of the epoch. The 0 ms point is S2 onset. The CNV of the AWS is shown in red, the CNV of the FS is presented in black. The EEG signal past 0 ms is not entirely visible because the scale of the y-axis was chosen to enable a clear, detailed display of the early and late CNV.

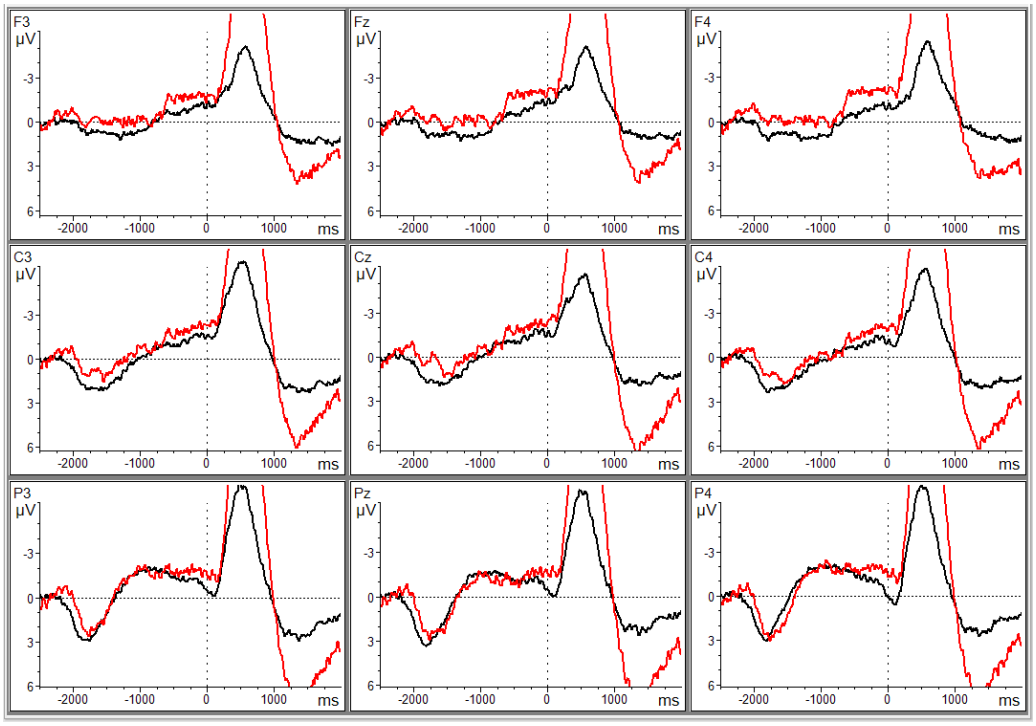

Figure 3: R-locked analysis for all electrodes of interest. Latency (x-axis) is represented in milliseconds (ms) and amplitude (y-axis) in microvolts (µV). Negative is plotted upwards. Baseline correction, which is only performed for the construction of the figures, is the first 500 ms of the epoch. The 0 ms point is EMG onset. The CNV of the AWS is shown in red, the CNV of the FS is presented in black. The EEG signal past 0 ms is not entirely visible because the scale of the y-axis was chosen to enable a clear, detailed display of the early and late CNV.

3. Results

3.1. Behavioural data

No significant difference in RT was observed (F(1,60)= 1.24; p= 0.27). AWS (M= 32.8 ms; SD= 177.7 ms) even tended to show earlier EMG onsets than FS (M= 60.9 ms; SD= 202.4 ms). Picture naming accuracy, however, was significantly lower in AWS (M= 95.0%; SD= 3.3%) than in FS (M= 96.9%; SD= 2.6%) (p= 0.008). A reduction of on average 2% was seen.

3.2. CNV slope analysis

A typical CNV wave was evoked, as can be seen in Figure 1C. After visual and linguistic processing of the pictures (S1), a clear increase in negativity occurred between 700 and 1000 ms following S1 (i.e. -1300 to -1000 ms with respect to S2 onset). This early CNV (blue bar in figure 1C) was seen over (pre)frontal, central and parietal electrodes. At 1000 ms, the early CNV was interrupted by a new phase of visual processing because at this point in time the picture disappeared from the screen. Shortly hereafter, a steep increase in negativity could be observed, peaking around the presentation of S2. This negativity is the late CNV (pink bar in figure 1C) and has a wide scalp distribution. Because the R-locked analysis is time-locked to response execution, activities related to stimulus processing are smeared out. As a result, the peaks related to visual and linguistic processing cannot be clearly seen in this grand average ERP (Figure 3).

Although in the S-locked analysis a significant Group*Hemisphere interaction (F(2, 60)= 5.59; p= 0.006) was seen, AWS were found to show a significantly steeper CNV slope than FS over the entire scalp (Left: p= 0.029; Midline: p= 0.004; Right: p=0.013). The significant Group*Hemisphere interaction (F(2, 60)= 3.23; p= 0.046) for the R-locked analysis did reveal an important difference between hemispheres. AWS had a significantly steeper CNV slope than FS over the right hemisphere (p= 0.050), but not over the left hemisphere (p= 0.350) and midline electrodes (p= 0.165).

For both the S- and R-locked analysis, the CNV slope had a centro-parietal maximum and showed no significant differences between left and right hemisphere (S-lock: F(2, 60)= 29.08; Left vs Right: p= 0.729; R-lock: F(2, 60)= 4.66; Left vs Right: p= 1.000).

3.3. Correlation

As none of the stutter measures were normally distributed (Kolmogorov-Smirnoff: p< 0.001), a Spearman correlation was calculated. A graphical depiction of all correlation analyses can be found in Figure 5. For the S-locked data, a correlation was performed with the CNV slope observed at Cz because here the largest CNV occurred. A significant and positive correlation was found for all variables: %SS during reading (r= 0.58; p= 0.001), during conversation (r= 0.59; p= 0.001), and stuttering severity (r= 0.58; p=0.001). For the R-locked data, only over the right hemisphere a

significant difference between FS and AWS was observed (see above). Therefore, the occurrence of a correlation was explored with the CNV slope at C4. No correlations were found with any variable: %SS during reading (r= 0.15; p= 0.24), during conversation (r=0.16; p= 0.22), stuttering severity (r= 0.12; p= 0.28).

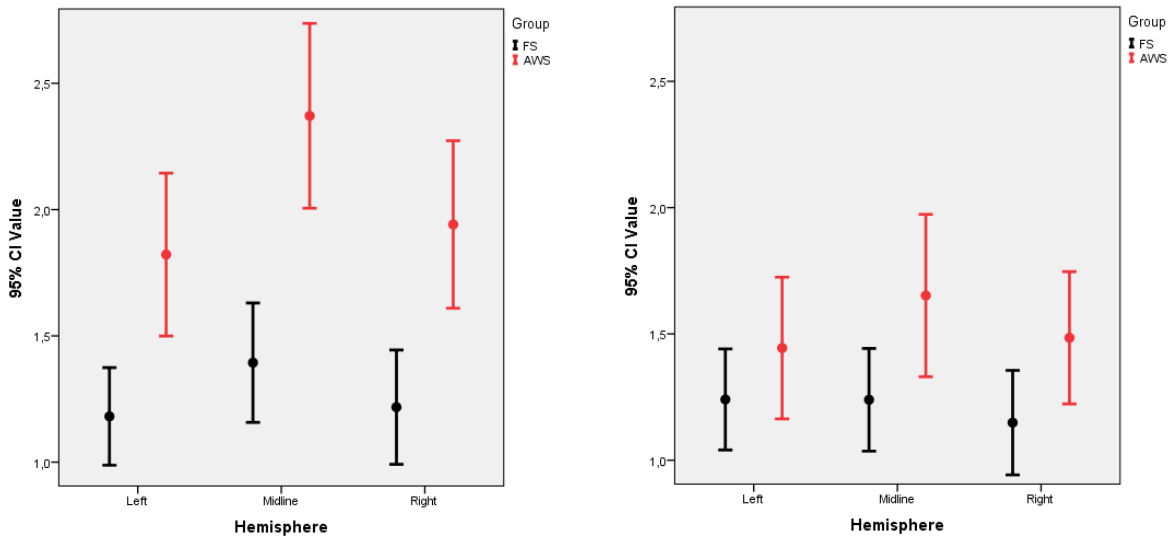

Figure 4: Graph depicting the 95% confidence interval of the CNV slope for all electrodes of interest (y-axis). S-locked analysis is shown on the left, R-locked analysis on the right. Results are split according to hemisphere (x-axis).

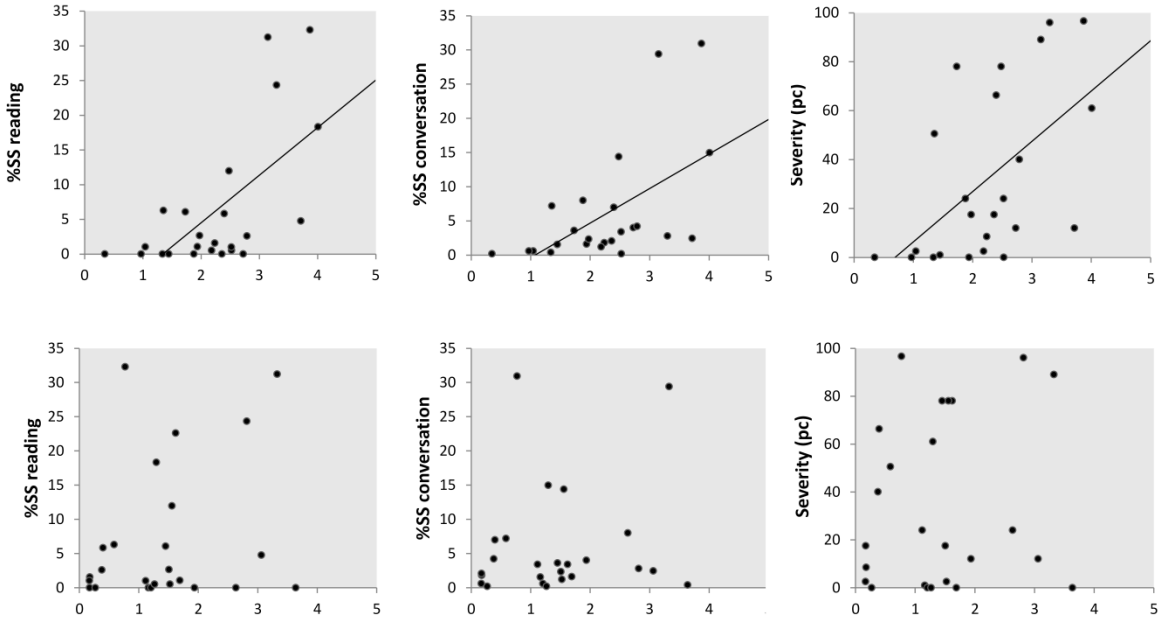

Figure 5: Scatter diagram of the correlation analyses between CNV slope (x-axis) and stuttering measures (y-axis): %SS during reading (A), % SS during conversation (B), and overall severity percentile score (C). The S-locked based correlations (CNV slope at CZ) are shown on top, the R-locked based correlations (CNV slope at C4) are shown below.

4. Discussion

Motor preparation was evaluated in AWS and FS by means of a CNV design during a picture naming task. A typical CNV wave was evoked: while an early CNV followed S1, a second and larger negativity over (pre)frontal, central, parietal and temporal areas preceded S2. The latter wave was largest over centro-parietal regions and is the late CNV related to motor preparation.

4.1. Increased CNV slope

The present findings show that AWS rely on more neural resources than FS during speech motor preparation. The late CNV was more steeply increased in AWS, which confirms previous studies reporting enlarged CNV amplitudes (Prescott, 1988; Prescott & Andrews, 1984). The increase was significant over the entire scalp for the S-locked analysis and only over the right hemisphere for the R-locked analysis. As the CNV reflects the neural activity in the BGTC – loop, the present results show that this cortico-subcortical network has markedly increased motor preparatory activity in DS.

The positive correlation between the CNV slope of the S-locked analysis and stuttering frequency/severity highlights that the more a person stutters, the more this network will be activated during motor preparation. Several neuroimaging studies have reported positive correlations between stuttering measures and neural activity in several structures of the BGTC – loop (Braun et al., 1997; Fox et al., 2000; Giraud et al., 2008; Chang et al., 2009; Kell et al., 2009; Ingham et al., 2012). Thus, the present results confirm that the more a person stutters, the more the BGTC – loop will be activated during motor preparation. As hypothesized, inter-individual variation in stuttering severity/frequency is reflected in the CNV slope.

Several theories on stuttering propose a key role for speech motor preparation (Howell, 2004; Alm, 2004; Giraud et al., 2008; Civier et al., 2013). Additionally, structural and functional alterations have been observed in bilateral cortical and subcortical structures related to motor preparation (e.g. Fox et al., 1996; Watkins et al., 2008; Kell et al., 2009). Also other neurophysiological studies evidenced the importance of motor preparation alterations in stuttering. A reversed activation pattern of left motor preparation and execution areas has been observed during a single word reading task (Salmelin et al., 2000). A recent Transcranial Magnetic Stimulation (TMS) study showed that an increased excitability in left primary motor cortex, prior to the execution of a speech gesture, is absent in AWS (Neef et al., 2015). Although this finding seems to contradict the increased CNV slope observed in the present study, some important methodological differences arise. The TMS study reports on the primary motor cortex during the transition phase, i.e. the moment when a fixed articulatory position moves to a variable subsequent speech movement. The CNV slope, on the contrary, reflects activity of the entire BGTC-loop when a participant's articulators are at rest.

4.2. Cause or compensation?

The present results are based on *fluently* produced words. As could be expected, the majority of the utterances were fluent. Isolated word production usually evokes no or very little stuttering (Brown, 1938; Adams et al., 1973) probably because it requires relatively little effort by the neural speech motor system (Bloodstein & Ratner, 2008). Nonetheless, even during fluent single word production, a substantial motor preparation alteration seems to be present in AWS. The lack of stuttered speech suggests that these motor programming disturbances can be overcome when only a limited load is imposed on the speech motor system. Motor preparation dysfunction alone seems insufficient to evoke stuttering. Future CNV studies may consider longer utterances to examine this hypothesis.

A reverse reasoning is possible as well: the words may have been produced fluently because an increase in brain activation was present. Maybe other neural disturbances in AWS are surmountable by increased motor preparation activation. These two opposite interpretations are related to a typical discussion in stuttering research: which altered neural activations cause stuttering and which are the consequence of lifelong stuttering? Several studies suggest that the left hemisphere would contain the primary anomaly (e.g. Cai et al., 2014b; Sommer et al., 2002), while right-sided alterations would reflect compensation strategies (Preibisch et al., 2003; Brown et al., 2005). The findings of the R-locked analysis are in favour of the compensation hypothesis as the significant increase in CNV slope was only observed over the right hemisphere. To clarify this cause-compensation issue, it might be interesting to have a look at the CNV amplitude preceding stuttered responses. An exploratory study on this matter is underway from our lab (Vanhoutte et al., under review). A recent case report demonstrated that large neural differences in motor preparation areas may present when comparing stuttered and fluent speech. Fluent words, as compared to blocks, evoked larger activation in left inferior frontal areas. An increase in its right counterpart was linked with the production of blocks (Sowman et al., 2012).

4.3. Dopamine

Dopamine, an important neurotransmitter in the BGTC – loop, may provide another explanation for the present results. The late CNV has been found to be modulated by dopamine availability (Kopell et al., 1974; Tecce, 1991; Tecce & Cole, 1974; Tecce et al., 1975) and its amplitude could even be a dopaminergic biomarker. Linsen et al., (2011) evaluated the CNV during a button press task after administration of placebo or 10, 20 or 40 mg of methylphenidate, a catecholamine re-uptake blocker which primarily enhances the synaptic concentration of dopamine. The authors reported an increase in CNV amplitude with increasing synaptic levels of dopamine. This is in accordance with CNV research in Parkinson's disease (PD), a neurodegenerative pathology characterized by dopamine

depletion in the central nervous system. PD patients have a decreased CNV amplitude (Ikeda et al., 1997) which increases after dopaminergic treatment (Amabile et al., 1986).

Dopamine is suggested to affect stuttering as well. The *excess dopamine theory of stuttering* hypothesizes that stuttering is related to or influenced by an increased level of dopamine in the brain. A three-fold increase was reported in left caudate and right medial prefrontal and auditory cortex as well as in some limbic structures (Wu et al., 1997). Moreover, dopamine antagonists have been shown to improve fluency (Lavid et al., 1999; Maguire et al., 2000). In this regard, the present increase in CNV slope may be a reflection of increased levels of dopamine in the brain of AWS.

The excess dopamine theory of stuttering is, however, not unequivocally supported by observations concerning stuttering-like dysfluencies in PD patients. These dysfluencies do not typically occur more frequently while on medication and less frequently while off medication. It rather seems that any change in dopamine level, an increase or a decrease, may affect the frequency of stuttering-like dysfluencies in both directions (Goberman & Blomgren, 2003; Goberman et al., 2010). This is in line with a recent suggestion by Civier et al., (2013) stating that both too low as well as too high levels of dopamine can cause stuttering moments.

The results of the NS case report are very intriguing in this respect. As this patient showed a decrease in CNV amplitude with increasing stuttering frequency, it seems that not only a hyperactivation (as observed in the present DS group) but also a hypoactivation during motor preparation can be associated with stuttering. Again, both too low as well as too high levels of a certain trait seem related to stuttering. Because the exact relationship between dopamine levels and stuttering has not been clarified, no strong hypothesis on the influence of dopamine on the CNV results can be made. Future research will be very interesting to elucidate the role of dopamine in stuttering in general and in aberrant motor preparation as measured by the CNV more specifically.

4.4. Reaction time (RT)

No significant difference was observed between FS and AWS concerning RT, i.e. time between S2 and EMG onset. Interestingly, the EMG onsets even tended to spread into earlier latencies in AWS than in FS. At first sight, this result contradicts the rather consistently found delayed RT in stuttering (for an overview, see Bloodstein and Ratner, 2008). However, an important methodological difference arises: previous research determined speech onset by phonation onset while the present study focused on EMG onset of the orbicularis oris muscle. McFarlane and Prins (1978) also evaluated RT based on the EMG of the orbicularis oris. No significant delay was found when the response cue was a visual cue. Moreover, the present RT results are in perfect agreement with Salmelin et al., (2000), who used a very similar method. Their participants performed a visual task with a CNV design while mouth movement onset was determined by EMG of the orbicularis oris muscle. No significant

difference in RT was found and AWS tended to show earlier latencies in EMG onset than FS. Thus, task design as well as the measure to determine 'speech onset' seem responsible for the lack in RT delay.

The difference in speed of phonation onset and lip movement onset might be a reflection of an important neurological observation. A recent meta-analysis showed that during fluent speech AWS have a reduced activation in left larynx motor area but an increased activation in right lip motor area (Belyk et al. 2014). The decreased larynx activation was suggested to be linked with the slower phonation initiation in stuttering. Maybe the increased lip activation can be associated with the somewhat faster lip movement onset.

4.5. Other influencing factors

Although language interference cannot be excluded, several arguments are in favour of the CNV results being mainly related to motor preparation. Indeed, AWS performed significantly less accurate on picture naming than FS. However, the difference is so small, on average 2%, that one can rightfully wonder if such a small difference could cause such large differences in neural activity. Previous language research using behavioural tasks evoked inconsistent results in stuttering (e.g. Prins et al., 1997; Bosshardt and Fransen, 1996). Recently, there is even a growing body of literature reporting null results (e.g. Prins et al., 1997; Burger and Wijnen, 1999; Melnick et al., 2003; Weber-Fox et al., 2004; Hennessey et al., 2008). Neurophysiological evaluation of picture naming on the other hand did report several alterations in both lexical-semantic and phonological processes (Maxfield et al., 2010, 2012, 2014). However, as there is an interstimulus interval of 2 sec in the present study, these processing stages occur well before motor preparation. Moreover, a slope analysis was performed which enables CNV evaluation independent from the preceding amplitude level. This reduces a possible influence from previous processes. Finally, the significant difference in CNV slope was also seen in the R-locked analysis which is time-locked to response onset and by consequence has little influence from stimulus processing. In sum, language interference is suggested to be small. Future CNV research using different language tasks could explore this matter.

A second possible confounding factor might be anticipation. Although the CNV primarily reflects motor preparation, an influence from stimulus anticipation has been reported as well (e.g. Bares et al., 2007). The S-locked analysis evoked larger significant results than the R-locked analysis. This might suggest that not only an increase in motor preparation, but also an increase in anticipation has contributed to the significant difference in the CNV slope. In the present study, EMG onset was visually determined. Future research might use a more objective method to evaluate the influence of anticipation. In any case, whether the CNV is purely affected by motor preparation or whether there

is an additional influence of stimulus anticipation, the present results show an increased BGTC – loop activity during fluent single word production in DS.

5. Conclusion

The present study evidences the presence of altered motor preparation in DS. Brain activity related to speech motor preparation was evaluated by use of a picture naming task with a CNV design. A significant increase in CNV slope confirms previous observations that report an increased activation of the BGTC-loop during speech motor preparation. A positive correlation between the CNV slope and stuttering frequency/severity was also observed. The more a person stutters, the more neural resources in this cortical-subcortical network seem to be activated. Remarkably, the present results are observed during fluent single word production. This suggests that motor preparation alterations are either (1) surmountable or insufficient to evoke stuttering or (2) a successful compensation strategy to overcome other neural disturbances. The observation that the increase in CNV slope is only significant over the right hemisphere in the response locked analysis, is in favour of the latter hypothesis. An exploratory study evaluating the CNV slope preceding stuttered responses might clarify this issue.

Chapter 10

When will a stutter occur?

The determining role of speech motor preparation

Vanhoutte Sarah, Cosyns Marjan, van Mierlo Pieter, Batens Katja, Corthals Paul

De Letter Miet, Van Borsel John, Santens Patrick

Neuropsychologia, under review

Abstract

Neurological research in developmental stuttering often faces the challenge to distinguish neural impairments related to the cause and related to the consequence of stuttering. In a recent electro-encephalography (EEG) study, speech motor preparatory activity generated by the basal ganglia-thalamo-cortical (BGTC) – loop was found to be significantly increased in AWS compared to fluent speakers (FS) (Vanhoutte et al., (2015a). Remarkably, this increase preceded fluent word production suggestive for two opposite explanations: (1) motor preparatory alterations are insufficient to evoke stuttering, or (2) enlarged motor preparatory activity enables fluent speech production. To elucidate this cause-compensation issue, the present study evaluated speech motor preparation preceding stuttered responses of the task reported in Vanhoutte et al., (2015a).

Speech motor preparation was evaluated by a contingent negative variation (CNV), i.e. a slow negative, event-related potential, evoked during a picture naming task. The CNV slope prior to stutters was compared to the CNV slope preceding fluent words in AWS and to the CNV slope obtained in the FS. The CNV prior to stutters did not differ from the CNV of the FS, but was significantly reduced compared to the CNV prior to fluent words of AWS. This confirms the compensation hypothesis: the increased CNV slope prior to fluent speech is a successful compensation strategy. The words are produced fluently because of an enlarged motor preparatory activity, especially over the right hemisphere. The left CNV slope prior to stutters correlated negatively with stuttering frequency and severity suggestive for a link between the left BGTC – network and the stuttering pathology. Overall, speech motor preparatory activity generated by the BGTC – loop seems to have a crucial role in stuttering. An important divergence between left and right hemisphere is hypothesized.

