"Psychiatry is a strange field because, unlike any other field of medicine, you never really finish. Your greatest instrument is you, yourself, and the work of self-understanding is endless. I'm still learning."

- Irvin D. Yalom

"It is more important to know what sort of person has a disease than to know what sort of disease a person has."

- Hippocrates

"For too long a time - for half a century, in fact - psychiatry tried to interpret the human mind merely as a mechanism, and consequently the therapy of mental disease merely in terms of technique. I believe this dream has been dreamt out. What now begins to loom on the horizon is not psychologized medicine but rather those of human psychiatry."

- Viktor E. Frankl

Content

Content

Foreword

Dear reader,

Before you lies the first issue of the 14th volume of the Journal of Neuroscience and Cognition. The theme of the journal is '*Rethinking the classification of neuropsychiatric disorders*'. When the Editorial Board presented their idea for this topic, I was immediately enthusiastic. For one, because I am personally very much interested in this theme. But more importantly, it shows that the Editorial Board consists of students who are not only eager to use their time as students to gather existing knowledge, but also to explore routes that may change an important field of study and ensure that it will progress in the future. I hope that the novel insights in this edition, for example in the interview with Professor Dr. Jim Van Os, will inspire you all to think outside the box when it comes to your own research areas. After all, the field will only progress if the future generation of students creates innovative ideas in their further careers.

For this issue, the Editorial Board has done a fantastic job of selecting content that fits the theme well. I especially want to draw your attention to a new aspect of the journal - the '*Personal perspective*' section. In this piece, a boy shares his personal experience with autism - a condition that most of us are familiar with mostly from a scientific perspective.

To be able to rethink the classification of neuropsychiatric disease, researchers need to team up and connect the dots from different research domains. At the time of writing, we are facing the global challenge of COVID-19 in which we need to rethink how we collaborate and relate to each other. Social isolation regulations, that are currently in effect, require us to quickly and suddenly make a radical change in the way we work together and connect with each other. Such an unexpected transformation is difficult and requires mental flexibility and innovative solutions.

Nevertheless, I dare to be optimistic and hope that this need for mental flexibility will also spark novel theories and research ideas. I hope that all of you will enjoy the rest of this semester in good health.

Yours sincerely,

Anouk Keizer
Senior supervisor Journal of Neuroscience & Cognition 2020

Editorial

Dear reader,

I am very happy to introduce you to the first issue of the Journal of Neuroscience and Cognition 2020! The whole board selected the theme for this edition with a lot of excitement - and it was difficult to contain our passion to talk about it! '*Rethinking the classification of neuropsychiatric disorders*' feels very important and special to all of us – some have experienced the burden of a neuropsychiatric diagnosis in their own families, others wish to contribute to the lives of patients in their professional capacity, but undoubtedly, all of us believe that this topic is more relevant than ever before. In times of rapid technological developments in neuroscience and psychiatry, the new generation of clinicians and researchers should use these advances to make the diagnostic and treatment process more individualised because each patient experiences their condition in a unique and highly personal way. This means that the categorical classification of patients to clusters of symptoms should be combined with targeted personalised approaches. It bears on the question of how medicine and healthcare can be made more humane and better suited to capture the complexity of the vivid and turbulent worlds that each psychiatric patient inhabits.

With this edition, we want to reveal some of this multifaceted complexity of neuropsychiatry to you - with its challenges and recent developments. Through a wide variety of sections, we want to show that the field has made considerable progress towards a better understanding of the human mind. Prof. dr. Jim van Os, Prof. dr. Hilleke Hulshoff pol and dr. Hugo Schnack – all experts with distinctive knowledge in the field of psychiatry – share in-depth insights about the diagnostic and treatment processes, but also the future developments and improvements that are underway. In addition, we have captured some of the experiential everyday aspects of living with a diagnosis by including a personal account of what it feels to be autistic in our newly incorporated '*Personal perspective*'. We are particularly proud of this piece due to the challenging nature of the interview, and the bravery required to share about such an experience.

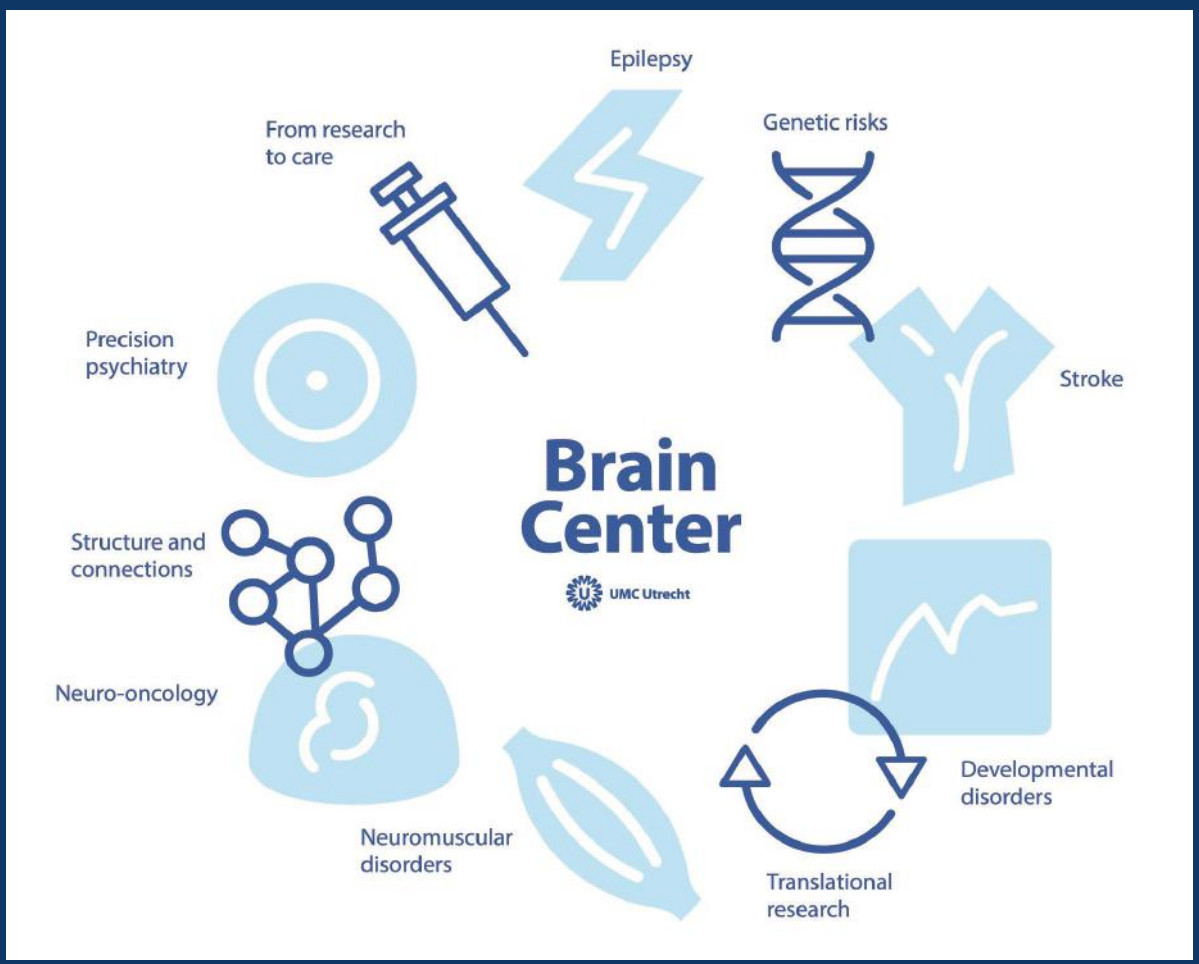
Further to this, we have two wise and light-hearted stories from students who went abroad – a Dutch student who studied in the US and a student from Brazil who came all the way to the Netherlands. We also offer a compelling book review and seminar section, as well as a first-hand story of what being a Ph.D. candidate feels like. From your fellow students, we have a handful of interesting pieces: a research article by Angelina, as well as review papers by Damian, Emma, Eveline, and Nick. Solée also shared about the challenges and enthusiastic preparations of the Mind the Brain Symposium.

We hope that you will enjoy reading this journal and that it will inspire you, and encourage you to think in novel ways about neuropsychiatry! On behalf of the whole board, we wish you all the best for your upcoming academic adventures!

Yours sincerely,

Ivana Kancheva
Editor in Chief Journal of Neuroscience & Cognition 2020

Sponsor of the Journal of Neuroscience & Cognition:



Rudolf Magnus Young Talent Fellowship

The UMC Utrecht Brain Center invests in junior scientific talent. The Rudolf Magnus Young Talent Fellowship ((€200,000 to be shared between the two applicants) allows junior researchers to develop a strong and recognizable research profile and set up interdisciplinary collaborations.

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Association between infarct location and post-stroke cognitive impairment: A large-scale lesion-symptom mapping study

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Ischemic stroke is a leading cause of chronic functional disability. Lesion location is an important determinant of one of its major consequences – post-stroke cognitive impairment. Although previous research has identified strategic regions for cognitive performance after stroke (i.e., regions where infarcts are most likely to occur), large-scale lesion-symptom mapping investigations of the relationship between lesion location and post-stroke cognition are still rare. In addition, current brain lesion coverage remains incomplete. Therefore, this study seeks to examine the association between acute infarct location and post-stroke cognitive impairment in a large cohort of 762 South Korean acute ischemic stroke patients using voxel-based and region of interest-based lesion-symptom mapping analyses. Furthermore, it aims to identify strategic lesion locations for both global cognition and distinct cognitive domains. Findings corroborate formerly established strategic infarct locations for global cognition, such as the basal ganglia, angular gyrus, and corona radiata. In addition, strategic regions involving both overlapping and domain-specific cortical and subcortical structures were identified for each of the cognitive domains, which illustrates the utility of employing a domain-specific approach to assess post-stroke cognition. Future studies should aim to improve brain lesion coverage and develop comprehensive prediction models based on infarct location in order to help explain how lesion burden in certain locations relates to impaired cognition following stroke.

Keywords: Ischemic Stroke; Post-Stroke Cognitive Impairment; Lesion Location; Voxel-Based Lesion-Symptom Mapping; Region of Interest Analysis

INTRODUCTION

Stroke is a leading cause of chronic functional disability worldwide (De Luca et al., 2018). More than 80% of all incident strokes are ischemic (Lloyd-Jones et al., 2010). Ischemic stroke is primarily caused by cerebrovascular disease, which includes both large vessel and small vessel disease (Hu et al., 2017). Its consequences are multidimensional, comprising deficits in cognitive, affective, behavioral, and motor domains (Chen et al., 2015).

Post-stroke cognitive impairment (PSCI), the prevalence of which ranges from 20% to 80%, is a critical determinant of health burden and loss of independence in daily life (Kosgallana et al., 2019; Sun et al., 2014). PSCI is a heterogeneous syndrome (Jokinen et al., 2015). It can be conceptualized as part of the vascular cognitive impairment (VCI) spectrum, which encompasses the full range of cognitive impairment (CI) associated with cerebrovascular disease (Dichgans & Leys, 2017). Common manifestations of PSCI include deficits in executive function, memory, language, and visuoconstructional abilities (Jokinen et al., 2015). Most stroke patients with PSCI will suffer from mild cognitive impairment (MCI), and up to a third will develop fully-fledged dementia (Barba et al., 2000).

Notably, lesion location is an important determinant of PSCI. To illustrate, the internal capsule, caudate nucleus and angular gyrus have been identified as strategic regions for global cognition (Gorelick et al., 2011), as well as the basal ganglia (Zhao et al., 2018), insula and opercular cortex (Cheng et al., 2014). Important infarct locations have also been found for separate cognitive functions, such as memory (Biesbroek et al., 2015), visuospatial abilities (Ten Brink et al., 2016), and executive function (Kalénine, et al., 2013). Recently, studies using lesion-symptom mapping (LSM), which examines the relationship between the location of a brain injury and behavioral outcomes, have further highlighted the importance of strategic infarct locations for PSCI occurrence (Rorden et al., 2009). For example, Munsch et al. (2016) found strokes in prefrontal and temporal cortex, hippocampus and thalamus, to independently predict cognitive outcomes at three-months post-stroke among a variety of factors, including age and neuropsychological assessment scores. Similarly, Puy et al. (2018) observed that, compared to other magnetic resonance imaging (MRI) markers, strokes in the left thalamus and middle frontal gyrus were the strongest predictors of six-month post-stroke global cognitive performance.

The increasing popularity of LSM over the past few decades has led to the development of a wide range of LSM methods (e.g., Sperber et al., 2019). Despite these advances, limited sample sizes used in LSM studies render current brain lesion coverage incomplete (Weaver et al., 2019). This is relevant because the impact of brain damage to a particular region on cognitive outcomes can only be assessed statistically if this region is affected in a sufficient number of patients (de Haan & Karnath, 2018). However, due to the structure of the brain vasculature, some regions are more commonly affected than others (Weaver et al., 2019). To illustrate, infarction in middle cerebral artery territory (MCA) is a lot more common compared to infarction in the orbitofrontal lobes in anterior cerebral artery territory (ACA) (Arboix et al., 2009). As a result, even LSM studies that have employed several hundred subjects have not considered rarely affected regions in statistical analyses, despite their potential criticality for cognition (Biesbroek et al., 2013; Zhao et al., 2018). Thus, a comprehensive map of strategic infarct locations for PSCI is still lacking.