Keywords

contingent negative variation, stuttering severity, stuttering frequency, compensation, motor preparation, basal ganglia

1. Introduction

Stuttering is a speech disorder in which the smooth succession of speech sounds is interrupted by the repeated occurrence of prolongations, blocks and repetitions of sounds and/or syllables. When stuttering is of developmental origin, manifesting itself for the first time during childhood, it is called developmental stuttering (DS) (Bloodstein & Ratner, 2008). About 95% of children who stutter (CWS) started stuttering by the age of 4 years (Yairi & Ambrose, 2005). Neurologically, DS is typically characterized by a hyperactivation in cortical and cerebellar motor structures and a hypoactivation in auditory areas (for a meta-analysis, see Brown et al., 2005). Alterations have also been described in several basal ganglia nuclei (e.g. Braun et al., 1997; Chang et al., 2009; Ingham et al., 2004; Kell et al., 2009; Loucks et al., 2011; Watkins et al., 2008) and their connections with cortical areas (Chang et al., 2011; Lu et al., 2010a, 2010b). By consequence, DS is characterized by cortical and subcortical abnormalities in speech motor planning, initiation, execution and monitoring.

Most of these neurological findings are based on research conducted in adults who stutter (AWS). However, because DS starts during childhood, neuroanatomical growth and maturation of CWS may follow an abnormal trajectory (Beal et al., 2013; Chang, 2011). Moreover, the brain will try to overcome these deficiencies causing neural adaptation and compensatory processes which will further shape structural development (Chang et al., 2015). As a result, the neural activity and morphology pattern observed in AWS is a combination of the cause of stuttering on the one hand and the consequence of lifelong stuttering and compensation strategies on the other hand. It is an ongoing discussion which neural anomalies are related to the cause and which to consequence/compensation. Particularly the relative role of left and right hemisphere has been addressed.

The increased cortical motor activations are often lateralized to the right hemisphere. Especially the right frontal operculum (RFO) is consistently reported to be overactivated in AWS (Brown et al., 2005). Anomalous increased brain activity in one hemisphere might reflect a compensation for disturbed signal transmission in the other hemisphere. Indeed, right inferior frontal gyrus (IFG) is involved in inhibiting speech acts that are generated in the left IFG (Xue et al., 2008) and would only interfere when left IFG experiences problems (Lu et al., 2010a). The most consistently reported neuroanatomical abnormality in stuttering concerns a decreased fractional anisotropy of the white matter underneath left ventral sensorimotor cortex, closely located to left IFG. This white matter anomaly will hamper cortical interactions and cortico-subcortical interactions between speech related regions (Chang et al., 2008; Connally et al., 2014; Cykowski et al., 2010; Sommer et al., 2002; Watkins et al., 2008). Because for a long time no structural deficits were found in subcortical structures, this white matter abnormality was suggested by many to be related to the primary cause of stuttering (Kell et al., 2009). Recently however, structural alterations have been identified in the

basal ganglia (Beal et al., 2013) and their connections, even in CWS close to stuttering onset (Chang & Zhu, 2013). Overall, though not generally believed (e.g. Connally et al., 2014; Kronfeld-Duenias et al., 2014; Watkins et al., 2008), there is increasing evidence for left hemisphere abnormalities to be associated with the neural basis of stuttering and right hemisphere deficits to be related to adaptation and compensation strategies (Preibisch et al., 2003). There is no consensus on whether the cortical or subcortical structures are the common basis for stuttering.

In a recent electro-encephalography (EEG) study from our laboratory, speech motor preparatory activity generated by the basal ganglia-thalamo-cortical (BGTC) loop was found to be significantly increased in AWS compared to fluent speakers (FS). This increase correlated positively with stuttering frequency and severity (Vanhoutte et al., 2015a). Several theories on stuttering propose a key role for speech motor preparation (Howell, 2004; Alm, 2004; Giraud et al., 2008; Civier et al., 2013). Additionally, many neuroimaging studies have reported positive correlations between stuttering measures and neural activity in several structures of the BGTC – loop (Braun et al., 1997; Fox et al., 2000; Giraud et al., 2008; Chang et al., 2009; Kell et al., 2009; Ingham et al., 2012). Also other neurophysiological studies evidenced the importance of motor preparation alterations in stuttering. They especially point at dysfunctions in the transfer of sensorimotor programs to the motor cortex, particularly in the left hemisphere (Neef et al., 2015; Salmelin et al., 2000).

Remarkably, the increase in BGTC – loop activity during speech motor preparation occurred preceding fluently produced single words. Two explanations may account for the fluent word production: (1) isolated word production is well known to evoke no or only a few stutters (Brown, 1938; Adams et al., 1973) probably due to its low demands on the neural speech motor system (Bloodstein & Ratner, 2008). Thus, when only a limited load is imposed on the speech motor system, motor preparation dysfunctions are either not enough to evoke stuttering or can be overcome by another system, or (2) the words were produced fluently because an enlarged speech motor preparatory activation in the BGTC – network was present.

These two opposite interpretations are again related to the cause-compensation issue of stuttering. The significant increase in speech motor preparatory activity was bilaterally observed with respect to stimulus onset, (i.e. stimulus-locked or S-locked analysis), but only over the right hemisphere with respect to lip movement onset as measured by electromyography (EMG) of the orbiculatoris oris muscle (i.e. response-locked or R-locked analysis). As the R-locked analysis takes reaction time into account, activities related to response execution would be more pronounced in the R- than in the S-locked analysis (Riès et al., 2013). The results of the R-locked analysis are thus slightly in favour for the compensation hypothesis. To clarify the cause-compensation issue, it may be very interesting to evaluate the speech motor preparation preceding stuttered responses. If the increased motor preparation activation prior to fluently produced words is related to successful compensation, the

speech motor preparation prior to stutters would be significantly lower than speech motor preparation preceding fluent words.

Neurological research on stuttered speech is extremely scarce because AWS speak mainly fluent in experimental settings. Recently, two meta-analyses of neuroimaging studies were performed in which stuttered speech was compared to natural fluent speech in AWS (Belyk et al., 2014) and natural and induced (e.g. choral speech) fluent speech in AWS (Budde et al., 2014). Both meta-analyses observed that stuttered speech is associated with an increased cerebellair and SMA activation and a decreased superior temporal gyrus activation. Unfortunately, the majority of the studies included in these meta-analyses referred to stuttered speech that is embedded in otherwise fluent speech with percentage stuttered syllables (% SS) starting from as low as 2.5%.

To our knowledge, only 4 studies compared 100% stuttered with 100% natural fluent speech in AWS (den Ouden et al., 2013; Jiang et al., 2012; Sowman et al., 2012; Whymbs et al., 2013). The findings of these studies seem very contradictory. While two case reports associated stutters with a decreased activation in left inferior frontal regions (den Ouden et al., 2013; Sowman et al., 2012), a group study found that stutters were associated with an increased activation in this region (Jiang et al., 2012). It should be noted, however, that the latter study reported on a comparison between typical stuttering-like dysfluencies (e.g. blocks and prolongations) and less typical stuttering-like dysfluencies (e.g. phrase repetitions). Moreover, the case in den Ouden et al., (2013) showed more activation during stuttered than during fluent speech in only a few regions, whereas in Whymbs et al., (2013) the majority of significant findings represented an increased activation during stuttered compared to fluent speech. Finally, distinctive differences in brain activation during stuttered word production were even found across the four participants of the latter study.

The present study aimed at evaluating speech motor preparatory activity preceding stuttered responses of the task reported in Vanhoutte et al., (2015a). By use of a picture naming task, a contingent negative variation (CNV) was evoked and recorded with EEG. A CNV is a slow, negative event-related potential occurring in between two successive stimuli. The first stimulus (S1) is a warning stimulus which precedes the second, called imperative, stimulus (S2). This S2 requires a motor response (Walter et al., 1964; Rohrbaugh & Gaillard, 1983; McCallum, 1988; Regan, 1989; Golob et al., 2005). When the interstimulus interval (ISI) is ≥ 2 seconds, two CNVs can be distinguished. The early CNV occurs within the first second following S1 and is related to orientation. The late CNV occurs just before S2 and primarily represents motor preparation (Walter et al., 1964; Loveless & Sanford, 1974; Rohrbaugh & Gaillard, 1983; McCallum, 1988; Regan, 1989). The late CNV is generally accepted to measure the neural activity within the BGTC – loop (Lamarche et al., 1995; Hamano et al., 1997; Gomez et al., 2003; Bares et al., 2007; Fan et al., 2007).

Although the stuttering participants in our previous study (in Vanhoutte et al., 2015a) produced the majority of the words fluently during the CNV picture naming task, a number of participants produced quite some stutters. It is hypothesized that if the increased motor preparation activation prior to fluent words is a successful compensation, the CNV prior to stutters would be significantly reduced compared to the CNV prior to fluent words. In addition, the CNV of the stuttered words is compared to the CNV of the FS included in the previous study as well. To our knowledge, this is the first EEG group study that compares brain activation related to pure fluent with pure stuttered utterances.

2. Method

2.1. Participants

25 AWS with developmental stuttering (M/F ratio: 19/6; mean age: 29.9 years; age range: 18 – 57 years; left/right handedness: 4/21) and 35 FS (M/F ratio: 24/11; mean age: 28.9 years; age range: 18 – 58 years; left/right handedness: 3/32) were included in the previous study analysing fluent responses (Vanhoutte et al., 2015a). Handedness was determined by the Edinburgh Handedness Inventory (Oldfield, 1971). As mentioned before, only a minority of the responses was stuttered by most AWS. However, at least 30 artefact free trials should be included to obtain a good signal-to-noise ratio (SNR) (Handy, 2005). Seven AWS (M/F ratio: 4/3; mean age: 33.4 years; age range: 19 – 57 years; left/right handedness: 1/6) produced enough stutters to retain a sufficient number of trials after all artefact rejections (see further on).

Table I: Detailed overview of the characteristics of the 7 AWS included in the measurement of the CNV slope preceding stutters.

Subject	Age	Gender	%SS		Severity		Included trials	
			Reading	Conversation	Percentile	Category	S-locked	R-locked
DA	27	M	32.3	30.9	97	very severe	31	30
DM	51	F	22.6	3.4	78	severe	80	79
DL	57	F	16.4	12.5	83	severe	89	87
HR	33	F	0.01	2.1	18	mild	32	31
MS	19	M	42.6	13.6	89	severe	89	85
VS	24	M	4.8	2.4	12	mild	35	31
VJ	23	M	1.0	3.4	24	mild	54	46

All AWS had already followed one or more treatments of variable duration and intensity. None of the control subjects had a history of stuttering nor had stuttering relatives. Both AWS and FS were monolingual native speakers of Dutch, and reported no history of hearing complaints, dyslexia or other speech-language problems, neurological or psychiatric disorders, and presented with normal or

corrected-to-normal vision. None of the participants was on psycho-active drugs. All participants gave their written informed consent in accordance with the declaration of Helsinki. This study was approved by the local ethics committee.

2.2. Speech assessment

For each participant, a conversational and a reading speech sample was collected. As a reading task, the participants were asked to read the Dutch translation of the text 'The north wind and the sun' (International Phonetic Association, 1974). During the conversation speech sample, every subject engaged in a conversation with the investigator about work/school/hobby/family. All samples were videotaped using a Canon ACV HD (1920 x 1080) camera and were audiotaped in Praat, a free software program for acoustical analysis (Boersma and Weenink, Phonetic Sciences, University of Amsterdam, Amsterdam, The Netherlands) using a Samsung CU01 microphone placed 50 cm in front of the participant.

Speech samples were judged for stuttering severity by means of the Stuttering Severity Instrument, fourth edition (SSI-4; Riley, 2008) and percent stuttered syllables (%SS) was calculated following the principles of the Stuttering Measurement System (Ingham & Ingham, 2011). Part-word (sound/syllable) repetitions, prolongations, blocks, broken words and tense pauses (American Speech-Language-Hearing Association, 1999; Yaruss, 1997) were counted as stuttered syllables. It is an ongoing debate whether to count monosyllabic word repetitions as stutters or not (Einarsdottir & Ingham, 2005). In the present study, repetitions of monosyllabic words were considered as stuttered dysfluencies when they were repeated at a high rate (Bezemer et al., 2010; Guitar, 2006), with apparent undue stress, tension or struggle (American Speech-Language-Hearing Association, 1999; Van Zaalen & Winkelman, 2009) or when the number of repetition units was 3 or more (Boey et al., 2009; Gregory, 1993). Stuttering was diagnosed by a certified speech-language pathologist (SLP) based on % SS (>3%) and/or the presence of significant speech-related struggle behaviour. Stuttering severity varied considerably between participants. In the group of 25 AWS included in the previous study analysing fluent responses (Vanhoutte et al., 2015a), 10 AWS presented with very mild, 7 with mild, 3 with moderate, 3 with severe and 2 with very severe stuttering.

All samples were scored off-line. 25% of samples were re-evaluated by a second rater (MC) to assess inter-rater reliability. Both raters are SLPs specialized in stuttering. An intraclass correlation coefficient (ICC) was calculated for overall percentile score on SSI-4 (Riley, 2008), %SS for reading and %SS for conversation. ICC's of 96.3; 99.7 and 99.5% respectively were obtained, which ensured excellent agreement.

2.3. Neurophysiological assessment

The same picture naming task and the same analysis as in Vanhoutte et al., (2015a) were used.

2.3.1. EEG data acquisition

EEG data were collected with Neuron-Spectrum-5 (4EPM) registration software (Neurosoft, Moscow, Russia). By use of an universal EEG cap (Haube S2), 21 Ag/AgCl electrodes (Fp1, Fpz, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, Oz, O2) were placed on the scalp according to the international 10/20 system. Two more electrodes were placed above the right side of the upper lip and underneath the left side of the lower lip to register the EMG of the orbicularis oris muscle in a bipolar fashion. An additional electrode on the forehead was used as ground. Neurophysiological data were recorded against a linked ears reference at a sampling rate of 500 Hz (0.01-75Hz band-pass filter). Impedance of each electrode was kept below 5kΩ. The participants were encouraged to avoid orofacial movements and to reduce eye-blinks as much as possible. Each EEG session was also videotaped using a Canon ACV HD (1920 x 1080) camera.

2.3.2. CNV paradigm

A picture naming task was made for which the pictures were selected from a picture naming norms database, provided by the Department of Experimental Psychology from the Ghent University, Belgium (Severens et al., 2005). For further analysis, speech onset had to be determined. Articulatory movements were shown to precede vocalization during a Bereitschaftspotential paradigm. Depending on the initial phoneme, the lips or the tongue were the first source (McArdle et al., 2009). Lip movements are easier to detect than tongue movements with EMG. Therefore, pictures were chosen that referred to a noun that had a bilabial (/m/, /w/, /b/, /p/) or labiodental (/f/, /v/) initial phoneme.

The picture was presented as S1 and was shown for 1 second. The S2, in the form of a short, black line, appeared 2 seconds after S1 onset (the ISI was 2 seconds) indicating that the participant should name the picture as quickly as possible. S2, shown for 2 seconds, was followed by a black screen for another 2 seconds. If the participant continued to stutter on a word once this black screen appeared, he/she was instructed to stop speaking in order not to contaminate the next trial with muscular artefacts (for a diagram of the CNV task, see figure 1A). One hundred and ten black and white line drawings were shown on a white background in the middle of a computer screen that was placed one meter in front of the participant. Participants were instructed to name the pictures using only one word or to say ‘pass’ if they did not know the noun.

Responses were excluded from further analysis if (1) the word was produced before S2 was shown, (2) the produced word did not have a labiodental or bilabial initial phoneme, or (3) the participant

swallowed or made an inappropriate lip movement within 1500 ms preceding S2 which was judged based on the videotape recordings and visual inspection of the EMG signal. The remaining responses were judged as fluent or stuttered by the first author following the same principles as the speech samples assessment (see 2.2). Only responses with a stutter on the initial phoneme were taken into account. 8 AWS produced more than 30 stuttered responses as required to obtain a satisfying SNR (Handy, 2005). Their utterances were re-evaluated by a second rater (MC). Responses that were not considered stuttered by both raters were also excluded. Stuttered responses were analysed separately from, but following the same procedure as the fluent responses.

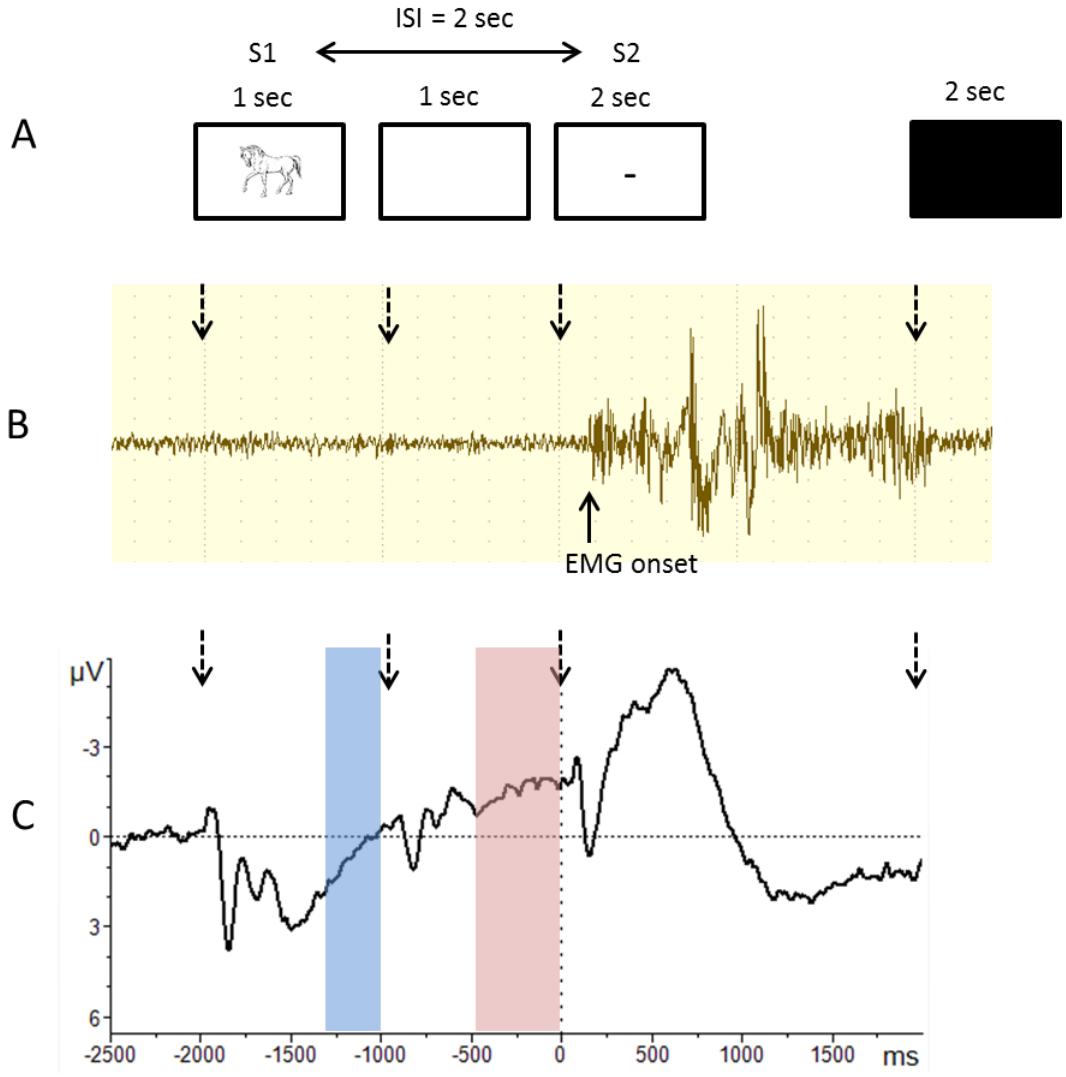

Figure 1: **(A)** Diagram of the picture naming CNV task. The warning stimulus (S1) consists of a picture, the imperative stimulus (S2) of a short, black line that prompts the participant to name the picture as quickly as possible. Interstimulus interval (ISI) is 2 sec. **(B)** EMG signal of the orbicularis oris muscle of one response. The dotted arrows represent the onset of the corresponding image in (A). The full arrow represents the onset of the EMG signal. **(C)** S-locked average at Cz for the FS. Latency (x-axis) is represented in milliseconds (ms) and amplitude (y-axis) in microvolts (µV). Negative is plotted upwards. Baseline is the first 500 ms op the epoch i.e. 500 ms before S1 onset. The 0 ms point is S2 onset. While the blue bar indicates the early CNV, the pink bar highlights the late CNV. Again, dotted arrows represent the onset of the corresponding image in (A).

2.3.3. Off-line EEG analysis

Off-line analysis was performed using BrainVision Analyzer 2 (Brain Products, Munich, Germany). After additional filtering (0.01-30 Hz band-pass filter, Notch filter 50 Hz), eye artefacts were removed by Independent Component Analysis (Mennes et al., 2010). Two components (eye blinks; left-right eye movements) were excluded based on inspection of the components' spatial distribution. It is recommended to analyse language related brain activities not only time-locked to stimulus onset, but also time locked to response onset. Activities linked to response execution emerge time-locked to the response and might consequently be reduced in the analysis time-locked to stimulus presentation (Riès et al., 2013). Therefore, data were analysed with respect to S2 and lip movement onset, referred to as stimulus (S-) and response (R-) locked respectively. Lip movement onset was detected by visual inspection of the EMG data of each trial (Van Boxtel et al., 1993; Hasbroucq et al., 1999; Carbonnell et al., 2004). The EMG data were separately band-pass filtered from 15 Hz to 100 Hz to reduce the contamination by motion artefacts and non-myogenic potentials (Van Boxtel, 2001). After segmenting the continuous EEG data into epochs, all trials containing artefacts were manually excluded (Cui et al., 2000; Bares et al., 2007; Mock et al., 2011). One AWS was additionally excluded because not enough artefact free trials remained. Thus, 7 AWS remained to be analysed. By averaging over corresponding epochs, the CNV potential was computed.

Due to methodological considerations of baseline correction (Luck, 2005), a slope analysis was performed on the CNV which is independent from the baseline (see 2.4. CNV analysis). As such, the baseline correction described in this paragraph is only performed for the construction of the figures (figure 1, 2 and 3) (Carbonnell et al., 2004). The continuous EEG data was segmented into epochs of 4500 ms for the S-locked analysis, starting 2500 ms prior to S2, and into epochs of 4600 ms for the R-locked analysis, starting 2600 ms prior to EMG onset. Baselines were taken from -2500 to -2000 ms for the S-locked and from -2600 to -2100 ms for the R-locked analysis. The baseline correction for the R-locked analysis was different in order to take a reaction time delay into account (see figure 1B). If the segmented epochs had been baseline corrected from -2500 to -2000 ms prior to EMG onset, a considerable number of trials would have a baseline that contained a part of the visual evoked potentials elicited by S1. Therefore, baseline correction was performed from -2600 to -2100 ms.

2.4. CNV analysis

The late CNV is usually measured by calculating its mean amplitude in the time window that contains its maximal variation. The time window of interest (TOI) for the present task was the 500 ms window preceding S2 and EMG onset. However, as seen in our previous study, mean amplitude appeared rather inappropriate for the present data. Instead, a slope measure was preferred. Slope analysis is more favourable than classic mean amplitude analysis (Carbonell et al., 2004) because (1) the slope is

independent of the amplitude level preceding the TOI and (2) the slope is independent of the baseline. This was of particular interest for the R-locked analysis. As reaction time varies largely between and within participants, a fixed baseline correction would be performed on a different part of the EEG epoch for every trial and participant. Therefore, an ERP analysis that is independent from this varying variable is advisable. A mean-to-mean amplitude was used in which the absolute value of the difference between the mean amplitude of the first (-500 to -400 ms) and the last (-100 to 0 ms) 100 ms of the TOI was computed (Handy, 2005; Luck, 2005). This was done for frontal (F3, Fz, F4), central (C3, Cz, C4) and parietal (P3, Pz, P4) electrodes as the late CNV was most pronounced at these sites.

2.5. Statistical analysis

Statistical analysis was performed in IBM SPSS Statistics 22.0 for both S- and R-locked analysis separately. The CNV slope prior to stuttered words was compared to (1) the CNV slope preceding fluent utterances of AWS, and (2) the CNV slope observed in the FS group. Both comparisons were separately performed using a linear mixed model approach. The comparison between stuttered and fluent responses contained Region (frontal, central, parietal), Hemisphere (left, midline, right) and Utterance (fluent, stuttered) as repeated variables. These were also inserted as factor. For the comparison between stuttered responses and FS, Region (frontal, central, parietal) and Hemisphere (left, midline, right) were included as repeated variable. Both were also inserted as factor, together with Group (FS, AWS) as a third factor. Significance values were set at $\alpha \leq 0.05$ for main and interaction effects. All further pairwise comparisons were Bonferroni corrected.

An additional correlation analysis was performed between the CNV slope and three stuttering measures: %SS during reading, %SS during conversation and stuttering severity. For the latter variable, the overall percentile score of the SSI-4 (Riley, 2008) was calculated.

3. Results

A typical CNV wave was seen preceding stuttered responses (figure 2). After visual and linguistic processing of the pictures (S1), a clear increase in negativity occurred between 700 and 1000 ms following S1. This early CNV (blue bar in figure 1C) was seen over (pre)frontal, central and parietal electrodes. At 1000 ms, the early CNV was interrupted by a new phase of visual processing because at this point in time the picture disappeared from the screen. Shortly hereafter, a steep increase in negativity could be observed, peaking around the presentation of S2. This negativity is the late CNV (pink bar in figure 1C) and has a wide scalp distribution. Since the R-locked analysis is time-locked to response execution, activities related to stimulus processing are smeared out. As a result, the peaks related to visual and linguistic processing cannot be clearly seen in this grand average ERP (figure 3).

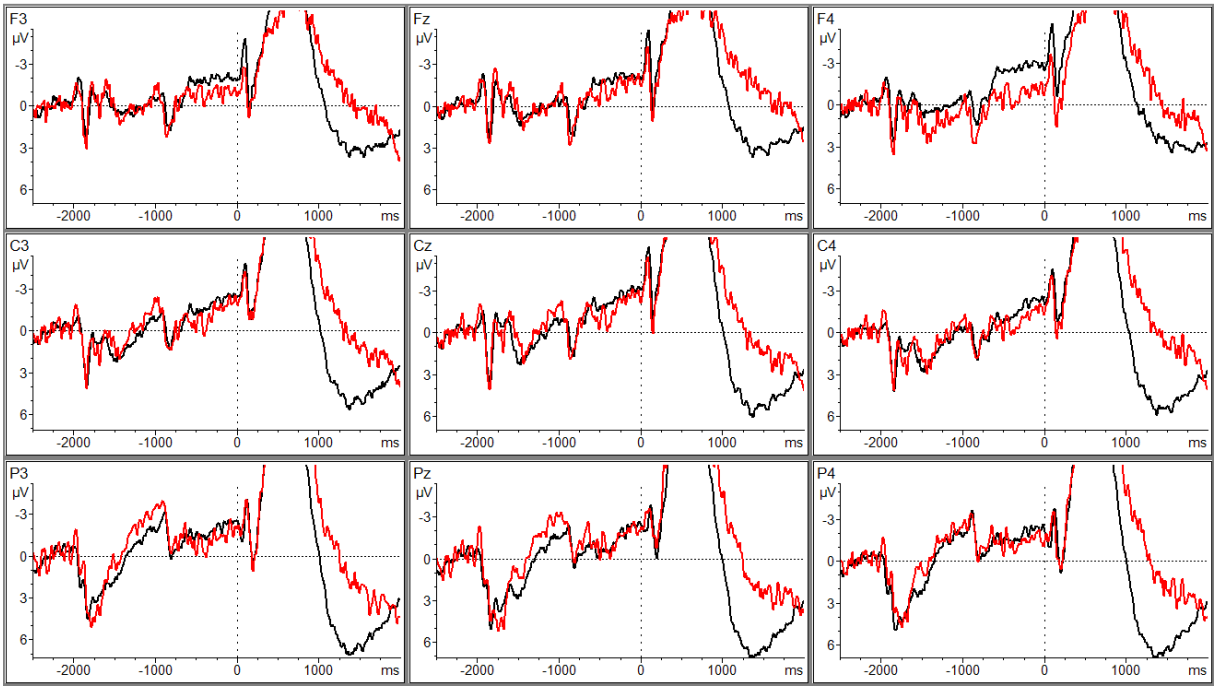

Figure 2: S-locked analysis for all electrodes of interest. Latency (x-axis) is represented in milliseconds (ms) and amplitude (y-axis) in microvolts (µV). Negative is plotted upwards. Baseline correction, which is only performed for the construction of the figures, is the first 500 ms of the epoch. The 0 ms point is S2 onset. The CNV of the stuttered words is shown in red, the CNV of the fluent words is presented in black. The EEG signal past 0 ms is not entirely visible because the scale of the y-axis was chosen to enable a clear, detailed display of the early and late CNV.

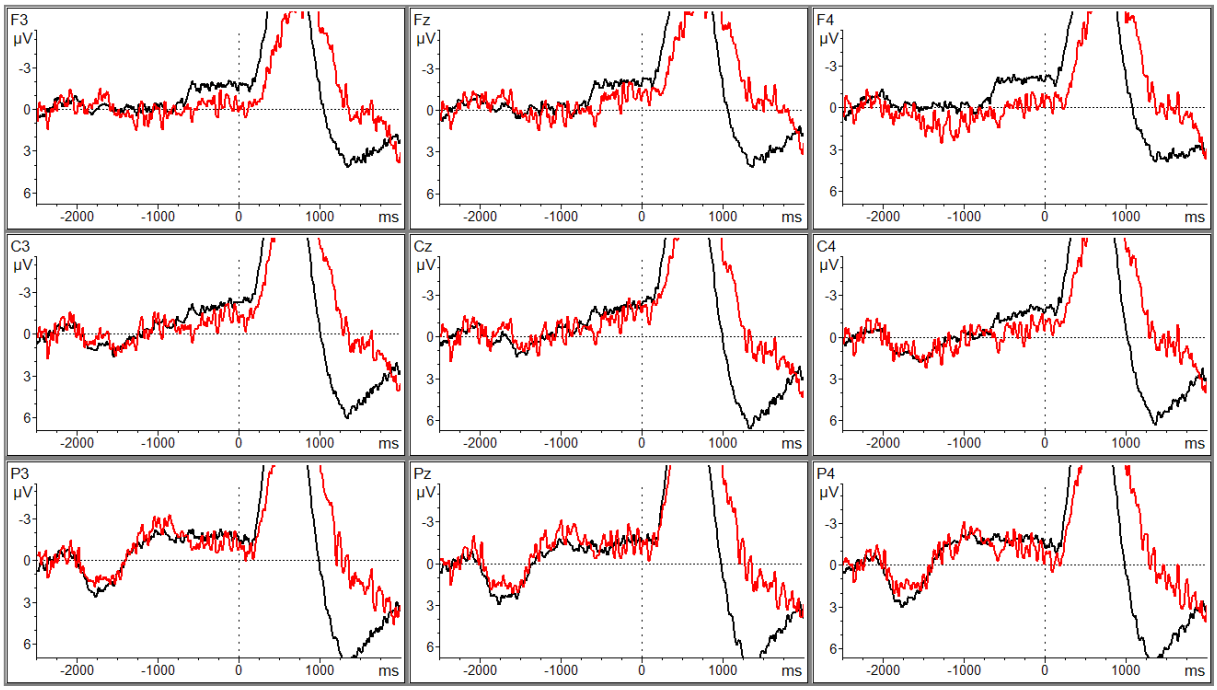

Figure 3: R-locked analysis for all electrodes of interest. Latency (x-axis) is represented in milliseconds (ms) and amplitude (y-axis) in microvolts (µV). Negative is plotted upwards. Baseline correction, which is only performed for the construction of the figures, is the first 500 ms of the epoch. The 0 ms point is EMG onset. The CNV of the stuttered words is shown in red, the CNV of the fluent words is presented in black. The EEG signal past 0 ms is not entirely visible because the scale of the y-axis was chosen to enable a clear, detailed display of the early and late CNV.

Both the S- and R-locked analysis displayed a main effect of Utterance (S-locked: F(1, 59)= 29.42; p< 0.001; R-locked: F(1, 70)= 5.75; p= 0.019). The CNV slope preceding stutters was significantly smaller than the CNV preceding fluent words in AWS (figure 4). No significant result was obtained when comparing the CNV preceding stutters in AWS with the CNV obtained in the FS (S-locked: F(1, 38)= 0.11; p= 0.746; R-locked: F(1, 41)= 0.93; p= 0.342). However, as can be seen in figure 4, the CNV slope of the R-locked analysis is markedly lower in AWS than in FS. The lack of a statistical significant difference is suggestive for large variation among the AWS. Therefore, an additional correlation analysis was performed between the CNV slope and the stuttering measures. Because the decrease in CNV was most pronounced over the left hemisphere, the CNV slope at C3 (R-locked) was used to explore a possible correlation. The association between CNV slope at C3 and the stuttering measures was best described by an exponential fit which was strongly significant for stuttering frequency during reading (R^2= 0.893; p< 0.001) and stuttering severity (R^2= 0.734; p= 0.007) (figure 5).

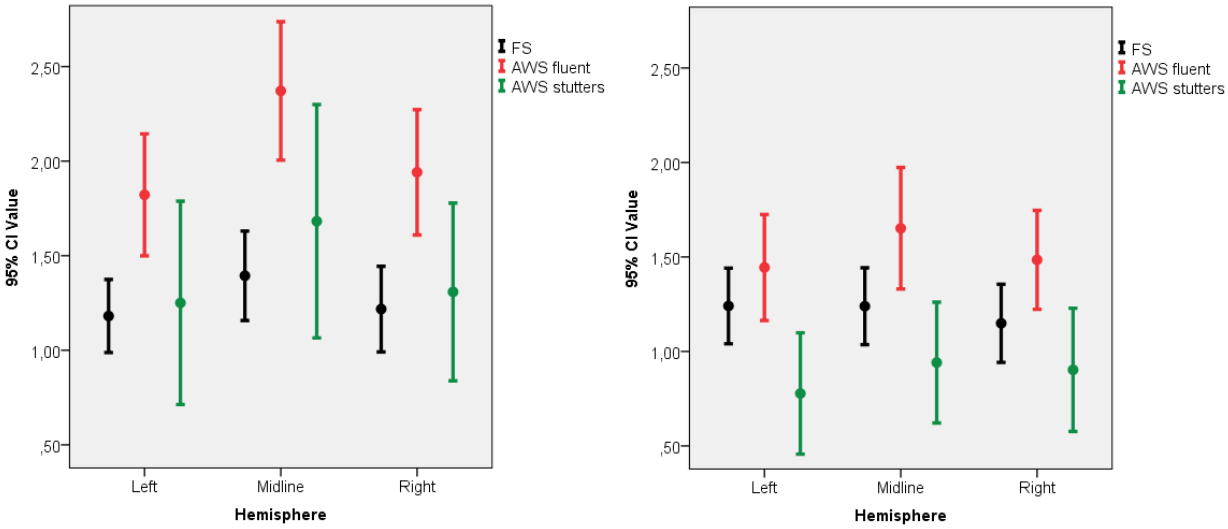

Figure 4: Graph depicting the 95% confidence interval of the CNV slope for all electrodes of interest (y-axis). S-locked analysis is shown on the left, R-locked analysis on the right. Results are split according to hemisphere (x-axis).

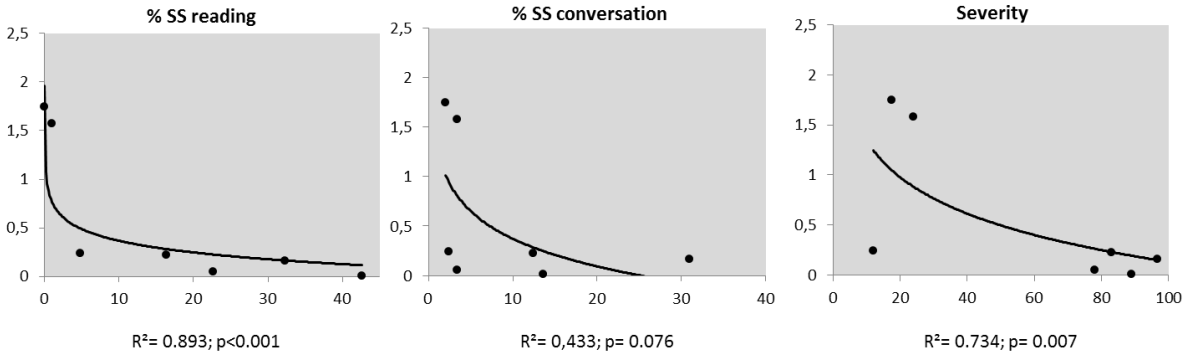

Figure 5: Scatter diagram of the correlation analysis between the R-locked CNV slope at C3 (y-axis) and the stuttering measures (x-axis). The correlation was best described by an exponential fit. The goodness of fit (R^2) and significance value (p) of each correlation are provided.

4. Discussion

The present study evaluated speech motor preparatory activity generated by the BGTC – loop preceding stuttered words. To our knowledge, this is the first group study using electrophysiology to compare 100% stuttered with 100% fluent speech. Motor preparation was evaluated by a CNV evoking picture naming task. A typical CNV could be identified consisting of an early CNV, occurring within 700 – 1000 ms following S1, and a late CNV, occurring prior to S2 and reflecting motor preparation. The late CNV preceding stuttered words was compared to (1) the CNV slope of the FS, and (2) the CNV slope preceding fluent words of AWS obtained in Vanhoutte et al., (2015a).

4.1. Increased CNV slope preceding fluent words: cause or compensation?

The CNV slope prior to stutters is significantly reduced compared to the CNV preceding fluent words. This reduction was observed over the entire scalp, for both the S- and R-locked analysis. Thus, motor preparatory activity generated in the BGTC – loops is significantly higher when a fluent words is produced compared to when a stutter is produced. This confirms the hypothesis that the marked increase in the CNV prior to a fluent word is a successful compensation strategy in AWS. The words are produced fluently because of an enlarged motor preparatory activity. The CNV slope prior to fluent words was previously found to correlate positively with stuttering frequency and severity (Vanhoutte et al., 2015a). Thus, the more a person stutters, the higher the motor preparatory activity must be to be successful and result in a fluently produced word. When this motor preparation increase does not occur, a stutter will be produced. Overall, the amount of motor preparatory activity in the BGTC – loop seems to be a determining factor whether or not a stutter will occur.

4.2. Exponential fit with left CNV slope

Despite a markedly lower CNV slope in AWS, no significant difference was found between the FS and the stuttered words of the AWS. This is suggestive for large variation among AWS. Indeed, an additional analysis revealed a negative correlation between the CNV slope at C3 and stuttering frequency during reading and stuttering severity. The more a person stutters in general, the lower his/her motor preparatory activity in left BGTC – loop preceding a stutter. This observation is in perfect agreement with a recent transcranial magnetic stimulation study showing reduced motor preparation excitability in left primary motor cortex just before a novel speech gesture was executed (Neef et al., 2015). Remarkably, also the magnitude of the excitability showed a negative, exponential fit with stuttering frequency. These findings suggest a link between motor preparatory activity in the left hemisphere and the stuttering pathology.

Alterations in left-sided motor preparation areas have repeatedly been reported in stuttering. Two case reports observed an association between reduced left IFG activation and failed initiation (den Ouden et al., 2013; Sowman et al., 2012). Left IFG activation has also been described to remain reduced after successful therapy, despite the normalization of other abnormal activations due to this therapy (Neumann et al., 2003). A reduction in grey matter volume of left IFG has been found to correlate positively with stuttering severity and to be independent from recovery (Kell et al., 2009). Moreover, the most consistently reported neuroanatomical abnormality in stuttering concerns reduced white matter density underneath left ventral sensorimotor cortex, closely located to left IFG (Chang et al., 2008; Chang et al., 2011; Connally et al., 2014; Cykowski et al., 2010; Sommer et al., 2002; Watkins et al., 2008). This reduction in white matter density is more pronounced, the more a person stutters (Cai et al., 2014b). All these findings suggest that left-sided motor preparation alterations are closely related to the origin of stuttering.

4.3. Left – right asymmetries

The brain is known to try to compensate deficits in one hemisphere by engaging the homologous region(s) in the other hemisphere. If left BGTC – loop has a link with the neural origin of stuttering, then compensation may primarily be provided by the right BGTC – network. Previous studies indeed suggested an important lateralization aspect in compensation strategies. Stuttering therapy especially reduces right-sided overactivations and shifts the activity pattern from a right to a more left lateralized engagement during speech production (De Nil et al., 2003; Neumann et al., 2003, 2005). An important region for compensation would be the RFO (Preibisch et al., 2003; Kell et al., 2009). RFO is consistently reported to be overactivated in stuttering (Brown et al., 2005). Moreover, its overactivation is strictly associated with fluent speech in AWS which confirms its hypothesized involvement in successful compensation (Belyk et al., 2014).

Also the CNV results suggest a more pronounced contribution of the right hemisphere to compensation. When comparing the CNV prior to fluent words in AWS to the CNV of the FS, the S-locked results were significant over the entire scalp while the R-locked results were only significant over the right hemisphere (Vanhoutte et al., 2015a). As the R-locked analysis takes reaction time into account, activities related to response execution are more pronounced in this analysis than in the S-locked analysis (Riès et al., 2013). This suggests that especially the increase in right BGTC – loop is involved in successful compensation.

The CNV data are not suited to make more detailed hypothesis on which structure(s) of the motor preparation network is(are) affected. Although EEG measures the electrical activity of the cortex, this cortical activity is a reflection of an underlying cortico-subcortical network (Fan et al., 2007). As such,

the present data neither favour a cortical nor a subcortical hypothesis as the primary cause and/or compensation strategy of stuttering.

4.4. Importance of temporal information

Previous studies on 100% stuttered speech provide conflicting results. These studies mainly focused on spatial localization of activations and deactivations (den Ouden et al., 2013; Jiang et al., 2012; Sowman et al., 2012; Whymbs et al., 2013). Sowman et al., (2012), using magneto-encephalography, found that depending on the time window, a different, even reversed, activation pattern can be observed. Stuttered syllables, compared to fluent syllables, were associated with a decreased inferior frontal activation from 300 to 600 ms post-stimulus onset and with an increased inferior frontal activation from 600 to 800 ms. Also the present EEG study shows that valuable information can be obtained by evaluating a particular processing stage of speech production in real time. Temporal information might be an important aspect in identifying neural characteristics of stuttered speech.

4.5. Additional considerations

Although a language interference cannot be excluded, several methodological arguments are in favour of the CNV results being mainly related to motor preparation. First, a slope analysis was performed which enables CNV evaluation independent from the preceding amplitude level. This reduces an influence from previous processes (Carbonell et al., 2004). Secondly, the group difference and negative correlation were significant for the R-locked CNV slope. In the R-locked results, activities related to response execution are more pronounced while activities related to stimulus processing are smeared out and will only have a limited influence (Riès et al., 2013).

Because isolated word production usually evokes no or only a few stutters (Brown, 1938; Adams et al., 1973), neurophysiological studies using single word production generally obtain too little stutters to have a satisfying SNR (e.g. Salmelin et al., 2000). Although the relatively high number of stutters in some participants in the present study might be surprising, several arguments can be found for their occurrence. First, due to its design, a CNV task evokes some time pressure and anticipation for the moment to speak which negatively influences fluency (Bloodstein & Ratner, 2008). Secondly, all words had a consonant as initial phoneme, a considerable amount of them even a stop consonant, which is more likely to evoke stuttering (Brown, 1945).

Finally, only a small group of AWS produced enough stutters to be included in the present analysis. The observed neurological differences between stuttered and fluent words warrant further neurological research that focusses on stuttered speech. As only ¼ of the present AWS produced enough stutters to obtain a good SNR, further research should include a high number of participants to enable a valuable analysis on stuttered speech.

5. Conclusion

To our knowledge, this is the first group study using electrophysiology to compare 100% stuttered with 100% fluent speech. Motor preparatory activity in BGTC – loop seems to have a crucial role in stuttering. Not only has the amount of activation a determining role in the actual moment of a stutter (i.e. proximal origin of stuttering: why does a stutter occur at a particular moment), its activation seems also related to the underlying stuttering pathology (i.e. distal origin of stuttering: what causes a person to be a stuttering speaker). An important divergence between left and right hemisphere is made in this respect. When motor preparatory activity in right BGTC – loop is markedly increased, no stutter will occur. The lower the left BGTC motor preparatory activity preceding a stutter, the more this person will stutter in general. These findings concur with a growing amount of studies stating that right hemisphere alterations are related to (successful) compensation strategies, while the left hemisphere would contain the primary cause of stuttering.

General discussion

Part III

Chapter 11

General discussion and future perspectives

1. General discussion and conclusion

Most neurological research on stuttering is restricted to the use of neuroimaging tools (e.g. fMRI), especially when it comes to speech motor control. As these tools have excellent spatial resolution, they provide important information on altered activations in specific brain regions. Speech production is, however, more than activating or deactivating certain brain areas. It is also a rapid and dynamic motor process that requires a timely, precise and sequential activation of these specific brain regions. These temporal aspects can be evaluated by use of neurophysiological tools like EEG and MEG which have excellent temporal resolution. EEG and MEG research on speech motor control is stuttering is very scarce. The present thesis aimed to identify neurophysiological characteristics of speech motor control in stuttering by use of EEG. Its temporal resolution enables the evaluation of:

(1) Temporal coordination and sequencing of neural activations
(2) Specific processing stages in real time by evoking an ERP

Both aspects were explored in DS. EEG was also applied in a case of NS. To exclude influences from auditory deficits, only visual tasks were designed.

Overall, the present studies evidence that neurophysiological research can reveal important neurological deficiencies and alterations related to motor control in stuttering. In what follows, a short summary of the present studies is given followed by some general observations and considerations. Finally, some suggestions for future work are presented.