The key objective of this investigation is to shed further light on the association between infarct location and occurrence of PSCI. It further seeks to provide a more comprehensive lesion map of the brain by exploring strategic lesion locations for both global cognition and five distinct cognitive domains. For this purpose, a large-scale LSM study will be conducted on an ischemic stroke cohort of 762 patients. PSCI will be defined using the Vascular Behavioral and Cognitive Disorder (VASCOG) criteria for vascular cognitive disorders (VCDs), according to which a diagnosis of VCD can be made if there is evidence of substantial cognitive decline from a documented or inferred previous level of performance in at least one cognitive domain (Sachdev et al., 2019). In addition, PSCI will be measured using cut-off scores on the Korean version of the Mini-Mental State Examination (K-MMSE) (Kang, 2006) and global cognitive performance scores derived from the Korean version of the Vascular Cognitive Impairment Harmonization Standards Neuropsychological Protocol (K-VCIHS-NP) (Hachinski et al., 2006). Two LSM approaches will be used to investigate the association between strategic infarct location and occurrence of PSCI. First, voxel-based LSM will be performed, where a statistical test is conducted to compare patients with and without a lesion at individual voxels (Sperber & Karnath, 2017). Second, a region of interest-based approach will be employed to relate pre-defined regional lesion volumes to cognitive outcomes (Bates et al., 2003).

METHODS

Participating centers

This retrospective study entails a collaboration between the Hallym University Sacred Heart Hospital, Anyang (Republic of Korea), the Seoul National University Bundang Hospital, Seongnam (Republic of Korea), and University Medical Center, Utrecht (the Netherlands). The study protocol was approved by the Institutional Review Board of the Hallym and Bundang hospitals.

Subjects and Inclusion Criteria

Participants were patients of either the Hallym University Sacred Heart Hospital or the Seoul National University Bundang Hospital, admitted with acute cerebral infarction between January 2007 and December 2018. A total of 3227 subjects were included in the original cohort. They underwent brain imaging and neuropsychological assessment within a year after stroke, as part of the study protocol (994 from the Hallym cohort and 2233 from the Bundang cohort). Of these 3227 patients, 1897 were initially selected for inclusion in this study based on availability of MRI imaging (diffusion-weighted imaging, DWI) or non-contrast computer tomography (CT) showing the acute symptomatic infarct, as well as assessment of post-stroke cognition at any follow-up time post-stroke, up to a maximum of one year (878 from the Hallym cohort and 1019 from the Bundang cohort). Patient selection then proceeded according to the inclusion criteria shown in Figure 1. The final study sample consisted of 762 patients.

Magnetic Resonance Imaging

Brain MRI, including T1-weighted, T2-weighted, DWI, and FLAIR sequences, was performed with a 3.0 Tesla MRI scanner (Achieva, Philips Healthcare) in the first week after stroke onset at both hospitals. Patients at the Hallym hospital were scanned at baseline (admission). Patients at the Bundang hospital were scanned at baseline (admission) and at one-week follow-up.

Imaging Processing Steps

Imaging processing steps included lesion segmentation, spatial normalization, and lesion map projection onto a brain template. First, acute lesions were manually delineated on each slice of patients' original MRI DWI sequences with an in-house developed software using MeVisLab (MeVis Medical Solutions AG, Bremen, Germany) (Ritter et al., 2011) by two trained investigators (A.K.K. and G.A.), according to the infarct segmentation protocol published by Biesbroek and colleagues (2019). They were subsequently checked and adapted (if necessary) by another rater (N.A.W.), and further revised by a fourth rater (J.M.B.) in the event of uncertainty regarding lesion location and/or classification. All four raters were blinded to patient medical history, diagnosis, and neuropsychological data during the segmentation process. Discrepancies between ratings were discussed in consensus meetings throughout the segmentation process. Acute lesions were delineated on FLAIR sequences on a subset of the scans (n= 11; 1.4 %) where delineation on DWI was not possible. FLAIR and T1-weighted sequences were used as reference sequences for scans from the Hallym VCI cohort. T1 and ADC sequences were used as reference sequences for scans from the Bundang VCI cohort.

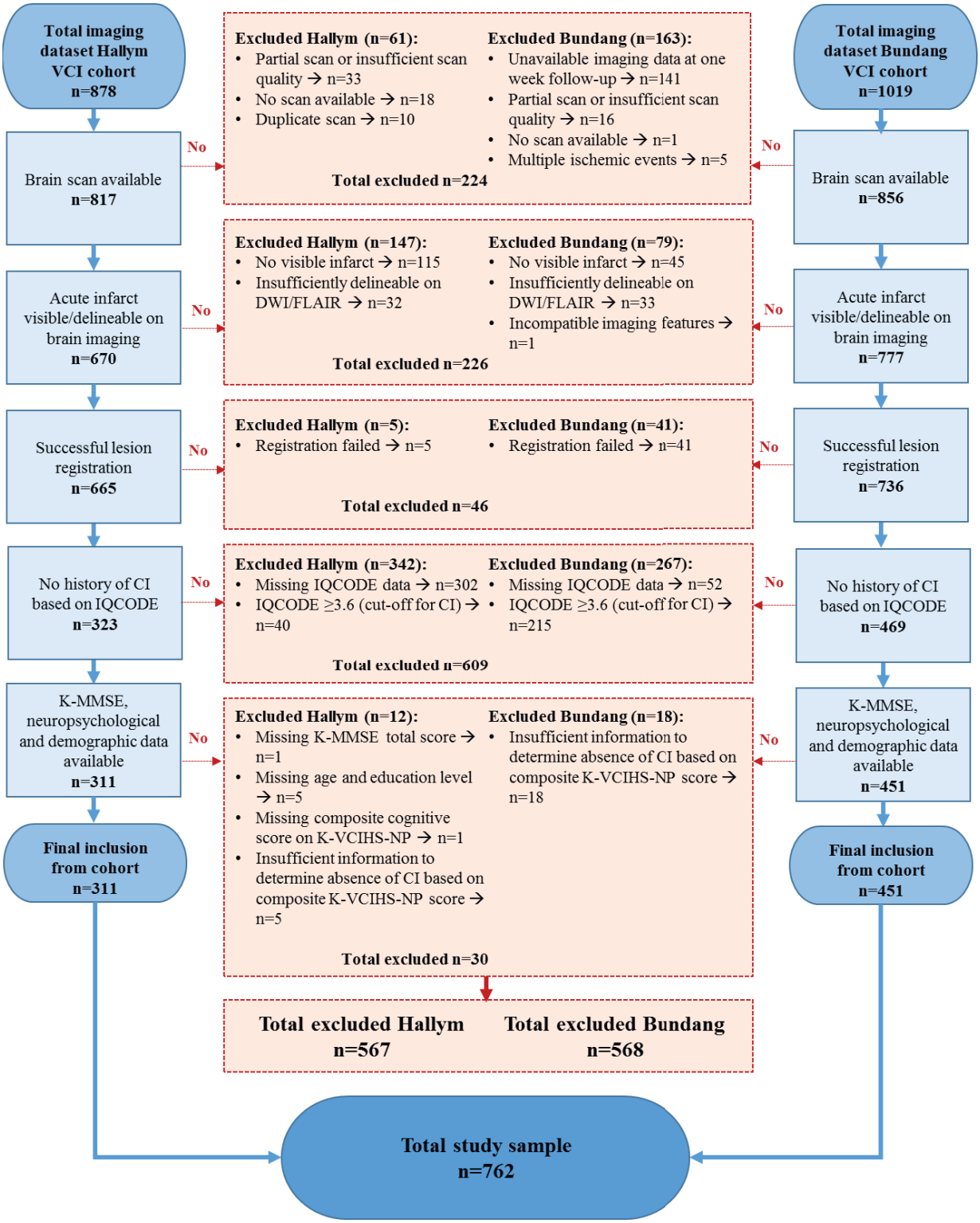

Figure 1. Flowchart of patient inclusion from the Hallym and Bundang VCI cohorts.

Following lesion segmentation, all scans and the corresponding lesion maps were transformed to fit the size and shape of a brain template (spatial normalization) using the RegLSM software package (https://metavcimap.org/features/software-tools; Biesbroek et al., 2019). RegLSM provides custom-fit settings for different imaging modalities and sequences, and corrects for anatomical variation (e.g., ethnicity-based morphometric differences) by performing intermediate transformation steps using population-adjusted templates to improve registration accuracy (Weaver et al., 2019). The final output is the same for each setting (i.e., lesion maps in standardized brain space), which allows lesion data from different imaging modalities (e.g., CT/MRI) and sequences (e.g., DWI/FLAIR/T1) to be combined into a single dataset.

In a final step, the resulting spatially normalized binary lesion maps were projected onto a Montreal Neurological Institute (MNI)-152 template (Fonov et al., 2009). The registered lesion maps were compared to the original scans to determine if lesion registration was successful. The main criteria were that key anatomical landmarks of the transformed scan and template corresponded, and that registered lesion maps accurately represented the original lesions in terms of size, shape, and location. Manual adaptations were made by N.A.W. in case of minor registration errors (44 % of cases; n= 522).

Neuropsychological Assessment

Each patient underwent neuropsychological assessment within a year after stroke onset. Cognitive performance was evaluated in the hospital setting using the K-MMSE (Kang, 2006) and the K-VCIHS-NP, adapted from the 60-minute VCIHS-NP proposed by the National Institute of Neurological Disorders and Stroke and Canadian Stroke Network (Gorelick et al., 2011; Hachinski et al., 2006). Table 1 provides an overview of the cognitive domains and neuropsychological tests included in the neuropsychological assessment protocol. Five cognitive domains were examined for the purposes of this study, namely executive function, processing speed, language, visuospatial abilities, and memory. Patients' history of pre-morbid CI was measured using the K-IQCODE: short form, where a score of ≥3.6 was used as a cut-off point, indicating pre-existing CI (Lee et al., 2005). All tests and scales in the original version were validated and standardized in a Korean population (Yu et al., 2013). The series of tests and questionnaires were administered by trained clinical psychometricians who were blinded to patients' clinical and neuroradiological profiles (Lim et al., 2014; Yu et al., 2013).

Outcome measures

The main outcome of this study was PSCI. Two measures were used to determine its occurrence. The first measure employed the definition of CI put forward in the VASCOG statement, which defines a VCD as substantial cognitive decline in at least one cognitive domain (Sachdev et al., 2019). For the purposes of this study, impairment in one of the five cognitive domains based on the K-VCIHS-NP was considered sufficient to signify CI, and only subjects unimpaired in all five cognitive domains were regarded as truly cognitively unimpaired. If a cognitive domain was evaluated with multiple tests, impairment on most of the subtests was required for the whole domain to be considered impaired. The executive function domain was measured with three subtests and impairment in at least two was required for a classification of CI. The memory domain was also assessed with three subtests and impairment in two was sufficient to determine CI. The processing speed domain comprised two subtests and impaired performance on at least one of them was necessary for the entire domain to be considered impaired. The second measure of PSCI was a K-MMSE cut-off score of ≤ 23, which was used to distinguish between patients with and without PSCI based on Korea-specific reference values (Kang et al., 2016). Patients who had scored ≤23 were considered cognitively impaired and patients who had scored above that cut-off were considered cognitively unimpaired (Kang, 2006). This cut-off has also been found as optimal to detect impaired cognition in other populations and across a variety of studies (e.g. Arevalo-Rodriguez et al., 2015; Kochhann et al., 2010; O'Bryant et al., 2008).

Data dichotomization and derivation of composite cognitive scores

Age- and education-corrected percentile scores based on normative data from a healthy Korean population (Lim et al., 2014; Yu et al., 2013) were dichotomized using < 5th percentile as a cut-off value for cognitive impairment for all neuropsychological tests, as this criterion has been used across a wide range of studies assessing post-stroke cognition (Wajer et al., 2019). The only exception was K-TMT ratio B/A, where dichotomization was carried out such that ratio scores ≥ 3 would indicate impaired cognitive performance and scores below that cut-off would signify unimpaired performance, as agreed by the research team (Sánchez-Cubillo et al., 2009). Composite scores per domain and global cognitive performance scores were then obtained according to the steps illustrated in Figure 2. Scores obtained on the K-MMSE were also dichotomized. Subjects who had scored below the cut-off point of ≤ 23 were considered cognitively impaired and assigned values of 1, while the rest of the participants were considered cognitively unimpaired and assigned values of 0.

Statistical Analysis

Two separate statistical analyses were performed, namely voxel-based lesion-symptom mapping (VLSM) and region of interest-based analyses (de Haan & Karnath, 2018). The full technical details are provided below.

Voxel-based lesion-symptom mapping

VLSM was performed using Non-Parametric Mapping (NPM) software implemented in MRIcron (Kimberg, 2009).

Table 1. Overview of the cognitive domains and neuropsychological tests included in the neuropsychological assessment protocol. K-VCIHS-NP: Korean version of the 60-minute Vascular Cognitive Impairment Harmonization Standards-Neuropsychological Protocol; K-MMSE: Korean version of the Mini-Mental State Examination; CI: Cognitive Impairment. Cognitive domains and neuropsychological tests used in this study were adapted from the K-VCIHS-NP, put forward by the National Institute of Neurological Disorders and Stroke and Canadian Stroke Network (Hachinski et al., 2006). The table provides information about the cognitive tests used to measure each of the five cognitive domains included in the analysis.