1.1. Summary of research findings

1.1.1. Perception paradigm

In the first part of this thesis, the temporal resolution of EEG was used to evaluate the temporal coordination and sequencing of motor related activation during a visual word recognition task. For this purpose, a well-known task from the action literature was used: silent reading of action verbs. Action verbs that denote a body movement (e.g. to throw) are suggested to activate, besides typical linguistic brain areas, also frontal motor areas related to the preparation and execution of the movement the verb refers to (e.g. Moseley et al., 2013). In the present thesis, action verb processing was evaluated from two points of view. From a linguistic perspective, in which the timing of motor activations related to hand action verb processing (as compared to non-action verb processing) was evaluated in healthy FS (*chapter 6*). From a motor perspective, in which these results were compared to the (timing of the) motor activations observed in AWS performing the same task (*chapter 7*). An overview of the results can be found in table I and II.

In FS, action verbs evoked stronger bilateral sensorimotor activation than non-action verbs from 155 to 174 ms post-stimulus onset. This sensorimotor differentiation was interpreted as a word-specific semantic difference suggestive for a contribution of sensorimotor cortex to early lexico-semantic

Table I: Overview of the ERP results of chapter 6 and 7.

Peak	Linguistic process	Action vs non-action	FS vs AWS
N1	Visual word form processes	No difference	No difference
P2	Early lexico-semantic and -syntactic processes	No difference	No difference
N400	Large scale semantic integration	Action > non-action	No difference

Table II: Overview of the results of the source reconstruction of chapter 6 and 7.

Time window	Brain area (left + right)	Comparison	Result
155 – 174 ms	Sensorimotor cortex	FS: action vs non-action	Action > non-action
		AWS: action vs non-action	No difference
		Action: AWS vs FS	No difference
		Non-action: AWS vs FS	AWS > FS
219 – 238 ms	DLPFC	Largest motor difference within FS group	Action > non-action
313 – 352 ms	Sensorimotor cortex	Largest motor difference within AWS group	Non-action > action

processing of hand action verbs. Hand action verbs thus seem to activate the motor programmes of the actions they refer to (Hauk et al., 2004). These results are in line with theories of embodied cognition which state that concepts are represented in specific language brain areas and in neural action and perception systems (e.g. Barsalou, 1999; Dove, 2009).

In AWS, temporal coordination of motor related activations was considerably altered. Due to motor abnormalities, the maximal motor difference between both verb types was delayed with about 100 ms (228 ms for FS in DLPFC, 332 ms for AWS in sensorimotor cortex). These motor abnormalities are hypothesized to encompass two different activation patterns observed in stuttering: a general motor hyperactivation and a specific hand motor deficit. Motor cortex is typically found to be hyperactive in stuttering (Brown et al., 2005), even during silent reading of nouns not specifically related to motor semantic features (De Nil et al., 2000, 2003). The hand motor area on the other hand is characterized by decreased excitability (Busan et al., 2011). Both motor characteristics of stuttering seem to present during the visual word recognition task. Non-action verb processing in AWS evoked increased sensorimotor activation compared to (1) non-action verb processing in FS from 155 to 174 ms, and (2) action verb processing in AWS from 313 to 352 ms. This sensorimotor increase is suggested to reflect the general motor hyperactivation typically seen in stuttering (Brown et al., 2005) and independent of overt speech requirements (De Nil et al., 2000, 2003). Hand action verb

processing is hypothesized not to follow this general motor increase because these verbs spark hand motor cortex, a region characterized by decreased excitability in stuttering (Busan et al., 2011). Consequently, action verbs cannot evoke stronger sensorimotor activity than non-action verbs in AWS.

In sum, due to motor abnormalities in general and in hand motor processing in particular, temporal coordination of motor related activations is altered in stuttering, even during a silent reading task. These findings confirm that neural motor abnormalities in stuttering are not restricted to deficits during (speech) movement execution (Chang et al., 2009; De Nil et al., 2000, 2003; Morgan et al., 2008; Neef et al., 2011).

1.1.2. Production paradigm

In the second part of this thesis, the temporal resolution of EEG was used to examine speech motor preparation in real time by use of ERP analysis. A picture naming task was developed to elicit a CNV as this ERP primarily reflects motor preparatory activity in BGTC – loops (e.g. Bares et al., 2007; Bender et al., 2004; Fan et al., 2007), which are known to be altered in stuttering (e.g. Chang et al., 2009; Ingham et al., 2004; Kell et al., 2009; Lu et al., 2010a, 2010b; Watkins et al., 2008). Although most participants produced the majority of the words fluently, 7 out of 28 AWS produced a substantial amount of stutters which allowed a separate analysis. As a result, the CNV preceding fluent speech in AWS could not only be compared to the CNV of FS (*chapter 9*), but also to the CNV preceding pure stuttered speech (*chapter 10*). Studies evaluating neurogenic aspects of stuttered speech are very scarce. Moreover, most of them address stuttered speech that is embedded in otherwise fluent speech (Braun et al., 1997; Fox et al., 2000; Ingham et al., 2004; Toyomura et al., 2011). Making a clear distinction between 100% stuttered and 100% fluent speech is, however, recommended as dysfluent and fluent speech are associated with different neural findings (Belyk et al., 2014; Budde et al., 2014; Fox et al., 2000; Ingham et al., 2000, 2004). To our knowledge, the present study is only the third group study which addresses pure stuttered speech and the first to evaluate this with EEG. An overview of the results of chapter 9 and 10 can be found in table III.

To interpret the CNV findings, a distinction should be made between the distal and proximal origin of stuttering. The distal origin refers to the aetiology of stuttering as a disorder (why is someone a stuttering speaker) whereas the proximal origin is related to the concrete stuttering moment (why does someone stutter at a particular moment). The CNV studies are related to both concepts.

The CNV preceding fluent words was not only significantly higher than the CNV of the FS, it was also significantly increased compared to the CNV preceding stutters. As an increase in CNV slope seems to be associated with fluent speech production in AWS, increased motor preparatory activity was interpreted to be a successful compensation strategy. The positive correlation between the CNV and

Table III: Overview of the CNV findings of both the NS case report (chapter 8) as well as the DS group (chapter 9 and 10).

	Speech	CNV observation	Interpretation
DS	Fluent	• **Increased** CNV, most pronounced over the **right** hemisphere (compared to FS) • Positive correlation with stuttering frequency/severity at the vertex	**Compensation:** • Increased speech motor preparation is a successful compensation strategy • Most pronounced over the *right* hemisphere
	Stuttered	• **Decreased** CNV, over the entire scalp (compared to fluent words of AWS) • No difference with FS • Negative correlation with stuttering frequency/severity over the **left** hemisphere	**Causal link:** • The more (severe) a person stutters, the lower his speech motor preparatory activity • Link with underlying stuttering pathology in the *left* hemisphere
NS	Fluent	• Midline and left-sided electrodes: decreasing CNV with increasing stuttering frequency during conversation • Right frontal electrodes: relative increase CNV at test moment with most severe stuttering	⇨ **Causal link:** the amount of disturbance in motor preparation determines stuttering frequency ⇨ **Compensation:** right-sided compensation for left-sided anomaly (in STG)

the stuttering measures suggests that the more a person stutters, the higher this increase is or must be to enable fluent speech production. Because the response-locked analysis (evaluation of the CNV with respect to lip movement onset) showed only a significant increase over the right hemisphere, especially an increase in right BGTC motor preparatory activity was hypothesized to aid fluent word production. This compensation strategy is linked to the proximal origin of stuttering: whether or not a stutter will occur at a particular moment, depends on the amount of speech motor preparation.

Although the left CNV preceding stutters was markedly lower than the left CNV of the FS, no significant difference was found. This is highly suggestive for large variation among the AWS. Indeed, a negative correlation was found between the left CNV preceding stutters and the stuttering measures. The more a person stutters, the lower the left BGTC – loop activation. Thus, the decrease in left-sided motor preparation prior to a stutter is associated with the general stuttering frequency in that person. This suggests a link with the stuttering pathology and thus with the distal origin of stuttering.

Overall, motor preparation activity in BGTC – loop seems to have a crucial role in stuttering. Not only has the amount of activation a determining role in the actual moment of a stutter (proximal origin), its activation seems also related to the underlying stuttering pathology (distal origin). An important divergence between left and right hemisphere is made in this respect. When motor preparatory activity in right BGTC – loop is markedly increased, no stutter will occur. The lower the left BGTC motor preparatory activity preceding a stutter, the more this person will stutter in general.

These findings concur with a growing amount of studies stating that right hemisphere alterations are related to (successful) compensation strategies (Preibisch et al., 2003), while the left hemisphere would contain the primary cause of stuttering (e.g. Cykowski et al., 2010; Sommer et al., 2002; Kell et al., 2009). The present thesis highlights the importance of the BGTC – loop. Although EEG mainly measures cortical activity, this cortical activity is a reflection of the underlying cortical-subcortical network. As such, the findings of the CNV studies neither favour a cortical nor a subcortical hypothesis as primary cause of stuttering.

1.2. Some general observations and considerations

1.2.1. The origin of stuttering

Despite decades of research, the origin of stuttering has still not been resolved. Two major theoretical perspectives have dominated the literature: theories pointing at deficits in speech motor control and theories primarily concerning impairments in linguistic planning. Little effort has been done to reconcile one with the other.

A) Speech motor control theories

Stuttering has been hypothesized to result from an imbalance between feedforward and feedback processing (Max et al., 2004). Because of an impaired readout of feedforward models, AWS are forced to rely more heavily on auditory feedback. Over-reliance on feedback control would lead to production errors which causes the motor system to "reset" and repeat the current syllable (Civier et al., 2010). Deficient feedforward modelling in stuttering has previously been suggested from a structural (Chang et al., 2011; Sommer et al., 2002), functional (Brown et al., 2005; Lu et al., 2010a) and even electrophysiological (Salmelin et al., 2000; Neef et al., 2015) perspective. Because the present thesis does not evaluate feedback processing, no statements on the over-reliance hypothesis can be made. However, the present studies do confirm that stuttering is associated with impaired feedforward processing.

First, dysfunctional forward modelling implies that other movements than speech are affected too (Chang et al., 2009). The perception paradigm shows that hand motor representations in the brain have a decreased excitability and thus confirms that motor skills beyond speech show altered neural control in AWS (Busan et al., 2011; Chang et al., 2009; Morgan et al., 2008; Neef et al., 2011). Secondly, because the CNV reflects motor preparatory activity generated by the BGTC – loop, the CNV is solely related to activity in feedforward modelling (Bohland et al., 2010; Guenther, 2006). As such, the CNV studies demonstrate the crucial role of feedforward processing in stuttering. Not only does it contribute to successful compensation, it also seems to have a link with the stuttering pathology.

Recently, especially the role of the 'motor loop', as part of feedforward processing, has been highlighted in stuttering. The GODIVA model (Bohland et al., 2010), a recent model on speech motor preparation which addresses the selection, sequencing and initiation of speech movements, has been adapted to account for stuttering. Two neural characteristics of stuttering have been simulated in the model: elevated dopamine levels and deficient WM underneath left precentral gyrus. Both are suggested to interfere in the motor loop, i.e. the connection between BG and vPMC (Civier et al., 2013). This loop has two main functionalities: 1) biasing competition in the cortex to select the appropriate syllable, and 2) initiating the next syllable based on contextual signals. It is tempting to

suggest that the CNV reflects (partly) motor loop activations. Not only does the CNV reflect activity of the neural structures of the motor loop (e.g. Fan et al., 2007), this potential is also related to motor preparation (e.g. Bender et al., 2004), the processing stage that is provided by the motor loop. As such, the present CNV findings may confirm that stuttering is related to an impairment in the motor loop during feedforward modelling.

Finally, the present thesis shows that these motor impairments in feedforward modelling can occur without simultaneous deficits in auditory processing. The repeatedly observed simultaneous deactivation of auditory cortices and hyperactivation of motor cortices suggest that auditory-motor integration during speech production is altered in stuttering (e.g. Braun et al., 1997; Chang et al., 2009; Fox et al., 1996; Watkins et al., 2008). Because it remains to be determined which aspects of auditory processing and/or auditory-motor integration are exactly altered (Belyk et al., 2014), the present thesis solely used visual tasks to exclude an influence from auditory deficits. As motor alterations were clearly present, the current thesis proves that abnormal recruitment of motor regions does not depend on aberrant auditory input or deficits in auditory-motor integration.

B) Linguistic impairments

No consensus has been achieved on whether or not PWS exhibit linguistic impairments. It is, however, sure that PWS take longer to formulate their utterances. According to the psycholinguistic theories, this slowness stems from weaknesses in phonological, lexical and/or syntactic encoding. Many psycholinguistic theories on stuttering exist (for an overview, see Bloodstein & Ratner, 2008). The most well-known theory is the Covert Repair Hypothesis which attributes fluency failures to phonological encoding errors that are detected and repaired in inner speech by an internal monitoring system (Postma & Kolk, 1993). Because little experimental evidence is available for the Covert Repair Hypothesis, two important alternatives have been proposed. The Vicious Circle Hypothesis suggests that dysfluencies do not result from linguistic planning impairments, but can only be attributed to an overactive internal monitoring system (Vasić & Wijnen, 2005). The EXPLAN theory ascribes dysfluencies to a discrepancy between PLANning (linguistic and/or phonetic planning) and EXecution. Because of either a slowness in planning or a high speech rate, speech plans have not achieved a sufficient degree of completeness by the time they are executed (Howell, 2004).

How do the CNV findings relate to the psycholinguistic theories?

The CNV stems from motor research. Only a few speech/oral studies have been performed which mainly focused on non-speech oral movements like jaw opening and lip rounding (e.g. Yoshida & Iizuka, 2005). To our knowledge, no attempts have been made to link the CNV to speech production

models. As a result, it is not clear which processing stages underlying overt speech production are reflected in the CNV.

Based on models of speech production (Bohland et al., 2010; Guenther, 2006; Indefrey, 2011) and on neural findings of CNV research in general (e.g. Fan et al., 2007), it is tempting to suggest that phonetic encoding (in which a phonological word is transferred into articulatory motor programs) and word initial motor initiation (provided by the BG) are reflected in this ERP. Unfortunately, it is unclear how the delayed naming design of the task influenced the timing of the different processing stages. Only one study compared the sequence and duration of the linguistic processing stages during immediate and delayed picture naming (Laganaro & Perret, 2011). Similar encoding processes were revealed until 300 – 400 ms post-stimulus onset. From the beginning of phonological encoding onwards, the neuronal network diverged. Subjects seemed to prolong this process during delayed naming. Unfortunately, the authors did not evaluate the processes later on. Therefore, it remains obscure how much the phonological encoding process was altered and prolonged and how this influenced phonetic encoding.

Consequently, it is difficult to interpret the CNV findings in light of the psycholinguistic theories of stuttering. However, the CNV might be linked to the EXPLAN theory (Howell, 2004), not in the least because this theory also acknowledges the existence of motor deficits. The CNV might reflect atypical planning and the deficient interface between planning and execution because the suggested neural substrates of both are similar to the neural substrates of the CNV (e.g. Fan et al., 2007). Bilateral IFG and right putamen would be related to atypical planning, a weak connectivity between left IFG and left PMC would reflect the deficient interface between planning and execution (Lu et al., 2010a). EXPLAN is a model for connected speech in which planning and execution occur contiguously. The present picture naming task on the other hand allows speakers to plan before execution starts. According to EXPLAN, slowness in planning may be overcome due to this extra time. If true, the increased CNV slope preceding fluent words may reflect a successful compensation to overcome this slowness. Unfortunately, the unknown impact of the delayed nature of the task again prevents making clear hypotheses on which processing stage is involved in the CNV.

C) Is stuttering a language, a motor or a multiple-deficit disorder?

The findings of the present thesis seem to suggest that motor alterations in AWS can present without (large) simultaneous linguistic deficiencies. Linguistic impairments did not or only limitedly appear. During the perception paradigm, the amplitude and latency of the ERP peaks were evaluated as these reflect visual and linguistic processes (e.g. Dambacher et al., 2006; Hauk et al., 2012; Palazova et al., 2001; Zhang et al., 2009). No significant difference between FS and AWS was found for any of them. Moreover, when comparing action and non-action verbs, FS and AWS obtained similar results

for all the peaks (see table I). In the CNV task, FS and AWS showed a picture naming accuracy of 97% and 95% respectively. This 2% difference is, though significant, extremely small. Additionally, several adaptations in the analysis were performed to limit the influence of preceding linguistic processes (e.g. both stimulus- as response-locked analysis, slope instead of amplitude measurement). Overall, interferences of possible language impairments on the present tasks seem to be small to even absent.

In general, there is at present insufficient evidence to argue strongly for or against the motor or the psycholinguistic origin of stuttering. It is, however, a fact that stuttering ultimately presents itself as a disruption of the speech motor system through its primary speech characteristics (Max et al., 2004). Any cause of stuttering will finally result in a disruption of the muscular contractions and the movements required for speech. Moreover, extensive neurological evidence exists for deficits in brain structures related to speech motor control. Finally, the psycholinguistic theories can often not explain some motor characteristics of stuttering (Lickley et al., 2005). How, for example, can alterations in manual movements be related to a linguistic encoding problem?

This does not exclude any influence from or interaction with other processes. On the contrary, note that although a strong significant correlation was observed between the CNV slope preceding fluent words and the stuttering frequency/severity, the stuttering measures can 'only' explain 34 - 35% of the variance. Still 65% of the variance remains unknown which may reflect the impact of other factors. As such, the motor impairments might be seen as a final common pathway shared by all PWS, but affected by other factors. Their influence may differ from person to person explaining the large clinical variability typically seen among stuttering speakers.

Emotional and linguistic variables are two important influential factors in stuttering. Linguistic and articulatory processing cannot be seen as two separate, successive aspects of overt speech, but rather as interacting and influencing one another (Hickok, 2012). Indeed, the occurrence of a stutter appears to be determined by several linguistic variables like grammatical complexity and word frequency (Bloodstein & Ratner, 2008). Moreover, from a neurological point of view, there is a growing body of literature suggesting a functional interaction between language and motor systems (Pulvermüller & Fadiga, 2010; Watson & Chatterjee, 2011). As shown in chapter 6 for example, motor related areas aid in the lexico-semantic processing of action verbs, and maybe even in the linguistic processing of non-action verbs.

Emotional arousal is another important factor in stuttering (Bloodstein & Ratner, 2008). Depending on the speaking situation and/or the collocutor(s), large intra-individual differences in stuttering frequency may occur. This highlights that the motor control findings of the present thesis should be placed in a broader perspective. Whether or not a stutter occurs, also depends on the emotional arousal of a person. As several studies obtained conflicting results, it remains to be determined how

emotional arousal and speech motor control interact (Dietrich et al., 2012; Hennessey et al., 2014; van Lieshout et al., 2014).

1.2.2. Neurogenic versus developmental stuttering

The attempts to describe distinguishing features of NS and DS are challenged by studies showing considerable overlap in both behavioural (for a review, see Van Borsel, 1997) and neurological characteristics (Theys et al., 2012). To our knowledge, the present case report is the first study on NS which evaluated a specific neurophysiological process by use of EEG (*chapter 8*). An overview of the results of this case report and the DS group can be found in table III. Concerning the CNV slope preceding fluent words, a remarkable difference is seen between the NS case report and the DS group. While in DS an increase in CNV with increasing stuttering frequency was seen, the NS case report displayed a decrease in CNV with increasing stuttering frequency. These results suggest that NS and DS may have inverse neurophysiological functions.

However, when taking the interpretation of the CNV results into account, it seems that this assertion should be slightly nuanced. In DS, the increased CNV preceding fluent word production is interpreted as a compensation strategy which is most pronounced over the right hemisphere. Also the NS case report showed at the moment of most frequent stuttering an increase in CNV amplitude over the right hemisphere which was cautiously suggested to be a compensation attempt. The negative association between CNV amplitude over left and midline electrodes and stuttering frequency in the NS case report is suggestive for a causal link. Indeed, MH's lesion site is located in the left hemisphere, namely left STG. Also in the DS group, a decrease in left CNV slope (preceding stutters) is observed with increasing stuttering frequency. This decrease is hypothesized to be related to the stuttering pathology as well. In sum, the CNV results of NS and DS seem not entirely opposite when the interpretation of the CNV changes are taken into account. However, important differences remain e.g. the decrease in left CNV is associated with stuttered speech in DS and with fluent speech in the NS case report.

Although NS and DS are related to disturbances in the same brain network (Theys et al., 2012) which may result in a non-distinguishable phenotypical appearance (Van Borsel, 1997; Van Borsel & Taillieu, 2001), large differences in the neurophysiological processes that precede overt speech may still be present. The underlying brain network shows different abnormalities in motor preparation, probably due to a difference in lesion localisation (left STG for the NS case report, somewhere in left BGTC – loop for the DS group). These results suggest that the 'common neural characteristics of DS and NS' (Theys et al., 2012) may be limited to neuroanatomical findings. DS and NS may show considerable variation in neurophysiological functioning.

1.2.3. Natural recovery and male/female ratio

Although a large percentage of CWS (68 % to 96%) will recover spontaneously (for a review, see Yairi and Ambrose, 2013), only a few neurological studies compared persistent and recovered PWS. These studies attributed recovery to 1) SMA maturation (Forster & Webster, 2001), 2) structural anomalies in left IFG that are restricted to grey matter (while persistent CWS also showed WM anomalies underneath this region) (Chang et al., 2008), and 3) increased activation in left frontal operculum (BA 47/12) (Kell et al., 2009). Both the SMA and IFG are part of the BGTC – circuit and important contributors to speech motor preparation and initiation (Bohland et al., 2010). These studies seem to confirm the importance of the BGTC – network in stuttering. Besides having a role in the distal and proximal origin of stuttering, this circuit also seems to support spontaneous recovery in stuttering.

Spontaneous recovery is also suggested to occur more frequently in girls than in boys (Yairi & Ambrose, 2005). At onset, the male/female ratio is nearly 1:1 (Månsson, 2005), while in adults this ratio increases to on average 3:1 (Bloodstein & Ratner, 2008). If the BGTC – loop has a role in natural recovery, gender related differences may be expected in this loop. In the general population, girls are found to show an earlier maturation of the BG than boys (Lenroot & Giedd, 2010). Also in DS, gender related differences in subcortical structures are reported. Stuttering rate was found to correlate positively with BG activity in women and with CB activity in men (Ingham et al., 2004). These findings suggest that a difference in the BGTC – network may contribute to gender differences in natural recovery. Unfortunately, no clear hypothesis can be formulated because gender studies in the general population show large inconsistencies (Giedd et al., 2012) and neural differences between men and women are only rarely explored in DS. Also the present thesis did not perform gender comparisons. Because the typical male/female ratio found in adulthood was reflected in our group of participants, the group of women was much smaller than the group of men. Future research might include larger groups and more women to evaluate potential gender differences in stuttering concerning neurophysiological functioning.