K-VCIHS-NP	**Cognitive domains and neuropsychological tests**	**Cut-off scores for CI**	**Sources**
	Executive function		
Animal naming	Animal naming (semantic fluency)	<5th percentile	Kang *et al.*, (2000)
Korean-COWAT	Controlled Oral Word Association Test (COWAT) (phonemic fluency)	<5th percentile	Kang *et al.*, (2000)
Korean-Trail Making Test-Elderly's version ratio B/A	Trail Making Test	Ratio scores (raw) ≥3	Yi *et al.*, (2007)
	Processing speed		
Korean-Trail Making Test-Elderly's version A (time in seconds)	Trail Making Test	<5th percentile	Yi *et al.*, (2007)
Rey Complex Figure Test: Copy Time (time in seconds)	Rey Complex Figure Test: Copy Time	<5th percentile	Kang & Na (2003)
	Language		
Short Form of the Korean-Boston Naming Test	Boston Naming Test	<5th percentile	Kang *et al.*, (1999)
	Visuospatial abilities		
Rey Complex Figure Test: Copy	Rey Complex Figure Test: Copy	<5th percentile	Kang & Na (2003)
	Memory		
Seoul Verbal Learning Test (Subtests: Immediate Recall, Delayed Recall and Recognition)	Hopkins Verbal Learning Test	<5th percentile	Kang & Na (2003)
	Others		
K-MMSE	Mini-Mental State Examination (MMSE)	≤23 (raw scores)	Kang (2006)

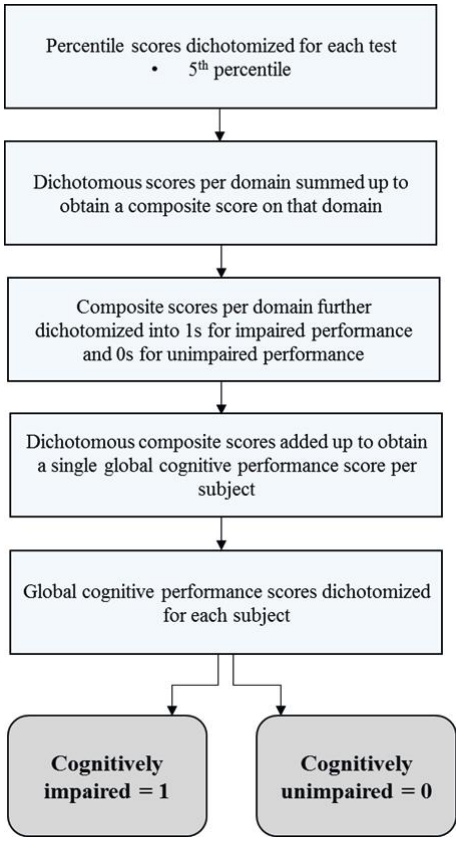

Figure 2. Overview of the applied dichotomization steps to obtain dichotomous global cognitive performance scores for each subject.

Settings were set to Leibermeister test, univariate analysis, only including voxels damaged in at least 8 patients (1% of the study sample). Correction for multiple testing was done using a false discovery rate threshold (FDR) with q < 0.05. Two independent analyses were carried out using the dichotomized K-MMSE scores and the global cognitive performance scores based on the K-VCIHS-NP as outcome measures. Additionally, five separate analyses were performed, where dichotomous composite scores for each of the five cognitive domains were included as outcome measures. The same settings and FDR thresholds were used for the main analysis.

Region of interest-based analysis

Next, region of interest (ROI)-based analyses were performed. For this purpose, point-biserial correlations were done between all cognitive outcomes of interest and regional volumes in SPSS, version 25.0.0.2. A point-biserial correlation is a special case of the Pearson's product-moment correlation, which measures the strength of association between a binary (categorical) and a continuous variable (Linacre & Rasch, 2008). In this study, grey and white matter regional volumes were continuous variables and all cognitive outcomes (dichotomized total K-MMSE scores, global cognitive performance scores, and composite scores per cognitive domain) were binary. Point-biserial values range from – 1.00 to + 1.00 and strong correlations typically have a value of 0.15 or higher (rpb ≥ 0.15; Varma, 2006). The ROIs were defined by the Harvard-Oxford grey matter atlas and ICBM-DTI-81 white matter atlas in MNI-152 space (Desikan et al., 2006; Mori et al., 2008, respectively). Regions affected in less than 8 people (1% of the study sample) were excluded (right frontal medial cortex volume for grey matter and column and body of fornix for white matter). All 141 regional volumes specified in the two atlases – 89 grey matter and 52 white matter – were included in the analyses. Dichotomized K-MMSE scores, global cognitive performance scores, and dichotomous composite scores for each cognitive domain were correlated with every grey and white matter regional volume. Subsequently, the p-values of all resulting point-biserial correlations were FDR corrected with q<0.05. Regions of interest that yielded the strongest correlations (rpb ≥ 0.15) were considered the most relevant and visualized results are based exclusively on them (Figure 6).

RESULTS

Study Population Baseline Characteristics

Table 2 contains the full baseline demographic and clinical characteristics of the study cohort (n=762). Participants' median age was 69 years (IQR=15) and 61.3% of them were men (n=467). Subjects' median level of education was 10 years (IQR=8.00) and 89.6% of them had not suffered a prior stroke. On average, brain imaging was performed within a week post-stroke (median=4 days, IQR=4, range=0 to 62 days). In addition, neuropsychological examination took place within half a year after stroke onset for most participants (median=99 days, IQR=61, range=1 to 365 days). One-hundred ninety-five (25.6%) of the included patients qualified as cognitively impaired based on the K-MMSE cut-off score (≤23). In turn, 403 subjects (52.9%) qualified as cognitively impaired based on the global cognitive performance scores. Overlap between the two measures in terms of number of subjects judged as cognitively impaired was observed for 551 patients (72.3%).

Voxel-Based Lesion-Symptom Mapping

The total distribution of significantly affected voxels in the study cohort is illustrated by the lesion prevalence map in Figure 3. Only voxels damaged in at least eight patients are displayed. Lesion prevalence was higher in the right hemisphere than in the left, and higher in subcortical regions than in cortical regions. Most commonly affected voxels in grey matter regions were mainly located in middle cerebral artery territory (MCA) and bilateral brainstem, as well as in white matter tracts, including the internal capsule and bilateral anterior and posterior corona radiata.

Two separate voxel-based analyses were conducted to explore associations between affected voxels and the two main cognitive outcomes of interest (Figure 4). VLSM identified significant associations between the dichotomized total K-MMSE scores and several clusters of voxels almost

Table 2. Baseline demographic characteristics and vascular risk factors of the study population.

Variable	Hallym VCI cohort	*n*	Bundang VCI cohort	*n*	Total	*n*
Demographic Characteristics						
Age (years), median (IQR)	65 (18)	311	69 (13)	451	69 (15)	762
Gender, n		311		451		762
Male, n (%)	184 (59.2)		283 (62.7)		467 (61.3)	
Female, n (%)	127 (40.8)		168 (37.3)		295 (38.7)	
Education (years), median (IQR)	9 (6)	311	12 (10)	451	10 (8)	762
BMI, median (IQR)	24.39 (3.95)	299	24.03 (3.66)	451	24.19 (3.72)	750
Handedness, n		311		449		746
Right, n (%)	292 (98.3)		432 (96.2)		724 (97.1)	
Left, n (%)	3 (1.0)		4 (0.9)		7 (0.9)	
Ambidextrous, n (%)	2 (0.7)		13 (2.9)		15 (2.0)	
Time interval between stroke onset and MRI (days), median (IQR)	1 (1)	310	5 (2)	451	4 (4)	761
Time interval between stroke onset and NPE (days), median (IQR)	96 (16)	310	108 (167)	451	99 (61)	761
IQCODE, median (IQR)	3.04 (0.21)	311	3.18 (0.29)	451	3.11 (0.29)	762
Vascular Risk Factors						
Smoking present, n (%)	82 (26.7)	307	97 (21.5)	451	179 (23.6)	758
Smoking past, n (%)	26 (8.7)	299	97 (21.5)	451	123 (16.4)	750
Hypertension, n (%)	186 (59.8)	311	337 (74.7)	451	523 (68.6)	762
Dyslipidemia, n (%)	121 (39.4)	307	104 (23.1)	451	225 (29.7)	758
Diabetes mellitus, n (%)	97 (31.3)	310	135 (29.9)	451	232 (30.5)	761
Prior TIA, n (%)	3 (1.1)	274	8 (1.8)	451	11 (1.5)	725
Prior stroke, n		306		451		757
No prior stroke, n (%)	264 (86.3)		414 (91.8)		678 (89.6)	
Ischemic, n (%)	28 (9.2)		30 (6.7)		58 (7.7)	
Hemorrhagic, n (%)	6 (2.0)		4 (0.9)		10 (1.3)	
Unknown, n (%)	8 (2.6)		3 (0.7)		11 (1.5)	
Atrial fibrillation, n (%)	39 (12.8)	305	76 (17.7)	451	115 (15.7)	734
Coronary heart disease, n (%)	12 (3.9)	307	31 (7.0)	451	43 (5.7)	749

BMI: Body Mass Index; NPE: Neuropsychological Examination; TIA: Transient Ischemic Attack.

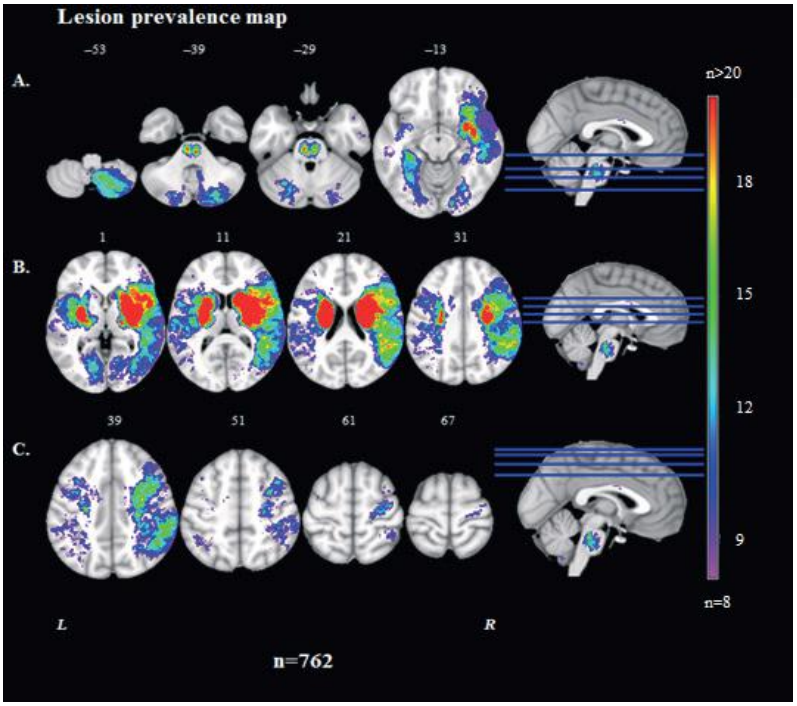

Figure 3. Lesion prevalence map showing an overview of the total distribution of affected voxels in the study cohort (n=762). Color bar indicates number of patients with a lesion for each voxel. Voxels affected in at least 8 people (pink) to 20 or more people (red) are displayed. Numbers above selected slices in A, B and C denote Z coordinates. L = left, R = right.

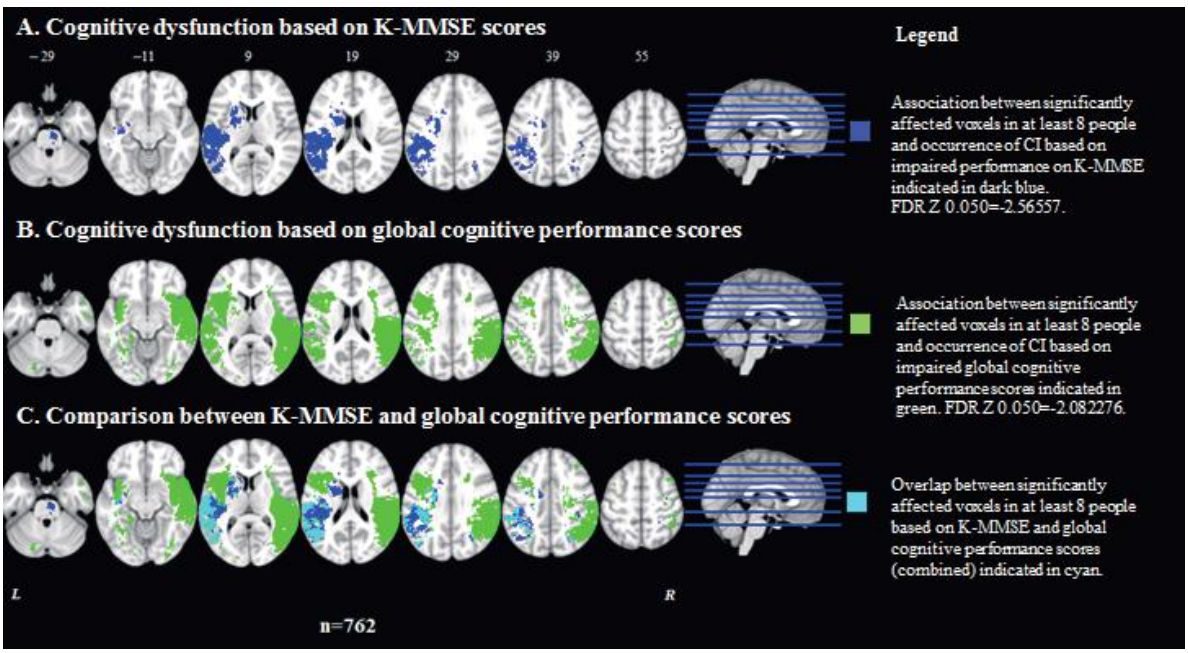

Figure 4. A. Association between voxels affected in at least 8 people (in blue) and occurrence of PSCI based on dichotomized total K-MMSE scores. B. Association between voxels affected in at least 8 people (in green) and occurrence of PSCI based on global cognitive performance scores. C. Comparison between significantly affected voxels in at least 8 people based on K-MMSE and global cognitive performance scores. Voxel overlap is indicated in cyan. Z coordinates for A, B and C: −29, −11, 9, 19, 29, 39, 55. L = left, R = right.