1.2.4. Why adults as participants and not children?

Neurological research is always more challenging in children than in adults. One of the major problems is to keep them sit still. For EEG specifically, another challenge concerns the analysis and interpretation of the EEG as the EEG of children is fundamentally different than the EEG of adults. Variability is the rule. This applies to the background rhythmic activity as well as to the presence of generalized irregular slow waves (Rowan & Tolunsky, 2003). For these reasons, electrophysiology would be quite challenging in children at stuttering onset. As we were a new research group and electrophysiology focusing on speech motor control in stuttering is a rather unexplored area, it was decided to avoid these problems and focus on adults instead of children at stuttering onset.

From the age of 7 – 8 years onwards, the EEG becomes more similar to an adult EEG. However, another difficulty in this age range concerns the duration of stuttering (since stuttering onset). This may vary largely and might have an (unknown) impact on the brain. E.g. a child of 7 years old that has stuttered for 4 – 5 years versus a child of 7 years old that has stuttered for 1 year. As the brain is very plastic, especially during childhood, one would expect that these 4 years difference will have an impact on the brain. The first child might have developed much more compensation strategies than the second child.

Moreover, the advantage of including adults is that it allowed finding a compensation strategy. This confirms that certain alterations found in neurological studies are related to compensation and not to the cause of stuttering.

1.3. Concerns and limitations

As mentioned in more detail in the separate research chapters, some concerns raise about the tasks used in the present thesis. For the perception paradigm, the main concern regards the limited spatial resolution of the source reconstruction. As motor involvement during verb processing is not linked to a certain ERP (e.g. Pulvermüller et al., 2001, 2005), source reconstruction had to be performed to be able to evaluate motor activations. The strength of EEG research is however its excellent temporal, not its spatial resolution. To meet this concern, no fine grained analyses nor interpretations were performed.

For the CNV task, the main concern involves the potential influence of other factors on the CNV like anticipation and linguistic processing. From a psycholinguistic point of view, it is not clear which processing stage(s) is(are) reflected in this ERP. Also the influence of the delayed nature of the task is unclear. Future research is necessary to address this issue.

A little elaboration is necessary on the statement that the CNV may be a neural correlate of stuttering frequency and severity. First, it should be noted that the stuttering measures were not performed during the CNV task itself. They were based on speech samples recorded *before* the EEG registration. Unfortunately, EEG is very sensitive to muscular artefacts making it difficult to analyze EEG trials *during* overt connected speech. Stuttering frequency/severity determined on a single-word production task is, however, not a valuable estimate of an individual's speech fluency abilities. Therefore, stuttering measures had to be determined based on speech samples not related to the experimental task similar to previous studies (Chang et al., 2009; Giraud et al., 2008; Kell et al., 2009; Preibisch et al., 2003). These studies also reported important correlations with neural activations. The single-word design of the CNV study is not without advantage. Although studies evaluating neural activity during discourse may give a reliable estimate of neural activity in daily life situations (e.g. Fox et al., 2000; Ingham et al., 2004, 2012), they present neural activity related to fluent ànd

stuttered speech which may be very different (e.g. Sowman et al., 2012). A single-word production task allows the comparison of pure fluent and pure stuttered speech without contaminating one with the other.

Secondly, stuttering is known to vary across speaking tasks and situations (Bloodstein & Ratner, 2008). By following the SSI-4 (Riley, 2008) and restricting the analysis to a reading and a conversation sample, the present stuttering measures cannot fully reflect an individual's stutter variability. Stuttering may also vary in time, with periods of more fluent or disfluent speech even within the same day (Bloodstein & Ratner, 2008). Therefore, speech samples and EEG were registered one after the other, within about an hour. As such, the stuttering measures provide a reflection of stuttering frequency/severity around the moment that the CNV was evaluated.

In general, even though EEG is not yet able to reflect stuttering frequency/severity measured online, it seems it will have the capacity to provide valuable information on stuttering measures in the future. The NS case report already showed that the CNV is able to reflect even small intra-individual differences in stuttering frequency. Moreover, as technology improves daily, some of the inherent EEG weaknesses like muscular artefact sensitivity may be overcome. Algorithms can be created which detect and delete speech muscle artefacts, even in the context of stuttering (Tran et al., 2004). Cortico-muscular coherence (CMC), a measure of efficient cortex-muscle neurocommunication, is recently suggested to be able to provide information on neural functioning *during* speech. CMC is a correlation measure between oscillatory signals like EEG and EMG. CMC is a rather unexplored electrophysiological measure in the context of speech production. Only recently, it has been cautiously investigated (Caviness et al., 2006; Ruspantini et al., 2012).

1.4. Conclusion

Based on the research aims of the present thesis, we can conclude:

(1) Considerable alterations in the temporal coordination of motor related activations occur in DS, even during a silent reading task in which no overt speech or any other movement is required. Neural motor abnormalities in DS are not restricted to deficits in overt speech production.

(2) Motor preparatory activity generated in the BGTC – loop has a crucial role in DS. Large differences occur preceding fluent and stuttered speech. While the amount of activation in right BGTC – loop seems to have a determining role in the actual moment of a stutter, the left BGTC – loop activity preceding a stutter seems to be related to the underlying stuttering pathology.

(3) The NS case report shows that intra-individual variability in stuttering frequency has a strong association with speech motor preparation. The results of the DS group add that the same accounts for inter-individual variability in stuttering measures.

(4) The proposed common neural characteristics of DS and NS seem to be limited to neuroanatomical findings. Both types of stuttering may show considerable variation in neurophysiological functioning.

Additionally, the present studies suggest that the observed motor alterations are related to deficiencies in the motor loop of feedforward processing. These motor alterations can present without simultaneous deficits in feedback processing or without obvious inferences of language impairments. In general, the present thesis evidences that neurophysiology is able to discover interesting and intriguing neural findings that may aid in unravelling the enigma of stuttering.

2. Future directions

The present thesis is a general plea to use neurophysiological tools more often in stuttering research. Due to its excellent temporal resolution, it is a very promising field of research to enlarge the neurological knowledge on stuttering.

2.1. CNV research

More fundamental research on the CNV in speech production tasks is necessary to elucidate which processing stages are reflected in this ERP. Moreover, the impact of the delayed nature of this kind of task should be clarified as well. For stuttering specifically, future research may focus more on the impact of other factors on speech motor control. Fluent speech does not only depend on normal motor processes. Emotional and linguistic variables may disturb these processes as well. The CNV can be of help in exploring these influences. By varying tasks and task demands, the effect on the CNV and on the difference in CNV between FS and AWS can be evaluated.

2.2. Stuttered speech

In research, collecting enough stuttered samples to perform a valid analysis is challenging as the majority of PWS mainly speak fluent in experimental settings. Many studies obtained too little stuttered speech and excluded these trials from further analysis. The present thesis, however, illustrates the importance of evaluating pure stuttered speech. Stuttered and fluent speech in PWS can be associated with very different neural findings. In this thesis, the inclusion of a large number of PWS and many trials per participant enabled a separate analysis for stuttered speech. Therefore, future neurological research might consider expanding their subject group and enlarging their tasks. Even if only one participant produced enough stutters to perform a valid analysis, it might be very interesting to report them in a case report, just as in Sowman et al., (2012).

2.3. Children who stutter

The neural activity and morphology pattern observed in adults reflects, besides core dysfunctions related to stuttering onset and development, also neuroplastic changes associated with compensatory and coping strategies that were developed over the years. This is shown in the present thesis as well, as the increased CNV slope preceding fluent speech production is hypothesized to be a successful compensation strategy. Because CWS show less to even none of these neuroplastic changes, research in children is of great importance to unravel the neurogenic cause of stuttering. Although neurophysiological research in CWS is still in its infancy, promising results have been obtained and most importantly, the feasibility of EEG and MEG studies in CWS has been demonstrated. Unfortunately, these studies have been limited to language (e.g. Weber-Fox et al.,

2008) and auditory processing (Jansson-Verkasalo et al., 2014). It might be of great interest to use neurophysiological tools to evaluate speech motor control in CWS as well.

2.4. Neurogenic stuttering

A final recommendation is to implement neurophysiological evaluations in research concerning acquired stuttering, and especially NS. Not only would this enlarge our knowledge on these other forms of stuttering and how they relate to DS, it would also provide information on which neurophysiological alterations can be associated with stuttering. Moreover, as the lesion site can usually be identified in NS patients, information on the neurophysiological impact of this lesion on the brain network involved in speech can be obtained.

References

Abe, K., Yokomay, R., & Yorifuji, S. (1993). Repetitive speech disorder resulting from infarcts in the paramedian thalami and midbrain. *Joural of Neurology, Neurosurgery, and Psychiatry*(56), 1024-1026.

Ackermann, H. (2008). Cerebellar contributions to speech production and speech perception: psycholinguistic and neurobiological perspectives. *Trends in Neurosciences*(31), 265-72.

Adams, M., Lewis, J., & Besozzi, T. (1973). The effect of reduced reading rate on stuttering frequency. *Journal of speech and hearing research*(16), 671-675.

Aerts, A., van Mierlo, P., Hartsuiker, R., Hallez, H., Santens, P., & De Letter, M. (2013). Neurophysiological investigation of phonological input: aging effects and development of normative data. *Brain and Language*(125), 253-263.

Alexander, G., & Crutcher, M. (1990). Functional architecture of basal ganglia circuits: neural substrates of parallel processing. *Trends in neuroscience*(13), 266-71.

Alexander, G., DeLong, M., & Strick, P. (1986). Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annual Review of Neuroscience*(9), 357-381.

Alm, P. (2004). Stuttering and the basal ganglia circuits: a critical review of possible relations. *Journal of Communication Disorders*(37), 325-69.

Amabile, G., Fattapposta, F., Pozzessere, G., Albani, G., Sanarelli, L., Rizzo, P., & Morocutti, C. (1986). Parkinson disease: electrophysiological (CNV) analysis related to pharmacological treatment. *Electroencephalography and Clinical Neurophysiology*(64), 521-4.

Ambrose, N., & Yairi, E. (1999). Normative fluency data for early childhood stuttering. *Journal of Speech, Language, and Hearing Research*(42), 895-909.

Ambrose, N., Cox, N., & Yairi, E. (1997). The genetic basis of persistence and recovery in stuttering. *Journal of Speech, Language and Hearing research*(40), 567-80.

American Speech-Language-Hearing Association. (1999). *Terminology pertaining to fluency and fluency disorders: guidelines.* www.asha.org/policy.

Andrews, G., & Harris, M. (1964). *The syndrome of stuttering, Clinics in developmental medicine, Vol. 17.* London: William Heineman Medical Books Ltd.

Andrews, G., Morris-Yates, A., Howie, P., & Martin, N. (1991). Genetic factors in stuttering confirmed. *Archives of General Psychiatry*(48), 1034-35.

Ardila, A., Bateman, J., & Nino, C. (1994). An epidemiologic study of stuttering. *Journal of Communication Disorders*(27), 37-48.

Arévalo, A., Baldo, J., & Dronkers, N. (2012). What do brain lesions tell us about theories of embodied semantics and the human mirror neuron system. *Cortex, 48,* 242-254.

Aziz-Zadeh, L., Wilson, S., Rizzolatti, G., & Iacoboni, M. (2006). Congruent embodied representations for visually presented actions and linguistic phrases describing actions. *Current Biology, 16,* 1818-1823.

Baayen, R., Piepenbrock, R., & van Rijn, H. (1995). The CELEX lexical data base. (U. o. Pennsylvania, Red.) Philadelphia: Linguistic Data Consortium.

Bak, T., & Hodges, J. (1999). Cognition, language and behaviour in motor neuron disease: evidence of frontotemporal dysfunction. *Dement Geriatr. Cogn. Disord*, *10,* 29-32.

Bak, T., & Hodges, J. (2004). The effects of motor neuron disease on language: further evidence. *Brain and Language*, *89,* 354-361.

Bak, T., O'Donovan, D., Xuereb, J., Boniface, S., & Hodges, J. (2001). Selective impairment of verb processing associated with pathological changes in Brodmann areas 44 and 45 in the motor neuron disease-dementia-aphasia syndrome. *Brain, 124,* 103-120.

Bak, T., Yancopoulou, D., Nestor, P., Xuereb, J., Spillantini, M., Pulvermüller, F., & Hodges, J. (2006). Clinical, imaging and pathological correlates of hereditary deficit in verb and action processing. *Brain, 129,* 321-32.

Barber, H., Otten, L., Kousta, S.-T., & Vigliocco, G. (2013). Concreteness in word processing: ERP and behavioral effects in a lexical decision task. *Brain and Language, 125,* 47-53.

Bares, M., Nestrasil, I., & Rektor, I. (2007). The effect of response type (motor output versus mental counting) on the intracerebral distribution of the slow cortical potentials in an externally cured (CNV) paradigm. *Brain Research Bulletin*(7), 428-435.

Barsalou, L. (1999). Perceptual symbol systems. *Behavioral Brain Sciences*(22), 577-660.

Bastiaanse, R., Bosje, M., & Visch-Brink, E. (1995). *Psycholinguïstische Testbatterij voor Onderzoek naar de Taalverwerking van Afasiepatiënten (PALPA).* Hove, East Sussex: Psychology Press.

Baumgartner, J. (1999). Acquired psychogenic stuttering. In R. Curlee, *Stuttering and related disorders of fluency, 2nd edition* (pp. 269-288). New York: Thieme Medical Publishers, Inc.

Beal, D., Cheyne, D., Gracco, V., Quraan, M., Taylor, M., & De Nil, L. (2010). Auditory evoked fields to vocalization during passive listening and active generation in adults who stutter. *NeuroImage*(52), 1645-53.

Beal, D., Gracco, V., Brettschneider, J., Kroll, R., & De Nil, L. (2013). A voxel-based morphometry (VBM) analysis of regional grey and white matter volume abnormalities within the speech production network of children who stutter. *Cortex*(49), 2151-61.

Beal, D., Quraan, M., Cheyne, D., Taylor, M., Gracco, V., & De Nil, L. (2011). Speech-induced suppression of evoked auditory fields in children who stutter. *NeuroImage*(54), 2994-3003.

Beck, C. (2000). *Literatuurstudie: stotteren en medicatie. Licentiaatsverhandeling.* Universiteit Gent: Faculteit Geneeskunde.

Belyk, M., Kraft, S., & Brown, S. (2014). Stuttering as a trait or state - an ALE meta-analysis of neuroimaging studies. *European Journal of Neuroscience*, 1-10.

Bender, S., Resch, F., Weisbrod, M., & Oelkers-Ax, R. (2004). Specific task anticipation versus unspecific orienting reaction during early contingent negative variation. *Clinical neurophysiology*(115), 1836-1845.

Bezemer, M., Bouwen, J., Winkelman, K., & Embrechts, M. (2010). *Stotteren: van theorie naar therapie.* Bussum: Coutinho.

Biermann-Ruben, K., Salmelin, R., & Schnitzler, A. (2005). Right rolandic activation during speech perception in stutterers: a MEG study. *NeuroImage*(25), 793-801.

Binder, J., Desai, R., Graves, W., & Conant, L. (2009). Where is the semantic system? A critical review and meta-analysis of 120 functional neuroimaging studies. *Cerebral Cortex, 19,* 2767-96.

Bishop, J., Williams, H., & Cooper, W. (1991). Age and task complexity variables in motor-performance of stuttering and nonstuttering children. *Journal of fluency disorders*(16), 207-217.

Blakemore, S., Wolpert, D., & Frith, C. (1998). Central cancellation of self-produced tickle sensation. *Nature neuroscience*(1), 635-40.

Bloodstein, O. (1970). Stuttering and normal nonfluency - a continuity hypothesis. *Britisch journal of disorders of communication*(5), 30-9.

Bloodstein, O. (1988). Verification of stuttering in a suspected malingerer. *Journal of Fluency Disorder*(13), 83-88.

Bloodstein, O., & Ratner, N. (2008). *A handbook on stuttering.* Delmar: Cengage Learning.

Bluemel, C. (1935). *Stammering an allied disorders.* New York: McMillan.

Boberg, E., Yeudall, L., Schopflocher, D., & Bo-Lassen, P. (1983). The effects of an intensive behavioral program on the distribution of EEG alpha power in stutterers during the processing of verbal and visuospatial information. *Journal of fluency disorders*(8), 245-63.

Boehler, C., Appeldaum, L., Krebs, R., Hopf, J., & Woldorff, M. (2010). Pinning down response inhibition in the brain - Conjunction analyses of the Stop-signal task. *NeuroImage*(52), 1621-32.

Boey, R., Wuyts, F., Van De Heyning, P., Heylen, L., & De Bodt, M. (2009). Characteristics of stuttering in Duth-speaking individuals. *Clinical Linguistics & Phonetics*(23), 241-254.

Bohland, J., & Guenther, F. (2006). An fMRI investigation of syllable sequence production. *NeuroImage*(32), 821-41.

Bohland, J., Bullock, D., & Guenther, F. (2010). Neural representations and mechanisms for the performance of simple speech sequences. *Journal of Cognitive Neuroscience*(22), 1504-29.

Borghi, A., & Cimatti, F. (2010). Embodied cognition and beyond: acting and sensing the body. *Neuropsychologia, 48,* 763-73.

Borghi, A., Flumini, A., Cimatti, F., Marocco, D., & Scorolli, C. (2011). Manipulating objects and telling words: a study on concrete and abstract words acquisition. *Frontiers in psychology*, 2, 1-14.

Bosshardt, H.-G., & Fransen, H. (1996). On-line sentence processing in adults who stutter and who do not stutter. *Journal of Speech and Hearing Research*(39), 785-97.

Boulenger, V., Hauk, O., & Pulvermüller, F. (2009). Grasping ideas with the motor system: semantic somatotopy in idiom comprehension. *Cerebral Cortex, 19,* 1905-1914.

Boulenger, V., Roy, A., Paulignan, Y., Deprez, V., Jeannerod, M., & Nazir, T. (2006). Cross-talk between language processes and overt motor behavior in the first 200 ms of processing. *Journal of Cognitive Neuroscience, 18,* 1607-15.

Boyd, A., Dworzynski, K., & Howell, P. (2011). Pharmacological agents for developmental stuttering in children and adolescents: a systematic review. *Journal of Clinical Psychopharmacology*(31), 740-4.

Brady, J. (1998). Drug-induced stuttering: a review of the literature. *Journal of Clinical Psychopharmacology*(18), 50-4.

Braun, A., Varga, M., Stager, S., Schulz, G., Selbie, S., Maisog, J., Carsun, R., & Ludlow, C. (1997). Altered patterns of cerebral activity during speech and language production in developmental stuttering: a H2 15O positron emission tomography study. *Brain*(120), 761-84.

Brem, S., Bucher, K., Halder, P., Summers, P., Dietrich, T., Martin, E., & Brandeis, D. (2006). Evidence for developmental changes in the visual word processing network beyond adolescence. *NeuroImage, 29,* 822-837.

Brown, S. (1938). A further study of stuttering in relation to various speech sounds. *Quarterly journal of speech*(24), 390-397.

Brown, S. (1945). The loci of stutterings in the speech sequence. *Journal of Speech Disorders*(10), 181-92.

Brown, S., Ingham, R., Ingham, J., Laird, A., & Fox, P. (2005). Stuttered and fluent speech production: an ALE meta-analysis of functional neuroimaging studies. *Human Brain Mapping*(25), 105-17.

Buccino, G., Riggio, L., Melli, G., Binkofski, F., Gallese, V., & Rizzolatti, G. (2005). Listening to action-related sentences modulates the activity of the motor system: a combined TMS and behavioral study. *Cognitive Brain Research*, *24,* 355-363.

Buck, S., Lees, R., & Cook, F. (2002). The influence of family history of stuttering on the onset of stuttering in young children. *Folia Phoniatrica et Logopaedica*(54), 117-124.

Budde, K., Barron, D., & Fox, P. (2014). Stuttering, induced fluency, and natural fluency: a hierarchical series of activation likelihood estimation meta-analyses. *Brain and Language*(139), 99-107.

Burger, R., & Wijnen, F. (1999). Phonological encoding and word stress in stuttering and nonstuttering subjects. *Journal of Fluency Disorders*(24), 91-106.

Busan, P., D'Ausilio, A., Borelli, M., Monti, F., Pelamatti, G., Pizzolato, G., & Fadiga, L. (2011). Motor excitability evaluation in developmental stuttering: a transcranial magnetic stimulation study. *Cortex*(49), 781-92.

Busse, E., & Clark, R. (1957). The use of the electroencephalogram in diagnosing speech disorders in children. *Folia Phoniatrica*(9), 182-7.

Buxbaum, L., & Saffran, E. (2002). Knowledge of object manipulation and object function: dissociations in apraxic and nonapraxic subjects. *Brain and Language*, *82,* 179-199.

Cai, S., Beal, D., Ghosh, S., Guenther, F., & Perkell, J. (2014a). Impaired timing adjustments in response to time-varying auditory perturbation during conneccted speech production in persons who stutter. *Brain and Language*(129), 24-9.

Cai, S., Beal, D., Ghosh, S., Tiede, M., Guenther, F., & Perkell, J. (2012). Weak responses to auditory feedback perturbation during articulation in persons who stutter: evidence for abnormal auditory-motor transformation. *Plos One*, doi:10.1371/journal.pone.0041830.

Cai, S., Tourville, J., Beal, D., Perkell, J., Guenther, F., & Ghosh, S. (2014b). Diffusion imaging of cerebral white matter in persons who sutter: evidence for network-level anomalies. *Frontiers in Human Neuroscience*, doi: 10.3389/fnhum.2014.00054.

Canevini, M., Chifferi, R., & Piazzino, A. (2002). Improvement of a patient with stuttering on levetiracetam. *Neurology*(59), 1288.

Canter, G. (1971). Observations on neurogenic stuttering: a contribution to differential diagnosis. *British journal of disorders of communication*(6), 139-43.

Cappa, S., Binetti, G., Pezzini, A., Padovani, A., Rozzini, L., & Trabucchi, M. (1998). Object and action naming in Alzheimer's disease and frontotemporal dementia. *Neurology*, *50,* 351-355.

Carbonnell, L., Hasbroucq, T., Grapperon, J., & Vidal, F. (2004). Response selection and motor areas: a behavioural and electrophysiological study. *Clinical Neurophysiology*(115), 2164-74.

Carota , F., Moseley, R., & Pulvermüller, F. (2012). Body-part-specific representations of semantic noun categories. *Journal of cognitive neuroscience*(24), 1492-1509.

Caviness, J., Liss, J., Adler, C., & Evidente, V. (2006). Analysis of high-frequency electroencephalographic-electromyographic coherence elicited by speech and oral nonspeech tasks in Parkinson's Disease. *Journal of speech, language and hearing research*(49), 424-38.

Cerella, J. (1985). Information processing rates in elderly. *Psychology Bulletin*, *98,* 67-83.

Chang, S. (2011). Using brain imaging to unravel the mysteries of stuttering. *Cerebrum*(12).

Chang, S.-E., & Zhu, D. (2013). Neural network connectivity differences in children who stutter. *Brain*, doi:10.1093/brain/awt275.

Chang, S.-E., Erickson, K., Ambrose, N., Hasegawa-Johnson, M., & Ludlow, C. (2008). Brain anatomy differences in childhood stuttering. *NeuroImage*(39), 1333-1344.

Chang, S.-E., Horwitz, B., Ostuni, J., Reynolds, R., & Ludlow, C. (2011). Evidence of left inferior frontal-premotor structural and functional connectivity deficits in adults who stutter. *Cerebral Cortex*(21), 2507-18.