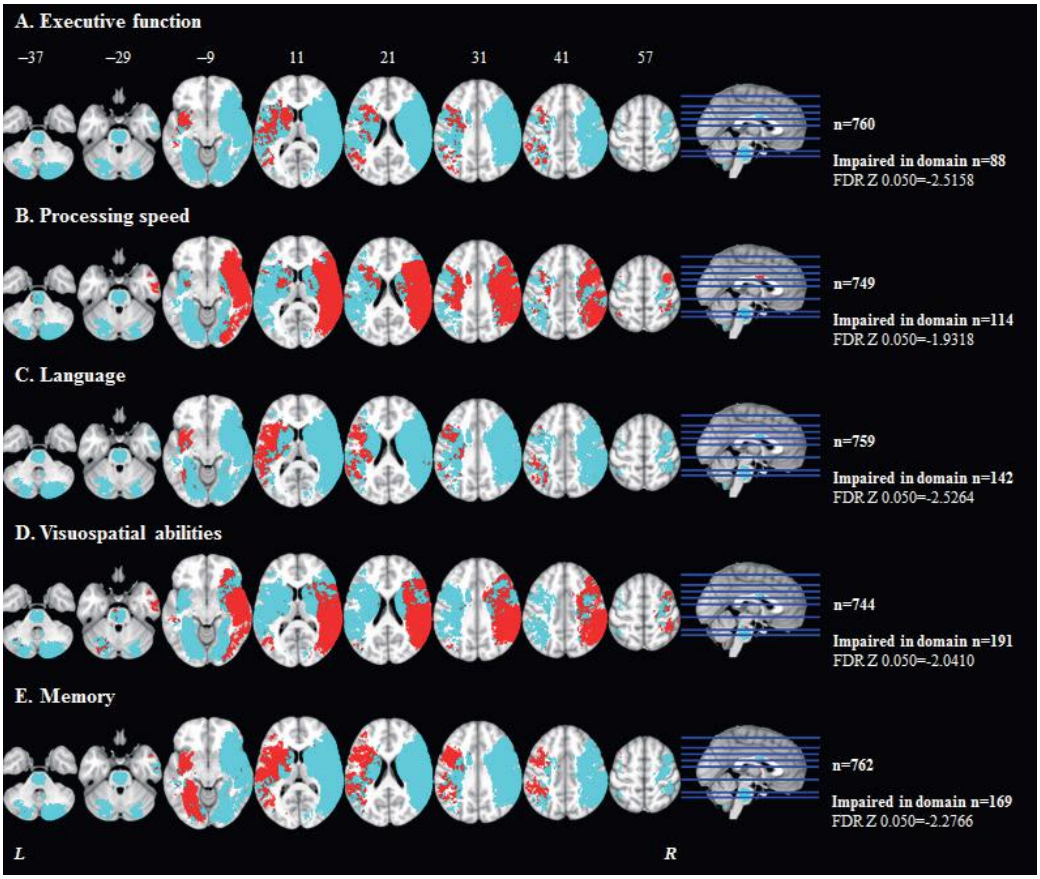

Figure 5. Overlay maps of all tested voxels (in cyan) compared against significantly affected voxels in at least 8 people (in red) for each cognitive domain (A-E). Total number of patients included in the separate analyses (A-E), number of impaired subjects per domain, and FDR thresholds are indicated on the right. Z coordinates for images A-E: –37, –29, –9, 11, 21, 31, 41, 57. L = left, R = right.

exclusive to the left hemisphere. They were predominantly located in left superior temporal lobe, left parietal cortex, left corona radiata, and right brainstem (pons). In turn, significant associations for the global cognitive performance scores were found in both hemispheres. Voxels were mostly located in bilateral parietal, temporal and occipital cortices, left and right corona radiata, and right sensory-motor cortices. Unlike for the K-MMSE, voxels in brainstem showed no significant involvement. When significantly affected voxels for the two outcomes were overlaid, overlap was mostly observed in left hemisphere regions, including portions of parietal operculum, superior and middle temporal gyrus, supramarginal and angular gyrus, as well as left anterior corona radiata.

In addition to exploring associations for the two main cognitive outcomes of interest, five separate VLSM analyses were performed, where dichotomous composite scores for each cognitive domain were entered as outcomes. Results demonstrated differential voxel involvement for different cognitive domains (Figure 5). First, executive function impairment was left hemisphere lateralized and most strongly related to damage in basal ganglia, insular cortex, and fronto-parietal regions. Processing speed impairment was right hemisphere lateralized and included extensive clusters of voxels in temporo-parietal and occipital cortices, as well as right corona radiata. For language, significantly affected voxels were located in left hemisphere regions, mostly including inferior frontal gyrus, superior temporal gyrus, and insular cortex. Impairment in visuospatial abilities was predominantly right lateralized, similar to processing speed. Significantly damaged voxels were located in extensive clusters spanning right frontal, parietal, and occipital regions. Voxels in right sensory-motor cortices were also affected. Several clusters of voxels in brainstem regions and cerebellum were exclusively associated with impairment in the visuospatial domain as well (left cerebellum, pons and medulla). Finally, memory was mostly linked to damage in voxels located in left basal ganglia and left occipital cortex, and was the only domain to reveal significant voxel associations in the hippocampus (left).

Region of Interest-Based Analysis

Point-biserial correlations were run between 141 regional volumes, 89 grey matter regional volumes defined in the Harvard-Oxford atlas (Desikan et al., 2006) and 52 white matter specified in the ICBM-DTI-81 atlas (Mori et al., 2008),

and all cognitive outcomes in SPSS, version 25.0.0.2. Figure 6 presents the strongest correlations for each cognitive outcome (threshold rpb≥ 0.15; Varma, 2006). Significant correlations for global cognitive performance scores did not exceed the specified threshold (rpb≥ 0.15) and are therefore not presented.

Strongest correlations for cognitive impairment based on dichotomized K-MMSE total scores were found in regional volumes in left parietal and temporal lobes, left lateral occipital cortex, and white matter tracts connecting frontal, temporal, parietal, and occipital lobes. Executive function impairment yielded strongest correlations in left fronto-parietal and lateral occipital cortices, left basal ganglia and left amygdala, as well as white matter tracts connecting parts of the limbic system with the frontal lobe. Strongest correlations for processing speed impairment were observed in right parietal and temporal lobes, right orbito-frontal cortex, frontal white matter and white matter tracts connecting the thalamus with the occipital and parietal lobes. Language impairment was most strongly associated with regional infarct volumes in left superior and inferior temporal gyrus, left inferior temporal cortex, and internal capsule. Impairment in visuospatial abilities correlated most strongly with regional volumes in right parietal and temporal lobes, and white matter bundles conveying fibers from parietal, occipital and temporal regions to subcortical destinations. Finally, the strongest correlations for memory impairment were with regional infarct volumes in left middle and inferior frontal gyrus, left hippocampus and parahippocampal gyrus, as well as corpus callosum and fronto-occipital white matter.

DISCUSSION

The key aim of this LSM study was to shed further light on the association between infarct location and occurrence of PSCI in a large acute ischemic stroke cohort of 762 patients. It further sought to improve current brain lesion coverage by exploring strategic lesion locations for both global cognition and five distinct cognitive domains using VLSM and ROI analyses.

Results confirm the relevance of infarct location for PSCI. Several strategic regions for both global cognitive dysfunction and impairment in the five cognitive domains were found. They were mostly located in MCA territory and included portions of bilateral parietal and temporal cortex, subcortical grey matter structures, and white matter tracts connecting the four lobes of the brain. Findings corroborate previously identified strategic infarct locations for cognitive impairment, such as left angular gyrus (Biesbroek et al., 2014), basal ganglia (Grau-Olivares et al., 2010; Narasimhalu et al., 2013), parietal operculum (Meyer et al., 2016), superior temporal gyrus (Leff et al., 2009; Mirman et al., 2015), left anterior corona radiata (Biesbroek et al., 2015; Munsch et al., 2016; Zhao et al., 2018), internal capsule (Biesbroek et al., 2017), and superior longitudinal fasciculus (Cristofori et al., 2015; Urger et al., 2015). Additionally, the left Heschl's gyrus (HG; containing human primary auditory cortex) and left planum temporale (the cortical area just posterior to the HG) (Warrier et al., 2009) were identified as strategic for global cognitive dysfunction.

Our findings also highlight the importance of previously observed cognitive domain-specific associations. For instance, executive function impairment was linked to a distributed network of grey and white matter spanning temporo-parietal cortical regions (Kalénine et al., 2013; Zhao et al., 2018), and visuospatial impairment was mostly related to damage in the parietal lobe and posterior thalamic radiation (e.g. Biesbroek et al., 2014, Ten Brink et al., 2016; Zhao et al., 2018). Further, results for the language domain agreed with well-established neural correlates of this cognitive function (e.g. Dronkers et al., 2004; Mirman et al., 2015). Notably, some clear distinctions between cognitive domains were found. For example, language impairment yielded the strongest correlations with left inferior frontal gyrus (IFG) and left superior temporal gyrus (STG). These anatomical locations correspond to Broca's (IFG) and Wernicke's areas (STG), which have consistently been implicated in language production and comprehension, respectively (e.g., Dronkers et al., 2004; Mirman et al., 2015; Weiss et al., 2016). Moreover, results for memory confirmed the importance of the area considered as the seat of this cognitive faculty – the hippocampus (McKenzie & Buzsáki, 2016). Strong associations with memory impairment were further revealed for parahippocampal gyrus, which plays a role in spatial memory (Aguirre et al., 1996), and left cingulum (hippocampus), the degeneration of which represents one of the earliest developmental changes in age-related dementia (Delano-Wood et al., 2012).

Interestingly, lesion prevalence for executive function, language, and memory was exclusively associated with left hemisphere regions. Overlap between the three domains was observed in regions including left inferior fronto-occipital fasciculus (IFOF), which has been implicated in language in earlier LSM studies (Almairac et al., 2015). These findings can be explained on the grounds that executive, language and memory domains were all measured with at least one language task that drew on verbal processing (e.g., vocabulary size for Animal naming and verbal memory for the Seoul Verbal Learning Test) (Kang et al., 2000; Kang & Na, 2003). On the contrary, lesion prevalence for processing speed and visuospatial abilities was mostly associated with right hemisphere cortical regions, including superior temporal gyrus and lateral occipital cortex (LOC). This can be explained with the fact that optimal performance on the Rey Complex Figure Test (and Rey Complex Figure Test: Copy Time for processing speed) has been shown to rely heavily on visual-perceptual processing, the neural seat of which is the LOC (Luck et al., 1997). Of note, processing speed was associated with the highest number of voxel associations and was the only domain to reveal a strong correlation with right orbitofrontal cortex (OFC), a brain region rarely considered in previous LSM studies (e.g., Arboix et al., 2009).

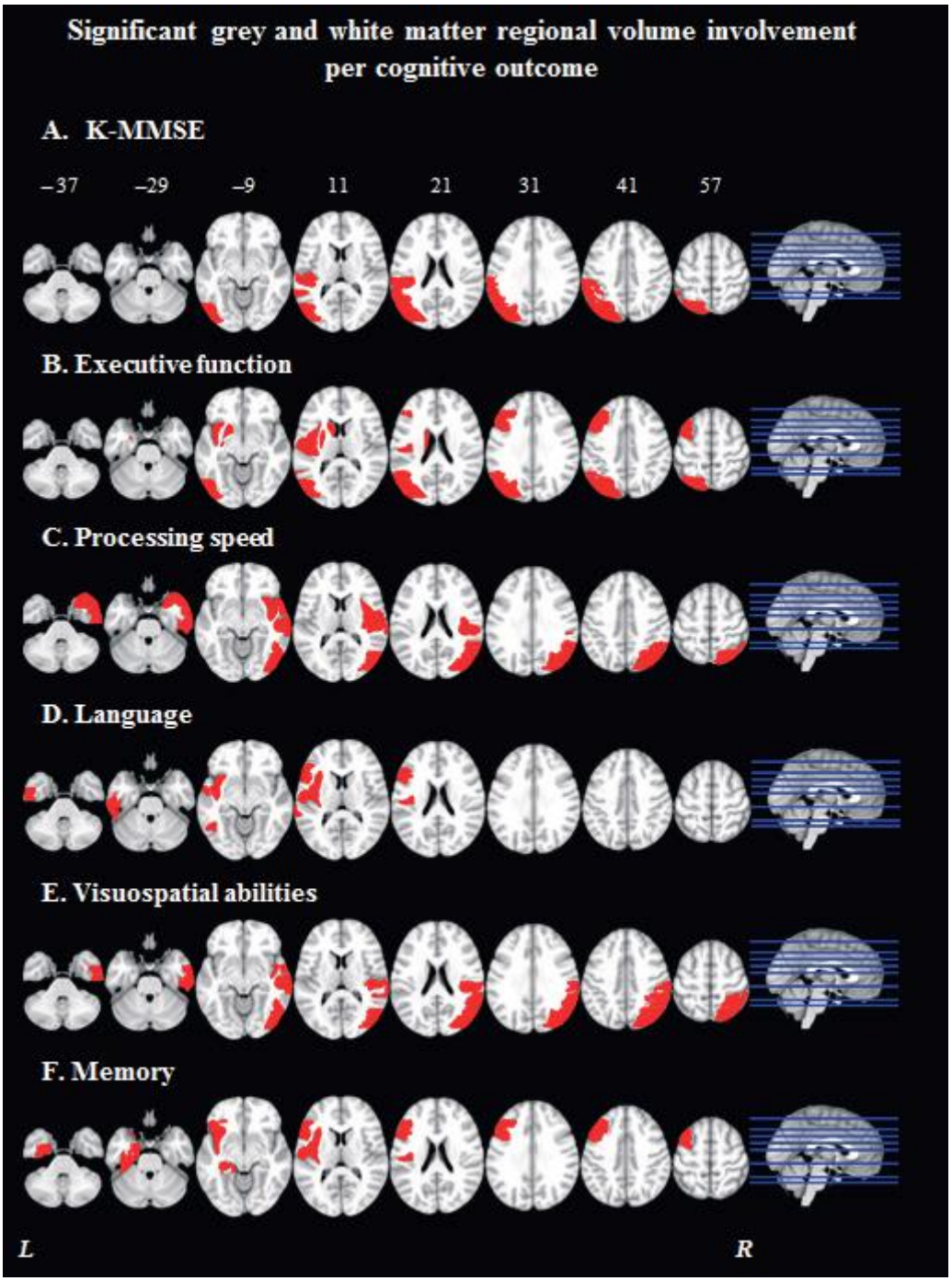

Figure 6. Significant associations between grey and white matter regional volumes and cognitive outcomes of interest based on point-biserial correlations (A-F). Only the strongest correlations (threshold rpb ≥0.15, p-values significant at the 0.01 level) are displayed for each domain (in red). Regional volumes affected in less than 8 people are not presented. Z coordinates for images A-F: –37, –29, –9, 11, 21, 31, 41, 57. L = left, R = right.

This could be due to the fact that the OFC connects to subcortical structures, many of which were common stroke targets in this study. Therefore, larger subcortical lesions that yielded significant associations with processing speed could have extended into this region.

The above findings illustrate that multiple brain areas appeared to be relevant for global cognitive function, including many regions established in previous LSM

studies. Global cognition seemed to integrate a variety of different brain functions, which justified the exploration of distinct cognitive domains in separate analyses. Indeed, when cognitive domains were analyzed separately, some important differences were observed. Each domain was related to the integrity of different regions, which highlighted the relevance of focusing on separate cognitive domains to uncover neural correlates of specific functions. Results also underscored that ischemic stroke lesions are not randomly distributed, but follow the vascular tree (i.e., voxels within the vascular territory of a specific artery are strongly related in terms of lesion status) (Zhao et al., 2018). For example, infarction in MCA territory was more commonly observed than infarction in ACA territory, in line with other investigations (Arboix et al., 2009). Such information is of clinical value, as it helps clinicians to understand where lesion burden has the most impact on cognition, and why patients with strategic lesions suffer from specific cognitive deficits (Zhao et al., 2018).

It should be noted that results based on the K-MMSE and the global cognitive performance scores differed. More specifically, 25.6% of the participants qualified as cognitively impaired based on the K-MMSE and twice as many based on the global cognitive performance scores (52.9%). Overlap between the two measures in terms of the number of subjects considered as impaired was found for 72.3%. This discrepancy can be explained on the grounds that global cognitive performance scores constituted a more sensitive and specific measure of PSCI, which was also illustrated by the higher number of significant voxel associations for that measure. At the same time, optimal performance on the K-MMSE appeared to depend heavily upon the integrity of language-dominant left hemisphere regions, such as the angular gyrus (Price et al., 2015; Weiss et al., 2016) and superior temporal gyrus (Leff et al., 2009; Zhao et al., 2018). These observations seem to cast doubt on the validity of the MMSE to measure global post-stroke dysfunction. Previous reports have already underscored that language items included in the MMSE are overrepresented (Bour et al., 2010; Kosgallana et al., 2019), while items assessing executive function and memory are relatively crude and non-specific (Mitchell, 2017). Thus, it could be that the K-MMSE failed to detect subtler changes underlying domain-specific CI, as well as the global cognitive performance scores in this study.

On a more general level, the above considerations resonate with the observation that domain-specific cognitive scores might be more suitable to detect strategic infarct locations for PSCI compared to global cognitive scores. To illustrate, no significant correlations with global cognitive performance scores exceeded the 0.15 threshold in ROI analyses, unlike for scores on the separate cognitive domains. Global cognition drew on a wide range of distributed networks across both hemispheres, such that no regions would reveal particularly strong associations. In fact, a recent systematic review of 22 PSCI studies reported that domain-specific screening tools are better suited to detect PSCI due to its heterogeneity (Kosgallana et al., 2019). That is, PSCI incorporates interrelated multidomain deficits, which may be easier to tease out with domain-specific cognitive measures (Dichgans & Leys, 2017). Results of this study complement such findings and further emphasize that focusing on distinct cognitive domains, rather than global cognitive dysfunction, might be more relevant in terms of revealing the neural correlates of specific cognitive functions.

Taken together, the discussed findings confirm previously established neural correlates of both global cognition and distinct cognitive functions, and improve the brain lesion coverage achieved by earlier monocenter LSM studies (e.g., Zhao et al., 2018). In doing so, they emphasize the validity of the LSM approach to uncover consistent lesion-deficit associations.

STRENGTHS AND LIMITATIONS

This study has several prominent strengths. First, it is one of the largest LSM studies to investigate the association between infarct location and occurrence of PSCI to date. A second strength is the use of complementary LSM techniques, where voxel-wise analyses provide high spatial resolution and ROI analyses take cumulative lesion burden in a specific structure into consideration (Sperber & Karnath, 2018). The inclusion of all grey and white matter regional volumes in correlational analyses constitutes an additional advantage, as it allowed for a large number of associations without a priori assumptions to be explored. As a result, a more complete map of strategic regions for the five cognitive domains was provided (Figures 5 and 6). Another strength is the use of two PSCI measures, K-MMSE scores and an extensive neuropsychological protocol, which made it possible to assess both global aspects of cognition and separate cognitive domains.

Nevertheless, an important limitation of the study is the use of a univariate LSM approach. This method does not take inter-voxel correlations into account, which makes it prone to some degree of displacement regarding the location of strategic brain regions (Sperber & Karnath, 2018). In addition, univariate LSM considers each voxel as a functional unit worth testing on its own (Sperber & Karnath, 2017). In reality, cognitive deficits arise when multiple voxels are damaged. Therefore, analyses that consider groups of voxels as the units sub-serving cognitive functions are required. Such multivariate analyses could not be performed in this investigation due to technical constraints. However, they have already been shown to provide higher sensitivity to detect lesion-deficit associations (e.g., support vector regression-based LSM) (Zhang et al., 2014; Zhao et al., 2018).

METHODOLOGICAL CONSIDERATIONS AND FUTURE RESEARCH

Regarding the methodology, it is worth mentioning that ROI results were based on point-biserial correlations. They were used to measure the strength of association between grey and white matter regional volumes (continuous variables) and all cognitive outcomes (dichotomized total K-MMSE scores, global cognitive performance scores, and composite scores per cognitive domain) (categorical variables). Dichotomization was carried out so that results are easier to interpret from a clinical perspective. Categorizing individuals into one of two groups made it possible to understand the relationship between infarct location and PSCI in terms of either being at risk or not being at risk for developing cognitive problems after stroke. However, binary variables were created artificially from continuous total K-MMSE and K-VCIHS-NP scores by grouping and re-coding cases. Albeit justified, the applied transformations are likely to have introduced some bias in the results, as continuous-level data contain more variance than categorical data and may provide more reliable correlations (Varma, 2006).

An additional selection bias is introduced by the stringent procedure used to select participants from the target population. Most notably, the IQCODE criterion led to the exclusion of a large number of subjects, which suggests that findings should be interpreted with caution and as population-specific. Also, results only display voxels and regions of interest affected in at least 8 people (1 % of the study sample). Thus, the impact of isolated defects occurring in fewer patients is not reflected in the study's findings.

Future LSM studies should aim to extend the current findings by including more patients with sufficient lesion coverage, and work towards translating them into clinical practice. This would require sophisticated infarct location-based prediction models for PSCI, which could be used to identify patients at risk for cognitive problems at early post-stroke stages. If rehabilitation strategies are initiated at such early points in time, patient outcomes could be significantly improved. The development of such prediction models would likely be feasible through collaborative investigations including thousands of patients. Such projects are already underway and demonstrate that multicenter data integration markedly improves brain lesion coverage (Weaver et al., 2019).

CONCLUSION

In conclusion, this study investigated the association between infarct location and PSCI in a large acute ischemic stroke cohort. It corroborated previously identified strategic lesion locations for both global cognition and five distinct cognitive domains, and confirmed the validity of the LSM approach to detect lesion-deficit associations. It also demonstrated the utility of a domain-specific approach to assessing PSCI instead of a global cognitive dysfunction approach. Future studies should further aim to improve brain lesion coverage and work towards developing comprehensive prediction models for PSCI based on infarct location through multicenter collaborations.

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CONFLICT OF INTEREST STATEMENT

The author declares no conflict of interest.

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Resting state brain activity in auditory verbal hallucinations: A systematic review

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Introduction: Approximately 70% of schizophrenia patients experience auditory verbal hallucinations (AVH). Most studies agree that the auditory and language-related brain regions are likely to be involved in the generation of AVHs, but the exact underlying mechanisms remain unclear. In this systematic review the fMRI findings of patients with AVHs of 7 studies (n=98 subjects) have been investigated.

Methods: Articles were eligible if they investigated the resting brain activity in schizophrenia or psychotic patients during the experience of AVHs with fMRI.

Results: Results regarding the activated brain areas during the experience of AVHs are inconsistent, but some areas are reported in the majority of studies investigated here. These areas include the inferior frontal gyrus, superior temporal gyrus, middle temporal gyrus, supramarginal gyrus, and insula.

Discussion: The studies included in this systematic review suggest that a dysfunctional activation and connectivity of the frontal language production area and the temporal language processing area are involved in the pathophysiological mechanism underlying AVHs. Future research should focus on the areas that seem to be most extensively activated during AVHs and larger sample sizes are required to produce consistent results. Once the mechanisms underlying AVHs are further unravelled, new treatment options for patients with AVHs can be developed.

Keywords: Auditory Verbal Hallucinations; Schizophrenia; Functional Magnetic Resonance Imaging; Resting State Brain Activity

INTRODUCTION

Roughly 70% of schizophrenia patients experience auditory verbal hallucinations (AVH) i.e. perception of voices in the absence of external stimuli, commonly referred to as hearing voices (Dierks et al., 1999; Zweerings et al., 2019). Schizophrenia patients are unable to function properly at school or work, and 5% of the patients eventually commit suicide, with the rest exhibiting severe problems in day-to-day functioning (Hyman & Cohen, 2013). Patients usually get diagnosed in their late adolescence and can experience cognitive, negative and positive symptoms (Hyman & Cohen, 2013). Cognitive symptoms are impairments in working memory and executive functions (Hyman & Cohen, 2013), whereas negative symptoms are impairments in normal functions, such as withdrawal from social interactions or a lack of motivation (Hyman & Cohen, 2013). Furthermore, positive symptoms are symptoms that are usually not present in healthy individuals, but are experienced by schizophrenia patients (Soares-Weiser et al., 2015). These positive symptoms include somatic hallucinations, delusions, and thought withdrawal. Having AVHs is one of the positive symptoms that schizophrenia patients can experience (Soares-Weiser et al., 2015). The antipsychotics that are used in schizophrenia to treat these symptoms act on the dopaminergic pathways (Hyman & Cohen, 2013). However, not every patient responds to this treatment, and it can cause a lot of side effects (Hyman & Cohen, 2013). Therefore, the treatment of AVHs is an area of active investigation.

Having AVHs is a predominant symptom of schizophrenia. It is generally uncontrollable and often causes disruption in daily life (Zweerings et al., 2019). This can decrease the quality of life and enhance the risk of suicide and acts of violence (van Lutterveld, Diederen, Otte, & Sommer, 2014). AVHs are not only present in schizophrenia patients, but can also be a symptom of other mental health disorders such as bipolar disorder or depression (Alderson-Day, McCarthy-Jones, & Fernyhough, 2015). Even healthy people can experience hearing voices without needing psychiatric care (Northoff & Qin, 2011). In schizophrenia patients, AVHs are the most common type of hallucinations (Curcic-Blake et al., 2013). These AVHs may sound very realistic, with the potential to lead to inappropriate and sometimes dangerous behaviour (Raij et al., 2009).

This perceptual quality is an interesting component of AVHs. Patients may be unable to distinguish the AVHs from real voices, because the AVHs sound like human voices, and seem to originate in the external physical environment (Kompus et al., 2013). For this reason, it appears particularly interesting that studies have implied that the brain regions responsible for auditory and speech processing, like the posterior superior temporal cortex and inferior frontal cortex (Thoma et al., 2016), may be involved in these hallucinations (Kompus et al., 2013). Indeed, multiple

studies seem to agree that the auditory and language-related brain regions are involved in the generation of AVHs. These studies are also called 'symptom capture' studies, because they try to capture the AVHs while measuring brain activity (Kompus et al., 2013). Even though plenty of research on this topic has already been done, there are still many unanswered questions concerning the neural mechanisms of AVHs.