Chang, S.-E., Kenney, M., Loucks, T., & Ludlow, C. (2009). Brain activation abnormalities during speech and non-speech in stuttering speakers. *NeuroImage*(46), 201-212.

Chang, S.-E., Zhu, D., Choo, A., & Angstadt, M. (2015). White matter neuroanatomical differences in young children who stutter. *Brain*, doi:10.1093/brain/awu400.

Chao, L., & Martin, A. (2000). Representation of manipulable man-made objects in the dorsal stream. *NeuroImage*(12), 478-84.

Choo, A., Kraft, S., Olivero, W., Ambrose, N., Sharma, H., Chang, S., & Loucks, T. (2011). Corpus callosum differences associated with persistent stuttering in adults. *Journal of communication disorders*(44), 470-7.

Christoffels, I., Formisano, E., & Schiller, N. (2007). Neural correlates of verbal feedback processing: an fMRI study employing overt speech. *Human Brain Mapping*(28), 868-79.

Cieslak, M., Ingham, R., Ingham, J., & Grafton, S. (2015). Anomalous white matter morphology in adults who stutter. *Journal of speech, language and hearing research*, doi: 10.1044/2015_JSLHR-S-14-0193.

Civier, O., Bullock, D., Max, L., & Guenther, F. (2013). Computational modeling of stuttering caused by impairments in a basal ganglia thalamo-cortical circuit involved in syllable selection and initiation. *Brain and Language*(126), 263-78.

Civier, O., Tasko, S., & Guenther, F. (2010). Overreliance on auditory feedback may lead to sound/syllable repititions: simulations of stuttering and fluency-inducing conditions with a neural model of speech production. *Journal of fluency disorders*(35), 246-79.

Connally, E., Ward, D., Howell, P., & Watkins, K. (2014). Disrupted white matter in language and motor tracts in developmental stuttering. *Brain and language*(131), 25-35.

Cooper, R., Todd, J., McGill, K., & Michie, P. (2006). Auditory sensory memory and the aging brain: a Mismatch Negativity study. *Neurobiology of Aging*(27), 752-762.

Corballis, M. (2002). *From hand to mouth: the origins of language.* Princeton (NJ): Princeton University Press.

Corbera, S., Corral, M.-J., Escera, C., & Idiazabal, M. (2005). Abnormal speech sound representation in persistent developmental stuttering. *Neurology*(65), 1246-52.

Craig, A., Hancock, K., Tran, Y., Craig, M., & Peters, K. (2002). Epidemiology of stuttering in the community across the entire life span. *Journal of Speech, Language and Hearing Research*(45), 1097-1105.

Cuadrado, E., & Weber-Fox, C. (2003). Atypical syntactic processing in individuals who stutter: evidence from event-related brain potentials and behavioral measures. *Journal of Speech, Language and Hearing Research*(46), 960-976.

Cui, R., Egkher, A., Huter, D., Lang, W., Lindinger, G., & Deecke, L. (2000). High resolution spatiotemporal analysis of the contingent negative variation in simple or complex motor tasks and a non-motor task. *Clinical Neurophysiology*(111), 1847-1859.

Cunnington, R., Iansek, R., Thickbroom, G., Laing, B., Mastaglia, F., Bradshaw, J., & Phillips, J. (1996). Effects of mangetic stimulation over supplementary motor area on movement in Parkinson's disease. *Brain*(119), 815-822.

Cunnington, R., Windischberger, C., Deecke, L., & Moser, E. (2002). The preparation and execution of self-initiated and externally-triggered movement: a study of event-related fMRI. *NeuroImage*(15), 373-385.

Curio, G., Neuloh, G., Numminen, J., Jousmäki, V., & Hari, R. (2000). Speaking modifies voice-evoked activity in the human auditory cortex. *Human Brain Mapping*(9), 183-91.

Cykowski, M., Fox, P., Ingham, R., Ingham, J., & Robin, D. (2010). A study of the reproducibility and etiology of diffusion anisotrophy differences in developmental stuttering: a potential role for imparied myelination. *NeuroImage*(52), 1495-504.

Dambacher, M., Kliegl, R., Howmann, M., & Jacobs, A. (2006). Frequency and predictability effects on event-related potentials during reading. *Brain Research*(1084), 89-103.

Daniele, A., Giustolisi, L., Silveri, M., Colosimo, C., & Gianotti, G. (1994). Evidence for a possible neuroanatomical basis for lexical processing of nouns and verbs. *Neuropsychologia, 32,* 1325-1341.

Dehaene, S., & Changeux, J. (2011). Experimental and theoretical approaches to conscious processing. *Neuron*, *2,* 200-27.

De Nil, L., Beal, D., Lafaille, S., Kroll, R., Crawley, A., & Gracco, V. (2008). The effects of simulated stuttering and prolonged speech on the neural activation patterns of stuttering and nonstuttering adults. *Brain and Language*(107), 114-23.

De Nil, L., Kroll, R., & Houle, S. (2001). Functional neuroimaging of cerebellar activation during single word reading and verb generation in stuttering and nonstuttering adults. *Neuroscience Letters*(302), 77-80.

De Nil, L., Kroll, R., Kapur, S., & Houle, S. (2000). A positron emission tomography study of silent and oral single word reading in stuttering and nonstuttering adults. *Journal of speech, language and hearing research*(43), 1038-1053.

De Nil, L., Kroll, R., Lafaille, S., & Houle, S. (2003). A positron emission tomography study of short- and long-term effects on functional brain activation in adults who stutter. *Journal of fluency disorders*(28), 357-380.

De Nil, L., Rochon, E., & Jokel, R. (2009). Adult-onset neurogenic stuttering. In M. McNeil, *Clinical management of sensorimotor speech disorders (2nd ed.)* (pp. 235-248). New York: Thieme.

den Ouden, D., Montgomery, A., & Adams, C. (2014). Simulating the neural correlates of stuttering. *Neurocase*(20), 434-45.

Deal, J., & Doro, J. (1987). Episodic hysterical stuttering. *Journal of Speech and Hearing Disorders*(52), 299-300.

Dell, G. (1986). A spreading activation theory of retrieval in language production. *Psychology Reviews*(93), 283-321.

Dietrich, M., Andreatta, R., Jiang, Y., Joshi, A., & Stemple, J. (2012). Preliminary findings on the relation between the personality trait of stress reaction and the central neural control of human vocalization. *International journal of speech-language pathology*(14), 377-89.

Di Russo, F., Martinez, A., Sereno, M., Pitzalis, S., & Hillyard, S. (2001). Cortical sources of the early components of the visual evoked potential. *Human Brain Mapping, 15,* 95-111.

Douglas, L. (1943). A study of bilateraly recorded electroencephalograms of adult stutterers. *Journal of experimental psychology*(32), 247-65.

Dove, G. (2009). Beyond perceptual symbols: a call for representational pluralism. *Cognition*(110), 412-431.

Druks, J. (2002). Verbs and nouns - a review of the literature. *Journal of Neurolinguistics*, *15,* 289-315.

Duffy, J. (2013). *Motor Speech Disorders. Substrates, Differential Diagnosis, and Management, 3rd edition.* St. Louis, Missouri: Elsevier Mosby.

Duncan, C., Barry, R., Connolly, J., Fischer, C., Michie, P., Näätänen, R., Polich, J., Reinvang, I., & Van Petten, C. (2009). Event-related potentials in clinical research: guidelines for eliciting, recording, and quentifying MMN, P300 and N400. *Clinical Neurophysiology*, *120,* 1883-908.

Duyck, W., Desmet, T., Verbeke, L., & Brysbaert, M. (2004). WordGen: a tool for word selection and nonword generation in Dutch, English, German, and French. *Behavior Research Methods, Instruments, & Computers*, *36,* 488-499.

Dworzynski, K., Remington, A., Rijsdijk, F., Howell, P., & Plomin, R. (2007). Genetic etiology in cases of recovered and persistent stuttering in an unselected longitudinal sample of young twins. *American Journal of Speech and Language Pathology*(16), 169-78.

Eggers, K. (2012). *Temporal characteristics of children with developmental stuttering.* Leuven - Tilburg: Doctoral thesis.

Eggers, K., De Nil, L., & Van den Bergh, B. (2013). Inhibitory control in childhood stuttering. *Journal of Fluency Disorders*(38), 1-13.

Egorova, N., Shtyrov, Y., & Pulvermüller, F. (2013). Early and parallel processing of pragmatic and semantic information in speech acts: neurophysiological evidence. *Frontiers in human neuroscience*, doi: 10.3389/fnhum.2013.00086.

Eickhoff, S.B., Paus, T., Caspers, S., Grosbras, M.-H., Evans, A.C., Zilles, K., Amunts, K., 2007. Assignment of functional activations to probabilistic cytoarchitectonic areas revisited. Neuroimage, *36,* 511-521.

Einarsdottir, J., & Ingham, R. (2005). Have disfluency tipe measures contributed to the understanding and treatment of developmental stuttering? *Americal Journal of Speech-Language pathology*(14), 260-273.

Etchell, A., Johnson, B., & Sowman, P. (2014). Behavioral and multimodal neuroimaging evidence for a deficit in brain timing networks in stuttering: a hypothesis and theory. *Frontiers in Human Neuroscience*(8), doi: 10.3389/fnhum.2014.00467.

Fan, J., Kolster, R., Ghajar, J., Suh, M., Knight, R., Sarkar, R., & McCandliss, B. (2007). Response anticipation and response conflict: an event-related potential and functional magnetic resonance imaging study. *The Journal of Neuroscience*(28), 2272-82.

Federmeier, K., & Kutas, M. (2001). Meaning and modality: influences of context, semantic momory organization and perceptual predictability on picture processing. *J. Exp. Psychol. Learn Mem. Cogn*, *27,* 202-224.

Felsenfeld, S., Kirk, K., Zhu, G., Statham, D., Neale, M., & Martin, N. (2000). A study of the genetic and environmental etiology of stuttering in a selected twin sample. *Behavioral Genetics*(30), 359-366.

Fernandino, L., Conant, L., Binder, J., Blindauer, K., Hiner, B., Spangler, K., & Desai, R. (2012). Parkinson's disease disrupts both automatic and controlled processing of action verbs. *Brain and Language*, http://dx.doi.org/10.1016/j.bandl.2012.07.008.

Field, A. (2009). *Discovering statistics using SPSS, third edition.* London: Sage.

Fodor, J. (1998). *Concepts. Where cognitive science went wrong.* Cambridge: MA: Harvard University Press.

Folstein, J., & Van Petten, C. (2008). Influence of cognitive control and mismatch on the N2 component of the ERP: a review. *Psychophysiology, 45,* 152-170.

Forster, D., & Webster, W. (2001). Speech-motor control and interhemispheric relations in recovered and persistent stuttering. *Developmental neuropsychology*(19), 125-45.

Fox, P., Ingham, R., Ingham, J., HirschT, Downs, J., & Martin, C. (1996). A PET study of the neural systems of stuttering. *Nature*(382), 158-62.

Fox, P., Ingham, R., Ingham, J., Zamarripa, F., Xiong, J.-H., & Lancaster, J. (2000). Brain correlates of stuttering and syllable production: a PET performance-correlation analysis. *Brain*(123), 1985-2004.

Freund, H. (1966). *Psychopathology and the problems of stuttering.* Springfield, IL: Charles C. Thomas.

Friederici, A., & Gierhan, S. (2012). The language network. *Current Opinion in Neurobiology*(23), 250-4.

Friston, K., Harrison, L., Daunizeau, J., Kiebel, S., Philips, C., Trujillo-Barreto, N., Henson, R., Flandin, G., & Mattout, J. (2008). Multiple sparse priors for the M/EEG inverse problem. *NeuroImage, 39*, 1104-20.

Funahashi, S. (2001). Neuronal mechanisms of executive control by the prefrontal cortex. *Neuroscience Research, 39,* 147-165.

Geschwind, N., & Galaburda, A. (1985). Cerebral lateralization: biological mechanisms, associations, and pathology: I. A hypothesis and a program for research. *Archives of Neurology*(42), 429-59.

Ghosh, S., Tourville, J., & Guenther, F. (2008). A neuroimaging study of premotor lateralization and cerebellar involvement in the production of phonemes and syllables. *Journal of Speech, Language, and Hearing Research*(51), 1183-1202.

Giaquinto, S., Ranghi, F., & Butler, S. (2007). Stability of word comprehension with age: an electrophysiological study. *Mechanisms of Aging and Development*(128), 628-38.

Giedd, J., Raznahan, A., Mills, K., & Lenroot, R. (2012). Review: magnetic resonance imaging of male/female differences in human adolescent brain anatomy. *Biology of sex differences*(21), doi: 10.1186/2042-6410-3-19.

Gierhan, S. (2013). Connections for auditory language in the human brain. *Brain and Language*(127), 205-21.

Giorgio, A., Watkins, K., Douaud, G., James, A., James, S., De Stefano, N., Matthews, P., Smith, S., & Johansen-Berg, H. (2008). Changes in white matter microstructure during adolescence. *NeuroImage*(39), 52-61.

Giraud, A.-L., Neumann, K., Bachoud-Levi, A.-C., von Gudenberg, A., Euler, H., Lanfermann, H., & Preibisch, C. (2008). Severity of dysfluency correlates with basal ganglia activity in persistent developmental stuttering. *Brain and Language*(104), 190-199.

Goberman, A., & Blomgren, M. (2003). Parkinsonian speech disfluencies: effects of L-dopa-related fluctuations. *Journal of Fluency Disorders*(28), 55-70.

Goberman, A., Blomgren, M., & Metzger, E. (2010). Characteristics of speech disfluency in Parkinson's disease. *Journal of Neurolinguistics*(23), 470-8.

Goldman, R. (1967). Cultural influences on the sex ratio in the incidence of stuttering. *American Antropologist*(69), 78-81.

Golfinopoulus, E., Tourville, J., & Guenther, F. (2010). The integration of large-scale neural network modeling and functional brain imaging in speech motor control. *NeuroImage*(52), 862-74.

Gölgeli, A., Süer, C., Özesmi, C., Dolu, N., Ascioglu, M., & Sahin, Ö. (1999). The effect of sex differences on event-related potentials in young adults. *International Journal of Neuroscience, 99,* 69-77.

Golob, E., Ovasapyan, V., & Starr, A. (2005). Event-related potentials accompanying motor preparation and stimulus expectancy in the young, young-old and oldest-old. *Neurobiology of Aging*(26), 531-542.

Gomez, C., Marco, J., & Grau, C. (2003). Preparatory visuo-motor cortical network of the contingent negative variation estimated by current density. *NeuroImage*(20), 216-224.

Grabski, K., Lamalle, L., Vilain, C., Schwartz, J., Vallee, N., Topres, I., Baciu, M., Le Bas, J., & Sato, M. (2011). Functional MRI assessment of orofacial articulators: neural correlates of lip, jaw, larynx, and tongue movements. *Human Brain Mapping*(33), 2306-21.

Graetz, P., de Bleser, R., & Willmes, K. (1991). *Akense Afasie Test, Nederlandstalige versie.* Lisse: Swets & Zeitlinger.

Graham, J. (1966). A neurologic and electroencephalographic study of adult stutterers and matched normal speakers. *Speech Monographs*(33), 290.

Gregory, H. (1993). A clinician's perspective: comments on identification of stuttering, prevention, and early intervention. *Journal of fluency disorders*(18), 389-402.

Grossman, M., Anderson, C., Khan, A., Avants, B., Elman, L., & McCluskey, L. (2008). Impaired action knowledge in amyotrophic lateral sclerosis. *Neurology*, *71,* 1396-1401.

Guenther, F. (2006). Cortical interactions underlying the production of speech sounds. *Journal of Communication Disorders*(39), 350-65.

Guenther, F., & Vladusich, T. (2012). A neural theory of speech acquisition and production. *Journal of neurolinguistics*(25), 408-22.

Guitar, B. (2006). *Stuttering: an integrated approach to its nature and treatment, third edition.* Baltimore - Philadelphia: Lippincott Williams & Wilkins.

Gullick, M., Mitra, P., & Coch, D. (2013). Imagining the truth and the moon: an electrophysiological study of abstract and concrete word processing. *Psychophysiology, 50,* 431-40.

Hamano, T., Lüders, H., Ikeda, A., Collura, T., Comair, Y., & Shibasaki, H. (1997). The cortical generators of the contingent negative variation in humans: a study with subdural electrodes. *Electroencephalography and clinical Neurophysiology*(104), 257-268.

Hampton, A., & Weber-Fox, C. (2008). Non-linguistic auditory processing in stuttering: evidence from behavior and event-related brain potentials. *Journal of fluency disorders*(33), 253-273.

Handy, T. (2005). *Event-related potentials: a methods handbook.* Massachusetts Institute of Technology: MIT Press Books.

Hasbroucq, T., Possamaï, C., Bonnet, M., & Vidal, F. (1999). Effect of the irrelevant location of the response signal on choice reaction time: an electromyographic study in humans. *Psychophysiology*(36), 522-6.

Hauk, O., & Pulvermüller, F. (2004a). Effects of word length and frequency on the human event-related potential. *Clinical Neurophysiologcy, 115,* 1090-110.

Hauk, O., & Pulvermüller, F. (2004b). Neurophysiological distinction of action words in the fronto-central cortex. *Human Brain Mapping, 21,* 191-201.

Hauk, O., & Pulvermüller, F. (2011). The lateralization of motor cortex activation to action-words. *Frontiers in human neuroscience, 5,* 1-10.

Hauk, O., Coutout, C., Holden, A., & Chen, Y. (2012). The time-course of single-word reading: evidence from fast behavioral and brain responses. *NeuroImage*, *60,* 1462-77.

Hauk, O., Davis, M., Ford, M., Pulvermüller, F., & Marslen-Wilson, W. (2006b). The time course of visual word recognition as revealed by linear regression analysis of ERP data. *NeuroImage*, *30,* 1383-1400.

Hauk, O., Johnsrude, I., & Pulvermüller, F. (2004). Somatotopic representation of action words in human motor and premotor cortex. *Neuron, 22,* 301-307.

Hauk, O., Patterson, K., Woollams, A., Watling, L., Pulvermüller, F., & Rogers, T. (2006a). [Q:] When would you prefer a SOSSAGE to a SAUSSAGE? [A:] At about 100 ms. ERP correlates of orthographic typicality and lexicality in written word recognition. *Journal of Cognitive Neuroscience*, *18,* 818-832.

Hauk, O., Shtyrov, Y., & Pulvermüller, F. (2008). The time course of action and action-word comprehension in the human brain as revelaed by neurophysiology. *Journal of Physiology, Paris*, *102,* 50-8.

Helm-Estabrooks, N. (1993). Stuttering associated with acquired neurological disorders. In R. Curlee, *Stuttering and related disorders of fluency* (pp. 205-218). New York: Thieme.

Helm-Estabrooks, N. (1999). Stuttering associated with acquired neurological disorders. In R. Curlee, *Stuttering and related disorders of fluency, 2nd edition* (pp. 255-68). New York: Thieme Medical Publishers.

Helm-Estabrooks, N., Yeo, R., Geschwind, N., & Freedman, M. (1986). Stuttering: disappearance and reappearance with acquired brain lesions. *Neurology*(36), 1109-1112.

Hennessey, N., Dourado, E., & Beilby, J. (2014). Anxiety and speaking in people who stutter: an investigation using the emotional Stroop task. *Journal of fluency disorders*(40), 44-57.

Hennessey, N., Nang, C., & Beilby, J. (2008). Speeded verbal responding in adults who stutter: are there deficits in linguistic encoding. *Journal of Fluency Disorders*(33), 180-202.

Hickok, G. (2010). The role of mirror neurons in speech perception and action word semantics. *Language and cognitive processes*, *6,* 749-776.

Hickok, G. (2012). Computational neuroanatomy of speech production. *Nature Reviews*(13), 135-145.

Hickok, G., & Poeppel, D. (2004). Dorsal and ventral streams: a framework for understanding aspects of the functional anatomy of language. *Cognition*(92), 67-99.

Hoshi, E. (2006). Functional specialization within the dorsolateral prefrontal cortex: a review of anatomical and physiological studies of non-human primates. *Neuroscience research*, *54,* 73-84.

Houde, J., Nagarajan, S., Sekihara, K., & Merzenich, M. (2002). Modulation of the auditory cortex during speech: an MEG study. *Journal of cognitive neuroscience*(14), 1125-38.

Hove, M., Fairhurst, M., Kotz, S., & Keller, P. (2013). Synchronizing with auditory and visual rhythms: an fMRI assessment of modality differences and modality appropriateness. *NeuroImage*(67), 313-21.

Howell, P. (2004). Assessment of some contemporary theories of stuttering that apply to spontaneous speech. *Contemporary Issues in Communication Sciences and Disorders*(31), 123-40.

Howie, P. (1981). Concordance for stuttering in monozygotic and dizygotic twin pairs. *Journal of Speech and Hearing Research*(24), 317-321.

Ikeda, A., Shibasaki, H., Kaji, R., Terada, K., Nagamine, T., Honda, M., & Kimura, J. (1997). Dissociation between contingent negative variation (CNV) and Bereitschaftspotential (BP) in patients with parkinsonism. *Electroencephalography and clinical neurophysiology*(102), 142-151.

Indefrey, P. (2011). The spatial and temporal signatures of word production components: a critical update. *Frontiers in psychology*(2), doi: 10.3389/fpsyg.2011.00255.

Indefrey, P., & Levelt, W. (2004). The spatial and temporal signatures of word production components. *Cognition*(92), 101-144.

Ingham, R., Fox, P., Ingham, J., & Zamarripa, F. (2000). Is overt stuttered speech a prerequisite for the neural activations associated with chronic developmental stuttering? *Brain and Language*(75), 163-94.

Ingham, R., Fox, P., Ingham, J., Xiong, J., Zamarripa, F., Hardies, L., & Lancaster, J. (2004). Brain correlates of stuttering and syllable production: gender comparison and replication. *Journal of Speech, Language and Hearing Research*(47), 321-41.

Ingham, R., Grafton, S., Bothe, A., & Ingham, J. (2012). Brain activity in adults who stutter: similarities across speaking tasks and correlations with stuttering frequency and speaking rate. *Brain and Language*(122), 11-24.

Ingham, J., & Ingham, R. (2011). *http://sms.id.ucsb.edu/downloads/SMS_Manual.pdf*. The Stuttering Measurement System.

Ingham, R., Wang, Y., Ingham, J., Bothe, A., & Grafton, S. (2013). Regional brain activity change predicts responsiveness to treatment for stuttering in adults. *Brain and Language*(127), 510-9.

International Phonetic Association. (1974). *The principles of the international phonetic association.* London: International Phonetic Association.

Jansson-Verkasalo, E., Eggers, K., Järvenpää, A., Suominen, K., Van Den Bergh, B., De Nil, L., & Kujala, T. (2014). Atypical central auditory speech-sound discrimination in children who stutter as indexed by the mismatch negativity. *Journal of fluency disorders*, doi: 10.1016/j.jfludis.2014.07.001.

Jasper, H. (1598). The ten twenty electrode system of the international federation. *Electroencephalography and clinical neurophysiology*(10), 371-5.