One of those questions is: how can AVHs occur spontaneously from intrinsic brain activity? This intrinsic brain activity is also known as resting state activity (Havlík, 2017). According to Havlík (2017), resting state activity reflects "the neural states that are produced spontaneously by the brain and not as responses to stimulation or immediate reactions to the environment". This resting state activity in the brain can be measured when participants are lying still in a functional magnetic resonance imaging (fMRI) scanner in the absence of any external task (Alderson-Day et al., 2016). This task-unrelated resting state fMRI is a non-invasive method of measuring brain activity during AVHs, and therefore an easy way to study the neural mechanisms underlying AVHs. As a result, the interest in resting state brain activity, i.e. the spontaneous activation of brain regions during AVHs, has grown and research attempts to explain the neural mechanisms underlying AVHs (Alderson-Day et al., 2015).

Functional neuroimaging has already been widely used in research in an attempt to understand the brain activity underlying AVHs. As reported by some studies, resting-state networks (RSNs) may be involved in the psychopathology of schizophrenia (Zhao et al., 2018). Nevertheless, the neural mechanisms underlying AVHs are still not completely clear. One of the RSNs that may play a role in these mechanisms is the default mode network (DMN) (Zhao et al., 2018). The DMN is a group of brain regions that show high levels of activity during rest. The DMN includes different brain regions that interact with each other, such as the anterior and posterior cortical midline structures, hippocampus and the lateral parietal cortex (Northoff & Qin, 2011). Moreover, recent studies have found dysfunctions in the DMN associated with AVHs in schizophrenia patients (Northoff & Qin, 2011; Zhao et al., 2018). However, study findings on connectivity and activation of the DMN are inconsistent (Zhao et al., 2018). As a result, the role of the DMN in AVHs in schizophrenia remains unclear. Some other possible explanations associated with the RSNs suggest that there is a disturbance in the interaction within speech-processing system (e.g. the inferior frontal gyrus) and potential abnormalities of the resting-state activity in the primary auditory cortex (PAC) (Cui et al., 2017). This review will attempt to address some of these uncertainties.

The aim of this review is to give a comprehensive overview of the recent findings on AVH-related resting state brain activity in schizophrenia and psychotic patients. Firstly, I will discuss the current findings on the increased activation of brain regions during AVHs. Secondly, I will discuss the main functions of the regions involved in AVHs, and which circuits could be affected. Revealing the involved brain areas in AVHs, may benefit the development of new treatment options for patients that suffer from AVHs.

METHODS

Search strategy

Searches for relevant publications were conducted following PRISMA guidelines, and relevant publications were identified in the PubMed database. A flow diagram of the selection process is shown in Fig. 1. The search string was: auditory verbal hallucinat* AND (schizophreni* OR psychosis) AND (fMRI OR default mode network (DMN)). Articles were included if written in English, and if research was conducted among human participants. Only studies that measured the resting state brain activity during AVHs with fMRI in schizophrenia and psychotic patients were included. Articles were excluded when they were a review, case report, letter, methodological study, editorial or when it did not mention the significance threshold for brain activity. Additionally, reference lists of selected articles were reviewed to make sure all relevant information was used. Review papers were also read to make sure all relevant publications were included.

Next, studies were screened based on title and abstract. For each relevant publication, the information was extracted (authors, year of publication and the journal) and each article was evaluated on full text and assessed for eligibility. From these articles, relevant citations were pursued and included.

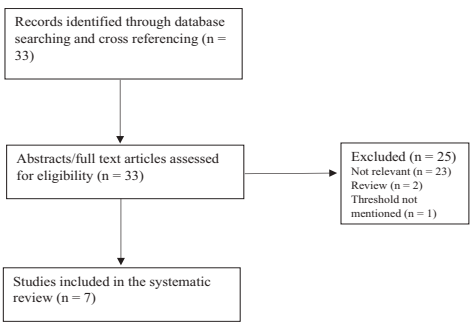

Figure 1. Flow diagram of the systematic selection process.

RESULTS

Functional imaging studies of AVHs look into the changes in blood flow that may be related to the development of AVHs (Allen, Larøi, McGuire, & Aleman, 2008). 'Activity studies' or 'symptom capture studies' try to measure the brain activity during the experience of AVHs. During the fMRI session, patients would go into the scanner and had to indicate the onset of an AVH with a button press or balloon squeeze and indicate the offset, usually, by releasing the button or balloon. Using the abovementioned search method, seven studies (n = 98 subjects) were found that assessed the resting brain activity with fMRI during an AVH experience in schizophrenia and/or psychotic patients. Detailed information about the increased activation of brain regions

Table 1. Summary of fMRI findings.

Study	*Sample group*	*Main findings (brain regions that show an increased activity during AVHs)*	*Significance threshold*	*Additional information*
Dierks et al. (1999)	3 paranoid schizophrenia patients	Heschl's gyrus, posterior superior temporal gyrus, middle temporal gyrus, frontoparietal operculum, hippocampus, and amygdala	$P < 0.01$	-
Raij et al. (2009)	11 patients with schizophrenia or schizoaffective disorder	Right parahippocampal cortex, bilateral inferior frontal gyrus, right posterior temporal lobe, left anterior temporal lobe, and right anterior cingulate cortex	$P < 0.05$	-
Mallikarjun et al. (2018)	9 First Episode Psychosis (FEP) patients	Bilateral auditory processing areas (superior temporal cortex), bilateral insula, parahippocampal complex, and the posterior regions of DMN (precuneus/posterior cingulate cortex)	$P < 0.01$	-
van de Ven et al. (2005)	6 paranoid schizophrenia patients	Primary auditory cortex (PAC), including the Heschl's gyrus (some patients show bilateral activation, some unilateral)	$P < 0.05$	-
Diederen et al. (2010)	24 patients with a psychotic disorder	Bilateral insula, inferior frontal gyrus (including Broca's homologue), middle temporal gyrus, superior temporal gyrus, supramarginal gyrus, bilateral inferior parietal lobule, precentral gyrus, postcentral gyrus, cerebellum, superior frontal gyrus, and middle frontal gyrus	$P < 0.05$	Deactivation in parahippocampal gyrus, left superior temporal gyrus, right inferior frontal gyrus, right insula, and left cerebellum before the AVH onset
Diederen et al. (2012)	21 psychotic patients	Bilateral inferior frontal gyrus, insula, superior temporal gyrus, supramarginal gyrus, postcentral gyrus, left precentral gyrus, inferior parietal lobule, and superior temporal pole	$P < 0.05$	-
Sommer et al. (2008)	24 chronically psychotic patients	Right inferior frontal area, including the right insula and Broca's homologue, the left insula, the bilateral supra marginal gyri, and the right superior temporal gyrus	$P < 0.05$	Most extensive activation in the right inferior frontal area

during AVHs can be found in Table 1.

DISCUSSION

This systematic review has given an overview of fMRI studies examining the resting brain activity during AVHs. The included studies show some inconsistent results regarding the brain activation during the generation of AVHs, and a lot of different brain regions are mentioned. Nonetheless, the majority of studies show an increased activation of the superior temporal gyrus (including the PAC and Heschl's gyrus), middle temporal gyrus, inferior frontal gyrus, supramarginal gyrus and insula during AVHs (Table 1). This suggests that these brain regions play an important role in the generation of AVHs, and different circuits in these brain regions could be altered.

The highest-associated brain region is the superior temporal gyrus, which is the top gyrus of the temporal lobe (Fig. 2) and consists of the auditory cortex, partly of the auditory association cortex (which includes the Heschl's gyrus) and Wernicke's area (Zevin, 2009). Lesions in the left Wernicke's area can result in Wernicke's aphasia, i.e. loss of comprehension of language (Zevin, 2009). The superior temporal gyrus or parts of this gyrus are mentioned in all 7 studies, which suggests a high association with the generation of AVHs. In addition, the supramarginal gyrus may also be important, as it lies just superior to the superior temporal gyrus, and is also part of Wernicke's area, which is involved in speech processing (Orlov et al., 2018).

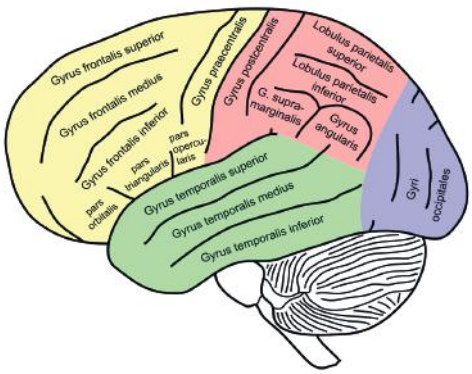

Figure 2. Lateral view of the human brain, with the main gyri labelled (Wikipedia (n.d.)).

Another gyrus in the temporal lobe is the middle temporal gyrus, which is located ventral to the superior temporal gyrus (Fig. 2) (Onitsuka et al., 2004). It is involved in language and semantic memory processing (Onitsuka et al., 2004). Functional deficits in this language and semantic memory processing has already been reported in schizophrenia (Onitsuka et al., 2004) and may therefore also play a role in the mechanisms underlying AVHs. In addition, the inferior frontal gyrus is also involved in language processing. It is part of the prefrontal cortex and has many functions, such as speech perception and comprehension (Rogers & Davis, 2017), and regulation of emotion and attention (Cha et al., 2016). Broca's area lies within the inferior frontal area (Rogers & Davis, 2017), which could explain why the inferior frontal area is involved in speech generation and may therefore also play a role in the generation of AVHs.

Lastly, the insula is a cortical language area that is located deeper in the brain and separates the temporal lobe from the frontal and parietal lobe (Sommer et al., 2008). It receives projections from the primary auditory cortex and auditory association cortex, and seems to be important for auditory processing (Kompus, Westerhausen, & Hugdahl, 2011). The insula has also been associated with awareness, which could imply that the activation of the insula during AVHs is part of the brain activity that has been associated with the conscious awareness of hearing voices. Furthermore, Mayer and colleagues (2009) concluded that the insula is involved in sensory gating, i.e. the regulation of the activity in the sensory cortices. An abnormal inhibition of the auditory cortex may be involved in the pathophysiology underlying AVHs.

The above-mentioned brain regions are all involved in language and auditory processing, and are also connected to each other, functionally and/or structurally. The altered connections in AVH patients that are mentioned in this review can be found in Figure 3. One of these connections is the functional connection between the superior temporal gyrus and the speech motor area (i.e. Broca's area) in the inferior frontal gyrus (Orlov et al., 2018). When this functional connectivity is impaired, there can be a deficit in the speech motor system that transports the information of self-generated actions to sensory brain regions (Orlov et al., 2018). One of these self-generated actions is inner speech. If this ability of the motor system is impaired, the sensory response in the superior temporal gyrus is hypothesized to not be attenuated to self-generated speech (Orlov et al., 2018). Incorrect interpretation of inner speech is thus caused by the failure of the speech motor area to attenuate the sensory activation in the superior temporal gyrus (Orlov et al., 2018). This can result in confusion of inner speech and externally generated stimuli like voices (Orlov et al., 2018). The failure of recognizing inner speech, is one of the most proposed models in AVHs, but is probably not the only cause underlying AVHs (Diederen et al., 2012). Nevertheless, inadequate functional connections between the superior temporal gyrus and inferior frontal gyrus can be an important part of the pathophysiology underlying AVHs.

Even though an inadequate functional connection between the superior temporal gyrus and inferior frontal gyrus is probably part of the generation of AVHs, this connection is not the only important connection between the previously mentioned brain regions. There is also functional connectivity between the superior temporal gyrus and the supramarginal gyrus, which is part of Wernicke's area and is involved in speech processing (Orlov et al., 2018). If this connection within speech and language perception is not working properly, it can be suggested that the latter may result in abnormal speech processing.

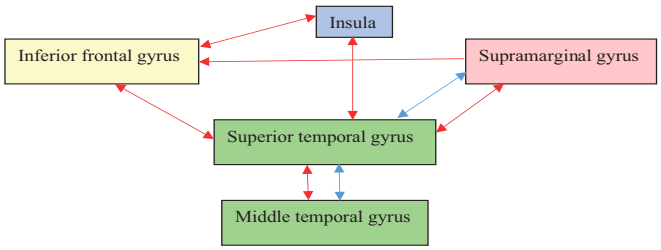

Figure 3. A schematic view of the mentioned connections of the activated brain regions during AVHs. Red arrows indicate functional connections. Blue arrows indicate structural connections. The arrow points in the way the connection goes.

Moreover, a study from Catani and colleagues (2011) already showed a decrease in structural connectivity, i.e. white matter, between the superior temporal gyrus and the supramarginal gyrus in patients with AVHs. This might indicate a reduced functional connectivity between these areas (Catani et al., 2011). Furthermore, Wernicke's area also seems to be functionally connected to the inferior frontal cortex in the left hemisphere (Li et al., 2017). This connection is stronger in schizophrenia patients with AVHs than in schizophrenia patients without AVHs (Li et al., 2017). This could suggest that an abnormal functional connectivity between the language-related brain regions in the frontal and temporal cortices is possibly related to the generation of AVHs.

Furthermore, an increased activation of the middle temporal gyrus is found in 3 out of 7 studies included in this review. This could suggest that the middle temporal gyrus only plays a small role in the pathophysiology underlying AVHs. Even so, Wolf and colleagues (2011) found an increase in the functional connectivity between the middle temporal gyrus and superior temporal gyrus in the left hemisphere in patients with AVHs. An altered connectivity within the temporal cortex may therefore also play a role in the generation of AVHs. Furthermore, some studies reported a correlation between volume reductions in the left superior and middle temporal gyri and the severity of AVHs (Jardri, Pouchet, Pins, & Thomas, 2011; Onitsuka et al., 2004). In addition, another interesting feature of the middle temporal gyrus is that the processing of meaning in text is related to this brain area (Kompus et al., 2011). This could suggest that the content of the AVHs might be influenced by the middle temporal gyrus.

The increased functional connectivity between the insula and the posterior superior temporal gyrus in patients with AVHs, is also of interest in the generation of AVHs (Orlov et al., 2018). This region is considered an extension of Broca's area, and is therefore an interesting area when investigating AVHs. Furthermore, it has been shown that a change in the volume of the insula or an altered resting connectivity is correlated with the severity of AVHs (Alderson-Day et al., 2016; Orlov et al., 2018). The grey matter loss in the insula, or the previously mentioned superior and middle temporal gyrus, could therefore be used as a predictor of the severity of AVHs. Furthermore, Clos et al. (2014) found an increased functional connectivity between the left inferior frontal gyrus and the left insula in patients with AVHs. This increased connectivity suggests an increased communication between language-related brain regions. Apart from the involvement of the insula in auditory processing and its connections to the auditory cortex, it is of special interest because it is thought to be a multimodal convergence zone that integrates sensory information from different brain areas with emotion (Kompus et al., 2011). One study suggests that the generation of AVHs starts in deeper brain structures and that the cortical activity is involved in the perceptual content of the AVHs (Allen et al., 2008).

LIMITATIONS

The results of this systematic review should be carefully interpreted because of some limitations. The pathophysiology of AVHs is still unknown, and one of the explanations for this and for the lack of consistency in this research field, could be the small sample size used in most studies. In fMRI studies, 20 to 25 subjects are needed to acquire a good reproducibility (Diederen et al., 2012; Sommer et al., 2008). To this point, almost none of the studies mentioned in this review, as well as many other fMRI studies in the field of schizophrenia, used this many participants. In addition, even though fMRI has clear advantages over other techniques, such as its non-invasiveness and its great spatial resolution, the use of fMRI also has disadvantages (Glover, 2011). Functional MRI does not demonstrate all types of neuronal activation, it is an indirect measure of brain activity, and has a low temporal resolution (Glover, 2011). Moreover, the scanner noise can interfere with studies involved in resting state brain activity (Glover, 2011). However, some studies resolve this issue by using noise cancelling headphones. Nevertheless, this raises the question if fMRI is the most suitable technique in the research of AVHs. However, there are no other techniques yet (e.g. EEG) that seem to be better suited for the research of AVHs than fMRI.

Another limitation is that most studies in this review used lenient thresholds ($p < 0.05$) for significance of brain activation, which could result in type I errors (i.e. false alarms) and could be an explanation for the inconsistent findings regarding the brain regions found to be active during AVHs. Interestingly, the fMRI studies with a lenient threshold in this review show an activation of the inferior frontal gyrus and the supramarginal gyrus, while the studies with a conservative threshold do not. This could mean that the reported activation of these brain regions might be a type I error.

Lastly, some patients were on medication at the time of the experiments. To explore the influence of this medication

on the brain activation during AVHs, future research should conduct experiments with medication-free patients. Another direction for future research would be to study the brain activity between patients when they are experiencing AVHs versus when they are not, instead of comparing them to other schizophrenia or psychotic patients without AVHs.

CONCLUSION

In conclusion, a dysfunctional activation and connectivity of the frontal and temporal lobes is likely to play a substantial role in the pathophysiological mechanism underlying the generation of AVHs. The functional and structural connectivity between the frontal language production area and the temporal language processing area seem to be altered. However, whether the connectivity in this frontal-temporal network is reduced or increased remains unclear. Besides the frontal and temporal connection, the connection of both areas to the insula seems to play a role in the perceptual content of the AVHs, because the sensory information is integrated with emotion in the insula. The development of new treatment strategies may focus on the areas that seem to be most extensively activated in AVHs, and larger sample sizes are required to produce consistent results. Even though this review may shine some new light on the involved brain regions in the generation of AVHs, future research is needed to unravel the mechanisms underlying AVHs even more before it can benefit the development of new treatment options for patients that suffer from AVHs.

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CONFLICT OF INTEREST STATEMENT

The author declares no competing interests.

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The Neuropeptide Y-ergic system and its implication in post-traumatic stress disorder

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Post-traumatic stress disorder (PTSD) is a specific anxiety disorder caused by a traumatic event. Several molecules play an important role in the pathogenesis of PTSD, e.g., neuropeptide Y (NPY), since it is related to the stress response and described in literature as a resilience molecule. This review elaborates on the way in which the system of NPY contributes to the stress response and the development of PTSD and how exogenous factors (lifestyle and environment) can influence the efficacy of the NPY-ergic system. Finally, it will be discussed that the molecular structure of NPY might change as a result of a traumatic event, potentially contributing to the onset of PTSD. Future research could focus on the contribution of NPY morphology to its anxiolytic effects, which could help to identify ways to recover from stress-related psychiatric diseases.

Keywords: Post-Traumatic Stress Disorder; Neuropeptide Y; NPY-ergic system; Resilience

INTRODUCTION

Human beings have adapted a successful fear response, which helps them react to threatening situations. During prehistoric times, the so-called fight-or-flight response helped us respond to our predators, flee from them, and, thus, survive. Anno 2020, we no longer need to fight-or-flight from predators that want to attack us. However, when a person experiences such stress responses for a prolonged period in absence of life-threatening situations, one might develop an anxiety disorder.

Anxiety disorders are a group of psychological diseases that can be defined by significant feelings of fear and anxiety (American Psychiatric Association, 2013). While different anxiety disorders have their commonalities (such as increased frequency of fear perception), they also differ in terms of the onset of the disease and the particular fearful stimuli. To name a few different anxiety disorders: generalized anxiety disorder (GAD) is a mental illness characterized by non-specific, long-lasting fear and worry for everyday matters (Stein & Sareen, 2015). People with a social anxiety disorder, as defined by the American Psychiatric Association, have extreme and persistent fear of embarrassment and humiliation.

Another well-known anxiety disorder is post-traumatic stress disorder (PTSD). PTSD can be defined as "a function-impairing, trauma-evoked syndrome" (Schmeltzer, Herman, & Sah, 2016). As Schmeltzer and colleagues (2016) also describe: "… experiencing or witnessing an intense traumatic event or events is a prerequisite to the development of PTSD." (p.197) This traumatic event is experienced directly and can, for example, be exposure to (many) deaths, sexual violation or life-threatening injury during, e.g., warfare (Zoladz & Diamond, 2013). An individual diagnosed with PTSD might experience, among others, intrusive memories, negative changes in thinking and mood, and changes in emotional and physical reactions after the traumatic event occurred (Mayo Clinic, 2018). Almost one in four persons develops some form of an anxiety disorder at some point in their lives, and nearly 8% of this group develops PTSD, making it a common mental disease (Martin, 2003).

In PTSD, the traumatic event is exogenous, occurring in the physical environment of the person, and triggers endogenous changes, among others, particularly in brain function. Research has shown that the symptoms of PTSD are mediated by many affected brain regions, which include the amygdala, the hippocampus, the prefrontal cortex (PFC), and the mid-anterior cingulate cortex (ACC) (Bremner, 2006; Vanelzakker & Shin, 2012). These regions either become either hyper- or hypoactive in individuals diagnosed with PTSD. For example, when the amygdala and the ACC are affected after trauma and become stimulated, this translates into an extreme fight-or-flight response in individuals with PTSD, since these behaviors partly depend on amygdala function (Hartley & Phelps, 2009). Everyday situations could then be perceived potentially dangerous. In contrast, the hippocampus, ventromedial PFC, and dorsolateral PFC all become hypoactive (Vanelzakker & Shin, 2012). This results in loss of memory (hippocampal dysfunction), reduced suppression of negative emotion, and lower quality of sleep (both PFC malfunctioning).

Damage to the hippocampus can also cause dysfunction in cortisol signaling (Sherin & Nemeroff, 2011). This stress hormone is, in general, produced via the hypothalamic-pituitary- adrenal (HPA) axis, the body's most important stress response system. Via secretion of corticotropin-releasing hormone (CRH), the hypothalamus ultimately triggers the release of cortisol at the adrenal glands. Cortisol then activates the sympathetic nervous system and results in a negative feedback response to the hypothalamus in order to cease the production of CRH. However, in patients with PTSD, this negative feedback system is impaired, causing a constantly stressed individual (e.g. Bremner, 2006; Vanelzakker & Shin, 2012).

Besides the hormone cortisol, there are other molecules

that play a role in (the development of) PTSD. One interesting example is neuropeptide Y (NPY). NPY consists of 36 amino acids, is a widely distributed peptide throughout the brain and the peripheral nervous system, and exerts many different functions, such as reducing pain perception, stimulating food intake, and coping with stress (Adrian et al., 1983; Allen, 1983). Growing evidence shows that NPY functions as a resilience factor: NPY presence is linked to stress resilience, and, vice versa, absence of NPY resilience is associated with stress vulnerability (Schmeltzer et al., 2016). On a fundamental level, NPY counteracts the activity of pro-stress mediators such as norepinephrine (Baker et al., 1999; Ulrich-Lai & Herman, 2009). The study of Bannon et al. (2000), compared wild type (WT) mice to NPY-knockout (NPY KO) mice and studied the effects on food intake and anxiety. To assess differences in anxiety, the authors investigated locomotor activity in open field chambers and observed less time spent in the center of the open area apparatus of NPY KO mice compared to WT mice. This can be interpreted as an anxiogenic effect, as a result of the NPY knockout (Bannon et al., 2000). In addition, the study of Heilig (2000) showed the effects of NPY overexpression in rats on behavior in the elevated plus maze paradigm. Results showed decreased anxiousness in rats with overexpressed NPY (Heilig, 2000). Studies in combat veterans have shown this effect in humans as well, demonstrating a negative correlation between NPY plasma concentration and occurrence of PTSD (Rasmusson et al., 2000). Therefore, NPY seems to be an essential element of the pathophysiology of PTSD. Thus, the remainder of this proposal will focus on the role of NPY in (the development of) PTSD.

Multiple factors contribute to the efficacy of the NPY-ergic system, including NPY endogenous expression levels, the expression of, and interaction with, its G-protein coupled receptors, NPY mechanism of release, and its downstream effects (Cohen et al., 2012). All of these can have their implications for (the development of) PTSD. In the next section of this review, the NPY-ergic system and its contribution to PTSD will be described in more detail at the molecular level (part 1). Thereafter, the factors (lifestyle and/or environmental factors) or traumatic events that alter the efficacy of the NPY-ergic system, will be discussed (part 2). Lastly, it will be discussed whether the molecular structure of NPY and the NPY-receptors is altered after a traumatic event, ultimately leading to reduced resilience or increased vulnerability to develop PTSD.

PART 1: THE LINK BETWEEN NPY AND PTSD ON A MOLECULAR LEVEL

As mentioned above, the NPY-ergic system is thought to play a critical role in the pathophysiology of PTSD. In this part, the molecular pathways by which NPY exerts its effects in relation to PTSD will be discussed. Unraveling the neurobiological mechanisms underlying stress resilience can help identify ways to recover from stress-related psychiatric diseases such as PTSD, making this an important field of research.

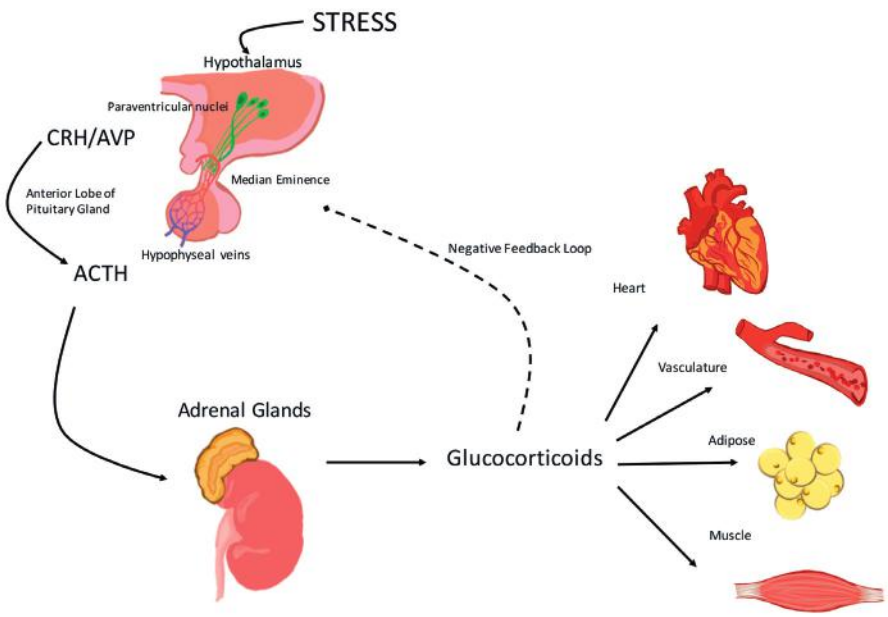

Figure 1. Schematic overview of the working mechanism of the hypothalamic-pituitary-adrenal (HPA) axis. This central stress response system is activated when an external stressor is perceived by an individual. This then activates a cascade of molecules being produced and secreted. Namely, the paraventricular nuclei (PVN) produce corticotropin releasing hormone (CRH), which in turn leads to the production and release of adreno-corticotropin (ACTH). ACTH then activates glucocorticoids production and release by the adrenal glands, which, in turn, leads to a physiological response to stress, such as an increased heart rate. The glucocorticoids also function as a negative feedback by deactivating the HPA axis at the level of the PVN. Figure obtained from Burford et al. (2017).