Jeon, H.-A., Lee, K.-M., Kim, Y.-B., & Cho, Z.-H. (2009). Neural substrates of semantic relationships: common and distinct left-frontal activities for generation of synonyms vs. antonyms. *NeuroImage*, *48,* 449-457.

Jiang, J., Lu, C., Peng, D., Zhu, C., & Howell, P. (2012). Classification of types of stuttering symptoms based on brain activity. *Plos One*, doi:10.1371/journal.pone.0039747.

Johnson, W., & Associates. (1959). *The onset of stuttering: Research findings and implications.* Minneapolis: University of Minnesota.

Joyce, C., Gorodnitsky, I., & Kutas, M. (2004). Automatic removal of eye movement and blink artifacts from EEG data using blind component separation. *Psychophysiology*, *42,* 313-25.

Kaganovich, N., Wray, A., & Weber-Fox, C. (2010). Non-linguistic auditory processing and working memeory update in pre-school children who stutter: an electrophysiological study. *Developmental Neuropsychology*(35), 712-36.

Kanske, P., & Kotz, S. (2007). Concreteness in emotional words: ERP evidence from a hemifield study. *Brain Research, 1148,* 138-48.

Karimi, H., Jones, M., O'Brian, S., & Onslow, M. (2013). Clinician percent syllables stuttered, clinician severity ratings and speaker severity ratings: are they interchangeable? *International journal of language and communication disorders*, doi: 10.1111/1460-6984.12069.

Kay, J., Lesser, R., & Coltheart, M. (1992). *PALPA: Psycholinguistic Assessments of Language Processing in Aphasia.* Hove, East Sussex: Psychology Press.

Kayser, J., Tenke, C.E., 2006. Principal components analysis of Laplacian waveforms as a generic method for identifying ERP generator patterns: II. Adequacy of low-density estimates. *Clinical Neurophysiology, 117,* 369-80.

Kell, C., Neumann, K., von Kriegstein, K., Posenenske, C., von Gudenberg, A., Euler, H., & Giraud, A.-L. (2009). How the brain repairs stuttering. *Brain*(132), 2747-2760.

Kellenbach, M., Wijers, A., Hovius, M., Mulder, J., & Mulder, G. (2002). Neural differentiation of lexico-syntactic categories or semantic features. *Journal of Cognitive Neuroscience*, *14,* 561-577.

Kemmerer, D., & Gonzalez-Castillo, J. (2010). The two-level theory of verb meaning: an approach to integrating the semantics of action with the mirror neuron system. *Brain and Language*, *112,* 54-76.

Kemmerer, D., Castillo, J., Talavage, T., Patterson, S., & Wiley, C. (2008). Neuroanatomical distribution of five semantic components of verbs: evidence from fMRI. *Brain and Language*, *107,* 16-43.

Kent, R. (2000). Research on speech motor control and its disorders: a review and prospective. *Journal of Communication Disorders*(33), 391-427.

Kidd, K. (1984). Stuttering as a genetic disorder. In R. Curlee, & W. Perkins, *Nature and Treatment of Stuttering* (pp. 149-69). San Diego: College Hill.

Kidd, K., Heimbuch, R., & Records, M. (1981). Vertical transmission of susceptibility to stuttering with sex-modified expression. *Proceedings of the National Academy of Sciences USA*(78), 606-10.

Knott, J., & Tjossem, T. (1943). Bilateral electroencephalograms from normal speakers and stutterers. *Journal of experimental psychology*(32), 357-62.

Koller, W. (1983). Dysfluency (stuttering) in extrapyramidal disease. *Archives of neurology*(40), 175-7.

Kopell, V., Wittner, W., Lunde, D., Wolcott, L., & Tinklenberg, J. (1974). The effects of methamphetamine and secobarbital on the contingent negative variation amplitude. *Psychopharmacologia*(34), 55-62.

Kraft, S., & Yairi, E. (2012). Genetic bases of stuttering: the state of the art, 2011. *FOlia Phoniatrica et Logopaedica*(64), 34-47.

Kronfeld-Duenias, V., Amir, O., Ezrati-Vinacour, R., Civier, O., & Ben-Shachar, M. (2014). The frontal aslant tract underlies speech fluency in persistent developmental stuttering. *Brain structure and function*.

Kropp, P., Kiewitt, A., Göbel, H., VetterP, & Gerber, W.-D. (2000). Reliability and stability of contingent negative variation. *Applied psychophysiology and biofeedback*(25), 33-41.

Kutas, M., & Federmeier, K. (2000). Electrophysiology reveals semantic memory use in language comprehension. *Trends in Cognitive Sciences*, *4,* 463-70.

Laganaro, M., & Perret, C. (2011). Comparing electrophysiological correlates of word production in immediate and delayed naming through the analysis of word age of acquisition effects. *Brain Topography*(24), 19-29.

Lamarche, M., Louvel, J., Buser, P., & Rektor, I. (1995). Intracerebral recordings of slow potentials in a contingent negative variation paradigm: an exploration in epileptic patients. *Electroencephalography and clinical neurophysiology*(95), 268-276.

Lavid, N., Franklin, D., & Maguire, G. (1999). Management of child and adolescent stuttering with olanzapine: three case reports. *Annals of Clinical Psychiatry*(11), 233-6.

Lenroot, R., & Giedd, J. (2010). Sex differneces in the adolescent brain. *Brain and cognition*(72), 46-55.

Levelt, W. (2004). Speech, gesture and the origins of language. *European Review*(12), 543-9.

Levelt, W., & Wheeldon, L. (1994). Do speakers have access to a mental syllabary. *Cognition*(50), 239-69.

Levelt, W., Roelofs, A., & Meyer, A. (1999). A theory of lexical access in speech production. *Behavioral and brain sciences*(22), 1-75.

Lezak, M., Howieson, D., & Loring, D. (2004). *Neurophychological assessment - fourth edition.* New York: Oxford Univerisity Press.

Lickley, R., Hartsuiker, R., Corley, M., Russell, M., & Nelson, R. (2005). Judgment of difluency in people who stutter and people who do not stutter: results from magnitude estimation. *Language and speech*(48), 299-312.

Linsen, A., Vuurman, E., Sambeth, A., Nave, S., Spooren, W., Vargas, G., Santorelli, L., & Riedel, W. (2011). Contingent negative variation as a dopaminergic biomarker: evidence from dose-related effects of methylphenidate. *Psychopharmacology*(218), 533-42.

Liotti, M., Ingham, J., Takai, O., Kothmann Paskos, D., Perez, R., & Ingham, R. (2010). Spatiotemporal dynamics of speech sound perception in chronic developmental stuttering. *Brain and Language*(115), 141-147.

Litvak, V., Mattout, J., Kiebel, S., Phillips, C., Henson, R., Kilner, J., Barnes, G., Oostenveld, R., Daunizeau, J., Flandin, G., Penny, W., & Friston, K. (2011). EEG and MEG data analysis in SPM8. *Comput Intell Neurosci, 2011*, doi: 10.1155/2011/852961

Loucks, T., Kraft, S., Choo, A., Sharma, H., & Ambrose, N. (2011). Functional brain activation differences in stuttering identified with a rapid fMRI sequence. *Journal of fluency disorders*(36), 302-7.

Louwerse, M., & Jeuniaux, P. (2010). The linguistic and embodied nature of conceptual processing. *Journal of Cognitive Neuroscience*, *114,* 96-104.

Loveless, N., & Sanford, A. (1974). Slow potential correlates of preparatory set. *Biological psychology*(1), 303-314.

Lu, C., Chen, C., Ning, N., Ding, G., Guo, T., Peng, D., Yang, Y., Li, K., & Lin, C. (2010a). The neural substrates for atypical planning and execution of word production in stuttering. *Experimental Neurology*, 146-156.

Lu, C., Chen, C., Peng, D., You, w., Zhang, X., Ding, G., Deng, X., Yan, Q., & Howell, P. (2012). Neural anomaly and reorganization in speakers who stutter: a short-term intervention study. *Neurology*(79), 625-32.

Lu, C., Peng, D., Chen, C., Ning, N., Ding, G., Li, K., Yang, Y., & Lin, C. (2010b). Altered effective connectivity and anomalous anatomy in the basal-ganglia-thalamocortical circuit of stuttering speakers. *Cortex*(46), 49-67.

Luck, S. (2005). *An introduction to the event-related potential technique.* Massachusetts, USA: The MIT Press.

Ludlow, C., & Loucks, T. (2003). Stuttering: a dynamic motor control disorder. *Journal of fluency disorders*(28), 273-95.

Ludlow, C., Rosenberg, J., Salazar, A., Grafman, J., & Smutok, M. (1987). Site of penetrating brain lesions causing chronic acquired stuttering. *Annals of Neurology*(22), 60-6.

Lundgren, K., Helm-Estabrooks, N., & Klein, R. (2010). Stuttering following acquired brain damage: a review of the literature. *Journal of Neurolinguistics*(23), 447-454.

Maguire, G., Riley, G., Franklin, D., & Gottschalk, L. (2000). Risperidone for the treatment of stuttering. *Journal of Clinical Psychopharmacology*(20), 479-82.

Maguire, G., Yu, B., Franklin, D., & Riley, G. (2004). Alleviating stuttering with pharmacological interventions. *Expert Opinion Pharmacotherapy*(5), 1565-71.

Mahr, G., & Leitz, W. (1992). Psychogenic stuttering of adult onset. *Journal of Speech and Hearing Research*(35), 283-6.

Månsson, H. (2005). Stammens kompleksitet og diversitet. *Dansk Audiologopaedi*(41), 13-33.

Marinkovic, K., Dhond, R., Dale, A., Glessner, M., Carr, V., & Helgren, E. (2003). Spatiotemporal dynamics of modality-specific and supramodal word processing. *Neuron*, *38,* 487-497.

Max, L., Guenther, F., Gracco, V., Ghosh, S., & Wallace, M. (2004). Unstable or insufficiently activated internal models and feedback-biased motor control as sources of dysfluency: a theoretical models of stuttering. *Contemporary issues in communication science and disorders*(31), 105-22.

Maxfield, N., Huffman, J., Frisch, S., & Hinckley, J. (2010). Neural correlates of semantic activation spreading on the path to picture naming in adults who stutter. *Clinical Neurophysiology*(121), 1447-63.

Maxfield, N., Morris, K., Frisch, S., Morphew, K., & Constantine, J. (2014). Real-time processing in picture naming in adults who stutter: ERP evidence. *Clinical Neurophysiology*, DOI: 10.1016/j.clinph.2014.05.009.

Maxfield, N., Pizon-Moore, A., Frisch, S., & Constantine, J. (2012). Exploring semantic and phonological picture-word priming in adults who stutter using event-related potentials. *Clinical Neurophysiology*(123), 1131-46.

Mazzucchi, A., Moretti, G., Carpeggiani, P., Parma, M., & Paini, P. (1981). Clinical observations on acquired stuttering. *British journal of disorders of communication*(16), 19-30.

McArdle, J., Mari, Z., Pursley, R., Schulz, G., & Braun, A. (2009). Electrophysiological evidence of functional integration between the language and motor systems in the brain: a study of the speech Bereitschaftspotential. *Clinical Neurophysiology*(120(2)), 275-84.

McCallum, W. (1988). Potentials related to expectancy, preparation and motor activity. In T. Picton, *Handbook of Electroencephalography and Clinical Neurophysiology: Revised series, vol.3: Human Event-related Potentials* (pp. 427-534). New York: Elsevier.

McClelland, J., & Elman, J. (1986). The TRACE model of speech perception. *Cognitive Psychology*(18), 1-86.

McFarlane, S., & Prins, D. (1978). Neural response time of stutteres and nonstutterers in selected oral motor tasks. *Journal of Speech and Hearing Research*(21), 768-78.

Melnick, K., Conture, E., & Ohde, R. (2003). Phonological priming in picture naming of young children who stutter. *Journal of Speech, Language and Hearing Research*(46), 1428-1443.

Meltzer, H. (1934). Personality differences between stuttering and nonstuttering children as indicated by the Rorschach Test. *Journal of Psychology*(17), 39-59.

Mennes, M., Wouters, H., Vanrumste, B., Lagae, L., & Stiers, P. (2010). Validation of ICA as a tool to remove eye movement artifacts from EEG/ERP. *Psychophysiology*, *6,* 1142-50.

Miall, R., & Wolpert, D. (1996). Forward models for psysiological motor control. *Neural network*(9), 1265-79.

Michalewski, H., & Weinberg, H. (1977). The contingent negative variation (CNV) and speech production: slow potentials and the area of Broca. *Biological Psychology*(5), 83-96.

Miller. (2000). The prefrontal cortex and cognitive control. *Nature Reviews Neuroscience, 1,* 59-65.

Miller, E., & Cohen, J. (2001). An integrative theory of prefrontal cortex function. *Annual Review Neuroscience, 24,* 167-202.

Mink, J. (1996). The basal ganglia: focused selection and inhibition of competing motor programs. *Progress in neurobiology*(50), 381-425.

Mitterer, H., & Müsseler, J. (2013). Regional accent variation in the shadowing task: evidence for a loose perception-action coupling in speech. *Atten Percept Psychophys*(75), 557-75.

Mock, J., Foundas, A., & Golob, E. (2011). Modulation of sensory and motor cortex activity during speech preparation. *European Journal of Neuroscience*(33), 1001-1011.

Möhring, N., Brandt, E., Mohr, B., Pulvermüller, F., & Neuhaus, A. (2014). ERP adaptation provides direct evidence for early mirror neuron activation in the inferior parietal lobule. *International journal of psychophysiology*(94), 76-83.

Moore, W., Craven, D., & Faber, M. (1982). Hemispheric alpha asymmetries of words with positive, negative, and neutral arousal values preceding tasks of recall and recognition: electrophysiological and behavioral results from stuttering males and nonstuttering males and females. *Brain and Language*(17), 211-24.

Morgan, A., Reilly, S., Anderson, A., Reutens, D., & Wood, A. (2008). *Functional brain activation differences for motor versus language regions in adults with and without stuttering: an fMRI study.* Oxford.

Moseley, R., Carota, F., Hauk, O., Mohr, B., & Pulvermüller, F. (2012). A role for the motor system in binding abstract emotional meaning. *Cerebral Cortex*, *22,* 1634-1647.

Moseley, R., Pulvermüller, F., & Shtyrov, Y. (2013). Sensorimotor semantics on the spot: brain activity dissociates between conceptual categories within 150 msec. *Scientific Reports*, doi: 10.1038/srep01928.

Möttönen, R., & Watkins, K. (2012). Using TMS to study the role of the articulatory motor system in speech perception. *Aphasiology*(26), 1103-1118.

Movsessian, P. (2005). Neuropharmacology of theophylline induced stuttering: the role of dopamine, adenosine and GABA. *Medical Hypotheses*(64), 290-7.

Mowrer, D., & Younts, J. (2001). Sudden onset of excessive repetitions in the speech of a patient with multiple sclerosis - a case report. *Journal of Fluency Disorders*(26), 269-309.

Mula, M., Trimble, M., Thompson, P., & Sander, J. (2003). Topiramate and word-finding difficulties in patients with epilepsy. *Neurology*(60), 1104-7.

Näätänen, R., Kujala, T., & Winkler, I. (2011). Auditory processing that leads to conscous perception: a unique window to central auditory processing opened by the Mismatch Negativity and related responses. *Psychophysiology*(48), 4-22.

Neef, N., Jung, K., Rothkegel, H., Pollok, B., von Gudenberg, A., Paulus, W., & Sommer, M. (2011). Right-shift for non-speech motor processing in adults who stutter. *Cortex*(47), 945-954.

Neef, N., Linh Hoang, T., Neef, A., Paulus, W., & Sommer, M. (2015). Speech dynamics are coded in the left motor cortex in fluent speakers but not in adults who stutter. *Brain*, doi:10.1093/brain/awu390.

Neininger, B., & Pulvermüller, F. (2003). Word-category specific deficits after lesions in the right hemisphere. *Neuropsychologia*(41), 53-70.

Neumann, K., Euler, H., von Gudenberg, A., Giraud, A.-L., Lanfermann, H., Gall, V., & Preibisch, C. (2003). The nature and treatment of stuttering as revealed by fMRI a within- and between-group comparison. *Journal of fluency disorders*(28), 381-410.

Neumann, K., Preibisch, C., Euler, H., von Gudenberg, A., Lanfermann, H., Gall, V., & Giraud, A. (2005). Cortical plasticity associated with stuttering therapy. *Journal of fluency disorders*(30), 23-39.

Numminen, J., Salmelin, R., & Hari, R. (1999). Subject's own speech reduces reactivity of the human auditory cortex. *Neuroscience Letters*(265), 119-22.

O'Brian, S., Packman, A., Onslow, M., & O'Brian, N. (2004). Measurement of stuttering in adults: comparison of stuttering-rate and severity-scaling methods. *Journal of speech, language and hearing research*(47), 1081-1087.

Ohashi, Y. (1977). Development of stuttering in children: a cross sectional study. *Japanese Journal of Child Psychiatry*(17), 57-68.

Okasha, A., Moneim, S., Bishry, Z., Kamel, M., & Moustafa, M. (1974). Electroencephalographic study of stammering. *The British journal of psychiatry*(124), 534-5.

Oldfield, R. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia, 9,* 97-112.

Oliveri, M., Finocchiaro, C., Shapiro, K., Gangitano, M., Caramazza, A., & Pascual-Leone, A. (2004). All talk and no action: a transcranial magnetic stimulation study of motor cortex activation during action word production. *Journal of Cognitive Neuroscience*, *16,* 374-381.

Onslow, M. (2000). Current Therapeutics. *Stuttering: treatment for adults*, 73-76.

Oostenveld, R., Fries, P., Maris, E., Schoffelen, J.M., 2011. FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. Comput Intell Neurosci 2011, 156869.

Osterhout, L., Bersick, M., & McKinnon, R. (1997). Brain potentials elicited by words: word length and frequency predict the latency of an early negativity. *Biological Psychology*, *46,* 143-68.

Özcan, Ö., Altinayar, S., Özcan, C., Ünal, S., & Karlidag, R. (2009). P50 sensory gating in children and adolescents with developmental stuttering. *Bulletin of clinical psychopharmacology*(19), 241-6.

Packman, A., Onslow, M., Richard, F., & van Doorn, J. (1996). Syllabic stress and variability: a model of stuttering. *Clinical Linguistics and Phonetics*(10), 235-63.

Paivio, A. (1986). *Mental representations: a dual coding approach.* New York: Oxford University.

Palazova, M., Manthill, K., Sommer, W., & Schacht, A. (2011). Are effects of emotion in single words non-lexical? Evidence from event-related brain potentials. *Neuropsychologia*(49), 2766-2775.

Papeo, L., Vallesi, A., Isaja, A., & Rumiati, R. (2009). Effects of TMS on different stages of motor and non-motor verb processing in the primary motor cortex. *PLoS One*, *4,* doi: 10.1371/journal.pone.0004508.

Papoutsi, M., de Zwart, J., Jansma, J., Pickering, M., Bednar, J., & Horwitz, B. (2009). From phonemes to articulatory codes: an fMRI study of the role of Broca's area in speech production. *Cerebral Cortex*(19), 2156-65.

Paus, T. (1999). Structural maturation of neural pathways in children and adulescents: in vivo study. *Science*(283), 1908-11.

Penolazzi, B., Hauk, O., & Pulvermüller, F. (2007). Early semantic context integration and lexical access as revealed by event-related brain potentials. *Biol. Psychol.*, *74,* 374-388.

Peters, H., Hulstijn, W., & Van Lieshout, P. (2000). Recent developments in speech motor research into stuttering. *Folia Phoniatrica et Logopaedica*(52), 103-19.

Pinsky, S., & McAdam, D. (1980). Electroencephalographic and dichotic indices of cerebral laterality in stutterers. *Brain and Language*(11), 374-97.

Poeppel, D., Idsardi, W., & van Wassenhove, V. (2008). Speech perception at the interface of neurobiology and linguistics. *Philosophical Transcriptions of the Royal Society of London. Series B, Biological Sciences*(363), 1071-1086.

Polich, J. (2007). Updating P300: an integrative theory of P3a and P3b. *Clinical Neurophysiology*, *118,* 2128-2148.

Postma, A., & Kolk, H. (1993). The covert repair hypothesis: prearticulatory repair processes in normal and stuttered disfluencies. *Journal of Speech and Hearing Research*(36), 472-87.

Postle, N., Ashton, R., McFarland, K., & de Zubicaray, G. (2013). No specific role for the manual motor system in processing the meanings of words related to the hand. *Frontiers in human neuroscience*, *7,* 1-17.

Preibisch, C., Neumann, K., Raab, P., Euler, H., von Gudenberg, A., Lanfermann, H., & Giraud, A.-L. (2003). Evidence for compensation for stuttering by the right frontal operculum. *NeuroImage*(20), 1356-1364.

Prescott, J. (1988). Event-related potential indices of speech motor programming in stutterers and non-stutterers. *Biological Psychology*(27), 259-273.

Prescott, J., & Andrews, G. (1984). Early and late components of the contingent negative variation prior to manual and speech responses in stutterers and non-stutterers. *International journal of psychophysiology*(2), 121-130.

Preus, A. (1981). *Identifying subgroups of stutterers.* Oslo, Norway: University of Oslo.

Price, C. (2009). The anatomy of language: a review of 100 fMRI studies published in 2009. *Annals of the New York Academy of Sciences*(1191), 62-88.

Price, C. (2012). A review and synthesis of the first 20 years of PET and fMRI studies of heard speech, spoken language and reading. *NeuroImage*(62), 816-847.

Prins, D., Main, V., & Wampler, S. (1997). Lexicalisation in adults who stutter. *Journal of Speech, Language and Hearing Research*(40), 373-84.

Pulvermüller, F. (2005). Brain mechanisms linking language and action. *Nature Reviews Neuroscience*, *6,* 576-582.

Pulvermüller, F., & Fadiga, L. (2010). Active perception: sensorimotor circuits as a cortical basis for language. *Nature reviews*(11), 351-360.

Pulvermüller, F., Härle, M., & Hummel, F. (2001). Walking or talking? behavioral and neurophysiological correlates of action verb processing. *Brain and Language*(78), 143-168.

Pulvermüller, F., Shtyrov, Y., & Imoniemi, R. (2005). Brain signatures of meaning access in action word recognition. *Journal of Cognitive Neuroscience*(17), 884-892.

Raposo, A., Moss, H., Stamatakis, E., & Tyler, L. (2009). Modulation of motor and premotor cortices by actions, action words and action sentences. *Neuropsychologia*, *47,* 388-396.

Reilly, S., Onslow, M., Packman, A., Wake, M., Bavin, E., Prior, M., Eadie, P., Cini, E., Bolzonello, C., & Ukoumunne, O. (2009). Predicting stuttering onset by the age of 3 years: a prospective, community cohort study. *Pediatrics*(123), 270-7.

Regan, D. (1989). Electrical activity that precedes motor action. In D. Regan, *Human brain electrophysiology: evoked potentials and evoked magnetic fiels in science and medicine* (pp. 217-227). New York: Elsevier.

Repetto, C., Colombo, B., Cipresso, P., & Giuseppe, R. (2013). The effects of rTMS over the primary motor cortex: The link between action and language. *Neuropsychologia, 51,* 8-13

Riaz, N., Steinberg, S., Ahmad, J., Pluzhnikov, A., Riazuddin, S., Cox, N., & Drayna, D. (2005). Genomewide significant linkage to stuttering on chromosome 12. *American Journal of Human Genetics*(76), 647-51.

Riès, S., Janssen, N., Burle, B., & Alario, F. (2013). Response-locked brain dynamics in word production. *PLoS one*(8), 1-14.

Riley, G. (2008). *Stuttering Severity Instrument for children and adults, fourth edition (SSI-4).* Austin: Pro-Ed.

Ringo, C., & Dietrich, S. (1995). Neurogenic stuttering: an analysis and critique. *Journal of Medical Speech-Language Pathology*(3), 111-22.

Rizzolatti, G., & Arbib, M. (1998). Language within our grasp. *Trends in Neuroscience*(21), 188-94.

Rodriguez-Ferreiro, J., Gennari, S., Davies, R., & Cuetos, F. (2010). Neural correlates of abstract verb processing. *Journal of Cognitive Neuroscience, 23,* 106-118.

Rohrbaugh, J., & Gaillard, A. (1983). Sensory and motor aspects of the contingent negative variation. In A. Gaillard, & W. Ritter, *Tutorials in Event-related Potential Research: Endogenous Components* (pp. 269-310). Amsterdam: North-Holland.

Rowan, A., & Tolunsky, E. (2003). *Primer of EEG.* Philadelphia, Pennsylvania: Elsevier.

Rüschemeyer, S.-A., Brass, M., & Friederici, A. (2007). Comprehending prehending: neural correlates of processing verbs with motor stems. *Journal of Cognitive Neuroscience*, *19,* 855-865.

Ruspantini, I., Saarinen, T., Belardinelli, P., Jalava, A., Parviainen, T., Kujala, J., & Salmelin, R. (2012). Corticomuscular coherence is tuned to the spontaneous rythmicity of speech at 2-3 Hz. *The journal of neuroscience*(32), 3786-90.

Sahin, H., Krespi, Y., Yilmaz, A., & Coban, O. (2005). Stutering due to ischemic stroke. *Behavioural neurology*(16), 37-9.

Sahin, N., Pinker, S., Cash, S., Schomer, D., & Halgren, E. (2009). Sequential processing of lexical, grammatical, and phonological information within Broca's area. *Science*(326), 445-9.

Sakreida, K., Scorolli, C., Menz, M., Heim, S., Borghi, A., & Binkofski, F. (2013). Are abstract action words embodied? An fMRI investigation at the interface between language and motor cognition. *Frontiers in human neuroscience*, *7,* 1-13.

Salmelin, R. (2007). Clinical neurophysiology of language: the MEG approach. *Clinical Neurophysiology*, *118,* 237-254.

Salmelin, R., Schnitzler, A., Schmitz, F., & Freund, H.-J. (2000). Single word reading in developmental stutterers and fluent speakers. *Brain*(123), 1184-1202.

Salthouse, T. (1991). *Theoretical perspectives on cognitive aging.* Hillsdale, New Jersey: Lawrence Erlbaum Associates.

Saltuklaroglu, T., Teulings, H., & Robbins, M. (2009). Differential levels of speech and manual dysfluency in adults who stutter during simultaneous drawing and speaking tasks. *Human Movement Science*(28), 1376-82.

Santens, P., & De Letter, M. (2010). *Neurowetenschappen in taal en spraak, boek 1: neuro-anatomie en neurofysiologie.* Antwerpen, België: Garant.

Saygin, A., Wilson, S., Dronkers, N., & Bates, E. (2004). Action comprehensionin aphasia: linguistic and non-linguistic deficits and their lesion correlates. *Neuropsychologia, 42,* 1788-1804.

Sayles, D. (1971). Cortical excitability, perseveration, and stuttering. *Journal of speech, language, and hearing research*(14), 462-75.

Sawyer, J., & Yairi, E. (2006). The effect of sample size on the assessment of stuttering severity. *American journal of speech-language pathology*(15), 36-44.

Scorolli, C., Jacquet, P., Binkofski, F., Nicoletti, R., Tessari, A., & Borghi, A. (2012). Abstract and concrete phrases processing differentially modulates cortico-spinal excitability. *Brain research*, *1488,* 60-71.

Sechi, G., Cocco, G., D'Onofrio, M., Deriu, M., & Rosati, G. (2006). Disfluent speech in patients with partial epilepsy: beneficial effect of levetiracetam. *Epilepsy & Behavior*(9), 521-3.

Sechi, G., Correddu, P., Deiana, G., Zucca, G., & Rosati, G. (1997). Expressive aphasia exacerbated by lamotrigine and gabapentin. *Epilepsia*(38(Suppl. 3)), A73.

Seery, C. (2005). Differential diagnosis of stuttering for forensic purposes. *American journal of speech language pathology*(14), 284-97.

Severens, E., Van Lommel, S., Ratinckx, E., & Hartsuiker, R. (2005). Tiimed picture naming norms for 590 pictures in Dutch. *Acta Psychologica*(119), 159-187.

Shin, S., Verstynen, T., Pathak, S., Jarbo, K., Hrick, A., Maserati, M., Beers, S., Puccio, A., Boada, F., Okonkwo, D., & Schneider, W. (2012). High-definition fiber tracking for assessment of neurological deficit in a case of traumatic brain injury: finding, visualizing, and interpreting small sites of damage. *Journal of neurosurgery*(116), 1062-9.

Shirkey, E. (1987). Forensic verification of stuttering. *Journal of Fluency Disorders*(12), 197-203.

Shtyrov, Y., Hauk, O., & Pulvermüller, F. (2004). Distributed neuronal networks for encoding category-specific semantic information: the mismatch negativity to action words. *European Journal of Neuroscience*, *19,* 1083-1092.

Smith, A., Sadagopan, N., Walsh, B., & Weber-Fox, C. (2010). Increasing phonological complexity reveals heighened instability in inter-articulatory coordination in adults who stutter. *Journal of fluency disorders*(35), 1-18.

Smits-Bandstra, S., & De Nil, L. (2007). Sequence skill learning in persons who stutter: implications for cortico-striato-thalamo-cortical dysfunction. *Journal of fluency disorders*(32), 251-78.

Smits-Bandstra, S., De Nil, L., & Saint-Cyr, J. (2006). Speech and nonspeech sequence skill learning in adults who stutter. *Journal of fluency disorders*(31), 116-136.

Smits-Bandstra, S., & Gracco, V. (2013). Verbal implicit sequence learning in persons who stutter and persons with Parkinson's Disease. *Journal of Motor Behavior*(45), 381-93.

Sommer, M., Koch, M., Paulus, W., Weiller, C., & Büchel, C. (2002). Disconnection of speech-relevant brain areas in persistent developmental stuttering. *Lancet*(360), 380-3.

Sowman, P., Crain, S., Harrison, E., & Johnson, B. (2012). Reduced activation of left orbitofrontal cortex precedes blocked vocalization: a magnetoencephalographic study. *Journal of fluency disorders*(37), 359-65.

Suresh, R., Ambrose, N., Roe, C., Pluzhnikov, A., Wittke-Thompson, J., Ng, M., Wu, X., Cook, E., Lindstrom, C., & Garsten, M. (2006). New complexities in the genetics of stuttering: significant sex-specific linkage analysis. *American Journal of Human Genetics*(78), 554-63.

Swink, S., & Stuart, A. (2012). The effect of gender on the N1-P2 auditory complex while listening and speaking with altered auditory feedback. *Brain and Language*, *122,* 25-33.

Takashima, A., Ohta, K., Matsushima, E., & Toru, M. (2001). The event-related potentials elicited by content and function words during the reading of sentences by patients with schizophrenia. *Psychiatry and Clnical Neurosciences*, *55,* 611-618.

Taniwaki, T., Okayama, A., Yoshiura, T., Togao, O., Nakamura, Y., Yamasaki, T., Ogata, K., Shigeto, H., Okyagi, Y., Kira, J., & Tobimatsu, S. (2006). Functional network of the basal ganglia and cerebellar motor loops in vivo: different activation patterns between self-initiated and externally triggered movements. *NeuroImage*(31), 745-53.

Tanji, J., & Hoshi, E. (2001). Behavioral planning in the prefrontal cortex. *Current Opinion Neurobiology, 11,* 782-7.

Tarkiainen, A., Helenius, P., Hansen, P., Cornelissen, P., & Salmelin, R. (1999). Dynamics of letter string perception in the human occipitotemporal cortex. *Brain*, *122,* 2119-2332.

Tecce, J. (1991). Dopamine and CNV: studies of drugs, disease and nutrition. *Electroencephalography and Clinical Neurophysiology: supplement*(42), 153-64.

Tecce, J., & Cole, J. (1974). Amphetamine effects in man: paradoxical drowsiness and lowered electrical brain activity (CNV). *Science*(185), 451-3.

Tecce, J., Cole, J., & Savignano-Bowman, J. (1975). Chlorpromazine effects on brain activity (contingent negative variation) and reaction time in normal women. *Psychopharmacologia*(43), 293-5.

Tettamanti, M., Buccino, G., Saccuman, M., Gallese, V., Danna, M., Scifo, P., Fazio, F., Rizzolatti, G., Cappa, S., & Perani, D. (2005). Listening to action-related sentences activates fronto-parietal motor circuits. *Journal of Cognitive Neuroscience*, *17,* 273-281.

Theys, C., De Nil, L., Thijs, V., van Wieringen, A., & Sunaert, S. (2012). A crucial role for the cortico-striato-cortical loop in the pathogenesis of stroke-related neurogenic stuttering. *Human Brain Mapping*(34), 2103-2112.

Theys, C., van Wieringen, A., & De Nil, L. (2008). A clinician survey of speech and non-speech characteristics of neurogenic stuttering. *Journal of Fluency Disorders*(33), 1-23.

Theys, C., van Wieringen, A., Sunaert, S., Thijs, V., & De Nil, L. (2011). A one year prospective study of neurogenic stuttering following stroke: incidence and co-occurring disorders. *Journal of Communication Disorders*(44), 678-87.

Tomasino, B., Fink, G., Sparing, R., Dafotakis, M., & Weiss, P. (2008). Action verbs and the primary motor cortex: a comparative TMS study of silent reading, frequency judgments, and motor imagery. *Neuropsychologia*, *46,* 1915-1926.

Tourville, J., Reilly, K., & Guenther, F. (2008). Neural mechanisms underlying auditory feedback contrl of speech. *NeuroImage*(39), 1429-43.

Toyomura, A., Fujii, T., & Kuriki, S. (2011). Effect on external auditory pacing on the neural activity of stuttering speakers. *NeuroImage*(57), 1507-16.

Toyomura, A., Fujii, T., & Kuriki, S. (2015). Effect of an 8-week practice of externally triggered speech on basal ganglia activity of stuttering and fluent speakers. *NeuroImage*, http://dx.doi.org/10.1016/j.neuroimage.2015.01.024.

Tran, Y., Craig, A., Boor, P., & Craig, D. (2004). Using independent component analysis to remove artifact from electroencephalographic measured during stuttering speech. *Medical and biological engineering and computing*(42), 627-33.

Travis, L. (1931). *Speech pathology.* New York: D. Appleton-Century.

Travis, L., & Knott, J. (1936). Brain potentials from normal speakers and stutterers. *Journal of psychology*(2), 137-50.

Tykalova, T., Rusz, J., Cmejla, R., Klempir, J., Ruzickova, H., Roth, J., & Ruzicka, E. (2015). Effect of dopaminergic medication on speech dysfluency in Parkinson's disease: a longitudinal study. *Journal of neural transmission*, DOI 10.1007/s00702-015-1363-y.

Van Borsel, J. (1997). Neurogenic stuttering: a review. *Journal of Clinical Speech & Language Studies*(7), 16-33.

Van Borsel, J. (2011). *Basisbegrippen Logopedie. Deel 2: communicatiestoornissen. Stotteren en broddelen.* Leuven, Belgium: Acco.

Van Borsel, J. (2014). Acquired stuttering: a note on terminology. *Journal of Neurolinguistics*(27), 41-9.

Van Borsel, J., & Taillieu, C. (2001). Neurogenic stuttering versus developmental stuttering: an observer judgement study. *Journal of communication disorders*(34), 835-95.

Van Borsel, J., Achten, E., Santens, P., Lahorte, P., & Voet, T. (2003a). fMRI of developmental stuttering: a pilot study. *Brain and Language*(85), 369-376.

Van Borsel, J., Moeyaert, E., Rosseel, M., Van Loo, M., & Van Renterghem, L. (2006). Prevalence of stuttering in regular and special school population in Belgium based on teacher perception. *Folia Phoniatrica et Logopaedica*(58), 289-302.

Van Borsel, J., Van Der Made, S., & Santens, P. (2003b). Thalamic stuttering: a distinct clinical entity? *Brain and Language*(85), 185-189.

Van Boxtel, A. (2001). Optimal signal bandwidth for the recording of surface EMG activity of facial, jaw, oral, and neck muscles. *Psychophysiology*(38), 22-34.

Van Boxtel, G., Geraats, L., Van den Berg-Lenssen, M., & Brunia, C. (1993). Detection of EMG onset in ERP research. *Psychophysiology*(30), 405-12.

Vanhoutte, S., Cosyns, M., van Mierlo, P., Batens, K., Corthals, P., De Letter, M., Van Borsel, J., Santens, P. (under review) When will a stutter occur? The determining role of speech motor preparation. *Neuropsychologia*.

Vanhoutte, S., Santens, P., Cosyns, M., van Mierlo, P., Batens, K., Corthals, P., De Letter, M., Van Borsel, J. (2015a). Increased motor preparation activity during fluent single word production in developmental stuttering: a correlate for stuttering frequency and severity. *Neuropsychologia* (75), 1-10.

Vanhoutte, S., Strobbe, G., van Mierlo, P., Cosyns, M., Batens, K., Corthals, P., De Letter, M., Van Borsel, J., Santens, P. (2015b). Early lexico-semantic modulation of motor related areas during action and non-action verb processing. *Journal of neurolinguistics* (34), 65-82.

Vanhoutte, S., Van Borsel, J., Cosyns, M., Batens, K., van Mierlo, P., Hemelsoet, D., Van Roost, D., Corthals, P., De Letter, M., Santens, P. (2014). CNV amplitude as a neural correlate for stuttering frequency: a case report of acquired stuttering. *Neuropsychologia* (64), 349-59.

van Lieshout, P., Ben-David, B., Lipski, M., & Namasivayam, A. (2014). The impact of threat and cognitive stress on speech motor control in people who stutter. *Journal of fluency disorders*(40), 93-109.

Van Riper, C. (1982). *The nature of stuttering (2nd ed.).* Prentice-Hall: Englewood Cliffs, NJ.

Van Zaalen, Y., & Winkelman, C. (2009). *Broddelen: een (on)begrepen stoornis.* Bossum: Coutinho.

Vasic, N., & Wijnen, F. (2005). Stuttering as a monitoring deficit. In R. Hartsuiker, R. Bastiaanse, A. Postma, & F. Wijnen, *Phonological encoding and monitoring in normal and pathological speech* (pp. 226-247). Hove : Psychology Press.

Venkatagiri, H. (2004). Slower and incomplete retreival of speech motor plans is the proximal source of stuttering: stutters occur when syllable motor plans stored in memory are concatenated to produce the utterance motor plan. *Medical hypotheses*(62), 401-5.

Vigliocco, G., Vinson, D., Druks, J., Barber, H., & Cappa, S. (2011). Nouns and verbs in the brain: a review of behavioral, electrophysiological, neuropsychological and imaging studies. *Neuroci Biobehav Rev*, *35,* 407-26.

Vigneau, M., Beaucousin, V., Hervé, P., Duffau, H., Crivello, F., Houdé, O., Mazoyer, B., & Tzourio-Mazoyer, N. (2006). Meta-analyzing left hemisphere language areas: phonology, semantics, and sentence processing. *NeuroImage*(30), 1414-1432.

Walla, P., Mayer, D., Deecke, L., & Thurner, S. (2004). The lack of focused anticipation of verbal information in stutterers: a magnetoencephalographic study. *NeuroImage*(22), 1321-1327.

Walter, W., Cooper, R., Aldridge, V., McCallum, C., & Winter, A. (1964). Contingent negative variation: an electric sign of sensorimotor association and expectancy in the human brain. *Nature*(203), 380-384.

Wang, J., Conder, J., Blitzer, D., & Shinkareva, S. (2010). Neural representation of abstract and concrete concepts: a meta-analysis of neuroimaging studies. *Human Brain Mapping*(31), 1459-68.

Ward, D. (2006). *Stuttering and cluttering: frameworks for understanding and treatment.* Hove and New York: Psychology Press.

Watkins, K., & Klein, D. (2011). Brain structure and function in developmental stuttering and bilingualism. In P. Howell, & J. Van Borsel, *Mulitlingual Aspects of fluency disorders* (pp. 63-89). Bristol, England: Multilingual Matters.

Watkins, K., Smith, S., Davis, S., & Howell, P. (2008). Structural and functional abnormalities of the motor system in developmental stuttering. *Brain*(131), 50-59.

Watson, C., & Chatterjee, A. (2011). The functional neuroanatomy of actions. *Neurology*(76), 1428-1434.

Weber-Fox, C. (2001). Neural systems for sentence processing in stuttering. *J. Speech Lang Hear Res*, *44,* 814-825.

Weber-Fox, C., & Hampton, A. (2008). Stuttering and natural speech processing of semantic and syntactic constraints on verbs. *Journal of Speech, Language and Hearing Research*(51), 1058-1071.

Weber-Fox, C., Hampton Wray, A., & Arnold, H. (2013). Early childhood stuttering and electrophysiological indices of language processing. *Journal of fluency disorders*(38), 206-21.

Weber-Fox, C., Spencer, R., Spruill, J., & Smith, A. (2004). Phonological processing in adults who stutter: electrophysiological and behavioral evidence. *Journal of Speech, Language and Hearing Research*(47), 1244-58.

Weber-Fox, C., Spruill III, J., Spencer, R., & Smith, A. (2008). Atypical neural functions underlying phonological processing and silent rehearsal in children who stutter. *Developmental Science*(11), 321-337.

Webster, W. (1997). Principles of human brain organization related to lateralization of language and speech motor functions in normal speakers and stutterers. In W. Hulstijn, H. Peters, & P. van Lieshout, *Speech production: motor control, brain research and fluency disorders: proceedings of the third international conference on speech motor production and fluency disorders* (pp. 119-139). Amsterdam: Elsevier.

Webster, W., & Ryan, C. (1991). Task complexity and manual reaction times in people who stutter. *Journal of Speech and Hearing Research*(34), 708-714.

Wedeen, V., Wang, R., Schmahmann, J., Benner, T., Tseng, W., Dai, G., Pandya, D., Hagmann, P., D'Arceuil, H., & de Crespigny, A. (2008). Diffusion spectrum magnetic resonance imaging (DSI) tractography of crossing fibers. *NeuroImage*(15), 1267-77.

Welcome, S., Paivio, A., McRae, K., & Joanisse, M. (2011). An electrophysiological study of task demands on concreteness effects: evidence for dual coding theory. *Experimental Brain Research, 212,* 347-58.

Wells, B., & Moore, W. (1990). EEG alpha asymmetries in stutterers and non-stutterers: effects of linguistic variables on hemispheric processing and fluency. *Neuropsychologia*(28), 1295-1305.

West, R. (1931). The phenomenology of stuttering. In R. West, *A symposium on Stuttering.* Madison, WI: College Typing Company.

West, R., & Ansberry, M. (1968). *The rehabilitation of speech (4th ed.).* New York: Harper & Row.

West, C., & Holcomb, P. (2000). Imaginal, semantic, and surface-level processing of concrete and abstract words: an electrophysiological investigation. *Journal of Cognitive Neuroscience*, *12,* 1024-37.

Whymbs, N., Ingham, R., Ingham, J., Paolini, K., & Grafton, S. (2013). Individual differences in neural regions functionally related to real and imagined stuttering. *Brain and Language*(124), 153-64.

Willems, R., Hagoort, P., & Casasanto, D. (2010). Body-specific representations of action verbs: neural evidence from right- and left-handers. *Psychological Science, 21,* 67-74.

Willems, R., Toni, I., Hagoort, P., & Casananto, D. (2009). Neural dissociations between action verb understanding and motor imagery. *Journal of Cognitive Neuroscience, 22,* 2387-2400.

Wirth, M., Horn, H., Koenig, T., Stein, M., Federspiel, A., Meier, B., Michel, C., & Strik, W. (2007). Sex differences in semantic processing: event-related brain potentials distinguish between lower and higher order semantic analysis during word reading. *Cerebral Cortex*, *17,* 1987-97.

Wolpert, D., Ghahramani, Z., & Jordan, M. (1995). An internal model for sensorimotor integration. *Science*(269), 1880-2.

World Health Organisation. (2007). *International Statistical Classification of Diseases and Related Health Problems. 10th Revision* http://apps.who.int/classifications/icd10/browse/2010/en#/F98.5

Wu, J., Maguire, G., Riley, G., Lee, A., Keator, D., Tang, C., Fallon., J., & Najafi, A. (1997). Increased dopamine activity associated with stuttering. *Clinical Neuroscience and Neuropsychology*(8), NeuroReport.

Xiao, X., Zhao, D., Zhang, Q., & Guo, C.-Y. (2012). Retrieval of concrete words involves more contextual information than abstract words: multiple components for the concreteness effect. *Brain and language*, *120,* 251-8.

Xuan, Y., Meng, C., Yang, Y., Zhu, C., Wang, L., Yan, Q., Lin, C., & Yu, C. (2012). Resting-state brain activity in adult males who stutter. *PLoS One*(2012), 1-11.

Xue, G., Aron, A., & Poldrack, R. (2008). Common neural substrates for inhibition of spoken and manual responses. *Cerebral Cortex*(18), 1923-1932.

Yairi, E. (1983). The onset of stuttering in two- and three-year-old-children: A preliminary report. *Journal of Speech and Hearing Disorders*(48), 171-177.

Yairi, E. (2007). Subtyping stuttering I: a review. *Journal of Fluency Disorders*(32), 165-96.

Yairi, E., & Ambrose, N. (2005). *Early childhood stuttering.* Austin, TX: Pro-Ed.

Yairi, E., & Ambrose, N. (2013). Epidemiology of stuttering: 21st century advances. *Journal of fluency disorders*(38), 66-87.

Yairi, E., Ambrose, N., & Cox, N. (1996). Genetics of stuttering: a critical review. *Journal of Speech and Hearing Research*(39), 771-84.

Yaruss, J. (1997). *Clinical measurement of stuttering behaviors.* www.asha.org.

Yoshida, K., & Iizuka, T. (2005). Contingent negative variation elicited before jaw and tongue movements. *Journal of Oral Rehabilitation*(32), 871-9.

Zhang, Q., Zhang, J., & Kong, L. (2009). An ERP study on the time course of phonological and semantic activation in Chinese word recognition. *International journal of psychophysiology*(73), 235-245.

Zimmerman, G., & Knott, J. (1974). Slow potentials of the brain related to speech processing in normal speakers and stutters. *Electroencephalography and clinical neurophysiology*(37), 599-607.