In the molecular biology of PTSD, the HPA axis plays an important role. As mentioned earlier, the HPA axis is responsible for the production of the stress-hormone cortisol and can therefore be seen as the central coordinator of the neuroendocrine stress response system. On a more detailed level, the HPA axis works as follows (Figure 1) (Burford, Webster, & Cruz-Topete, 2017). Upon stress exposure, the hypothalamic paraventricular nucleus (PVN) is activated and releases corticotropin-releasing hormone into the hypophyseal veins. CRH then stimulates the production and release of adreno-corticotropin (ACTH) from the anterior pituitary gland. ACTH in the circulation, travels to the adrenal glands where glucocorticoids are produced (e.g. cortisol). These molecules cause stress-induced changes in the body, including an increased heart rate and glycogenolysis, which guarantees a sufficient glucose concentration in the blood to "fight-or-flight". These glucocorticoids also have an inhibiting function on their own circuit, i.e. making a negative feedback loop to the PVN neurons, inhibiting the release of CRH.

When an individual experiences a prolonged period of high stress levels or an acute traumatic event, as is the case in people diagnosed with PTSD, this HPA axis is adjusted. Many studies have been executed in order to explore the role of a distorted HPA axis in PTSD. There is some disagreement concerning the actual alterations in the HPA axis in PTSD patients (Dunlop & Wong, 2019). Yet, a conclusion that many studies did draw is a counterintuitive one, namely, the baseline levels of the stress-hormone cortisol in the blood does not differ from individuals with PTSD compared to non-traumatized controls (Dunlop & Wong, 2019). The underlying mechanism for this effect remains elusive.

Furthermore, it has been consistently shown that a reduced negative feedback signal from cortisol causes CRH and ACTH levels to remain high, even after the cessation of stress. These molecules can then still exert stress-promoting actions, independently of the presence of a stressor, which could potentially manifest in PTSD symptoms (Dunlop & Wong, 2019).

Another stress-response pathway is the sympathoadrenal system (SAS) comprising the locus coeruleus, norepinephrine (NE), and the sympathetic nervous system (Vanitallie, 2002). This system reacts to a stressor by increasing the release of NE and NPY from sympathetic nerves, resulting in a stress response. The SAS and the HPA axis are interrelated with each other because NPY has been shown to inhibit CRH release (Sherin & Nemeroff, 2011). Inhibition of CRH causes less production of cortisol and ACTH resulting in reduced arousal. Therefore, NPY has been described as a resilience molecule. Several studies report plasma NPY to be reduced in PTSD patients (Enman, Sabban, Mcgonigle, & Bockstaele, 2015). Furthermore, it has been shown that decreased NPY activity contributes to noradrenergic hyperactivity, and that, administration of NPY led to a recovered negative feedback in the HPA axis (Enman, Sabban, Mcgonigle, & Bockstaele, 2015; Sherin & Nemeroff, 2011). The study of Morgan and colleagues (2002), executed in active duty men, also concluded that presence of NPY relates to greater resilience, as levels of NPY release were negatively correlated with feelings of mental distress after stress exposure, also suggesting NPY's anxiolytic activity (Morgan et al., 2002).

PART 2: NPY SYSTEM, STRESS, AND EXOGENOUS FACTORS ASSOCIATED WITH DEVELOPING PTSD

NPY efficacy is the result of collaboration between several components of the NPY-ergic system. The system's efficacy depends on NPY, peptide YY (PYY), and pancreatic polypeptide (PP) expression levels, as well as the function of G-protein coupled receptors Y1, Y2, Y4, Y5, and Y6, via which the peptides exert their downstream effects (Zhang, Bijker, & Herzog, 2011). PYY is a molecule found in the gut and mostly responsible for the control of body weight and appetite. Similarly, PP is localized in the pancreas where it regulates its secretion activities after meals. Therefore, neither of the latter peptides has a direct link with the development of anxiety related disorders like PTSD. That is why further focus will be on NPY and the NPY-receptors. This second part will investigate whether or not the efficacy of the NPY-ergic system can change over time due to lifestyle aspects or environmental factors.

Transcription of the NPY gene is regulated by a wide variety of second messenger cascades. Previous studies have found that the interaction of the signaling pathways of protein kinase C and adenylate cyclase positively regulate the transcription of neuropeptide Y (Lerchen & Minth-Worby, 1995). In the context of stress, transcription of NPY is induced when a stressor activates this cascade. After transcription, NPY is found to be subjected to posttranslational modification of its mRNA (Lomazzo, König, Abassi, Jelinek, & Lutz, 2017). After NPY synthesis, NPY exerts its effects through the five different NPY receptors. Binding of NPY causes several downstream effects in its molecular pathway (Figure 2) (Sah & Geracioti, 2015). First, binding to the receptor triggers hyperpolarization, especially by inhibition of the calcium channels. Thereafter, adenylate cyclase is inhibited, causing reduction of cyclic adenosine monophosphate (cAMP) leading to transcriptional regulation. Simultaneously, calcium is mobilized through phospholipase C/phosphatidylinositol 3-kinase (PLC/PI3K) activity. This molecular pathway ultimately leads to anxiolytic effects in the organism (Sah & Geracioti, 2015). How exactly this works, remains to be discovered.

The NPY receptors are classified within the family of G-protein coupled receptors. Receptors Y4, Y5, and Y6 are either less involved in the stress response or not particularly functional (Sabban, Alaluf, & Serova, 2016). However, receptor Y1 and Y2 are highly interesting in the context of stress. These two receptors are expressed to a great extent in brain regions implicated in anxiety, such as the amygdala,

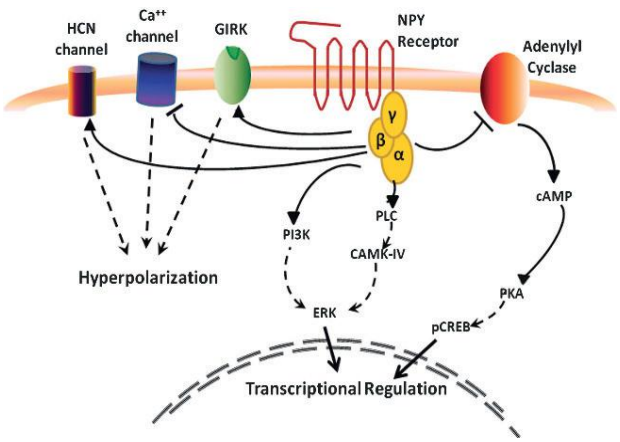

Figure 2. Downstream effects of NPY binding to its G-protein coupled receptors. Abbreviations: hyperpolarization-activated cyclic nucleotide-gated (HCN); G protein-coupled inwardly-rectifying potassium (GIRK); phospholipase C/phosphatidylinositol 3-kinase (PI3K); extracellular signal-regulated kinase (ERK); phospholipase C (PLC); Ca^{2+}/calmodulin-dependent protein kinase IV (CAMK-IV); cyclic AMP (cAMP); protein kinase A (PKA); cAMP response element-binding protein (CREB); phospho-CREB (pCREB). Picture obtained from Sah & Geracioti (2015).

locus coeruleus, hippocampus, and hypothalamus (Sabban et al., 2016). Activation of Y1 and Y2 receptors has different effects: activation of Y1 (specifically in the basolateral nucleus of the amygdala) induces anxiolytic effects, meaning that its activation reduces anxiety and decreases re-experiencing traumatic memories in PTSD patients (Kautz, Charney, & Murrough, 2017). On the other hand, binding of NPY to receptor Y2, localized in the central extended nucleus of the amygdala, results in induced fear and, therefore, has angiogenic downstream effects (Kautz, Charney, & Murrough, 2017). However, it must be considered that the role of the Y2 receptor has been less well described in the literature compared to Y1.

A very extensive literature research performed for the current review gave insights in the expression of NPY and NPY receptors in the context of stress. Lomazzo, et al. (2017) executed an experiment in which rodents were exposed to chronic unpredictable stress (CUS) in order to find the underlying molecular processes by which stress induces long-term effects (Lomazzo et al., 2017). Their hypothesis was that epigenetic changes could possibly alter the expression of NPY and functionality of NPY Y1 receptors, having anxiogenic consequences. For a period of eleven weeks, the mice were exposed to different stress-inducing factors (e.g. isolation, a dirty cage, and food deprivation). The authors showed that CUS resulted in epigenetic changes of NPY expression. They found reduced levels of histone acetylation on H3K9, associated with diminished NPY gene transcription. Lomazzo and colleagues also confirmed this association in their study. They found reduced NPY expression and lowered downstream signaling of NPY type 1 receptor. Therefore, these epigenetic changes may be involved in the dysregulation shown at NPY expression level and of Y1 receptor signaling, causing emotional imbalance induced by CUS in mice.

The study of Cohen et al. (2015) investigated the effects of an acute trauma on the behavioral responses in rats, modeling the onset of PTSD. Both NPY and Y1 receptor expression were assessed as well as the rats' behavior in an elevated plus-maze, seven days after stress exposure. The authors reported increased anxious behavior and stress-induced reduction in expression of both NPY and Y1 receptors (Cohen et al., 2015).

Based on the previously mentioned studies, the opposite seems plausible, i.e., that higher levels of NPY and NPY Y1 receptors could lead to decreased anxiety. This hypothesis was confirmed by Serova et al. (2013) who examined the effects of intranasal NPY administration in a rat model of PTSD. Their results demonstrated that rats pretreated with intranasal NPY, showed less anxious behavior as measured by the elevated plus maze paradigm compared to controls (Serova et al., 2013). In addition, the authors reported reduced immobility in the forced swim test in rats treated with intranasal NPY versus the control group, indicating reduced behavioral despair. These findings support the resilience-inducing effects of the NPY-ergic system in response to traumatic stress. Therefore, changes in NPY signaling may contribute to at least some of the fundamental causes of (long-term) PTSD symptoms.

When examining possible influences on the functioning of the NPY-ergic system, a question that arises is whether lifestyle and other environmental factors have (epigenetic) influences on the NPY-ergic system. In terms of lifestyle, the use of nicotine on NPY levels has been examined. A relative old study of Frankish et al. (1995) studied whether administration of 12 mg nicotine/kg/day (for a total of 12

days) affected NPY levels in rats. Interestingly, the study found that cigarette smoke inhibited NPY synthesis in the hypothalamus (Frankish et al., 1995). However, the authors investigated the effects of nicotine in regard to NPY-mediated food uptake regulation, and, therefore, no strong conclusion about its effects in relation to stress coping behaviors can be drawn. Still, this finding remains compelling and it might be interesting for further research to investigate the relationship between smokers and non-smokers on (the development of) PTSD.

In addition, it has been consistently shown that physical exercise directly decreases stress and anxiety in both rodents and humans, and that it provides relief from PTSD symptoms (Hoffman, Ostfeld, Kaplan, Zohar, & Cohen, 2015; Rosenbaum et al., 2015). It remains under investigation whether these effects were due to alterations at the level of NPY. In support of this notion, one study described the effects of exercise on NPY plasma levels in humans (Rämson, Jürimäe, Jürimäe, & Mäestu, 2012). This study showed that high-volume low intensity resistance training combined with endurance training in a four-week program increased NPY concentration. The authors concluded that greater NPY levels ensures faster training recovery potential of the rowers (i.e. energy levels returned to baseline). It would be an interesting follow-up study to examine whether regular physical activity induces anxiolytic effects via the manifestation of increased NPY levels (in patients with PTSD). In addition, the study of Melas and colleagues (2013) showed increased H3 acetylation in the NPY gene in a genetic rat model of depression after five weeks of wheel running experiments, suggesting that exercise has a stress-coping effect in an animal model (Melas et al., 2013).

Lastly, the study of Varman and Rajan (2015) studied wild-type mice housed in enriched conditions (to mimic safe environments and potentiation of social interactions, learning, and motor stimulation) and compared them with wild-type mice housed under standard conditions. Both groups got exposed to their natural predator (field rat Rattus rattus). The investigators observed that mice staying in enriched conditions displayed less-anxiety-like behavior when assessed in an elevated plus maze. On a more fundamental level, their results displayed that mice exposed to enriched conditions had increased histone acetylation of H3 and H4, as well as upregulated transcription of neuropeptide Y and its Y1 receptor. Furthermore, Y2 receptor expression was down-regulated compared to the mice housed under standard conditions. Thus, these results show that living in a safe, enriched environment facilitates a stress- coping response in an animal model for PTSD. Future research should further investigate whether increased resilience can also be observed in humans with PTSD, when staying in safe environments. This could then be promising for behavioral therapies in humans.

In summary, extensive evidence shows that stress has a direct effect on the expression levels of NPY and its receptors. Furthermore, it can be assumed that there is at least some evidence that external factors, such as variances in lifestyle (nicotine use and exercise) and enriched environments, effectively alter the function of the NPY-ergic system and its stress-relieving properties. Still, further research is needed in order to provide concise conclusions about the interaction between external factors and the NPY-ergic system on resilience in patients with PTSD.

CONCLUSION AND DISCUSSION

This review aimed to provide an overview of what is currently known about the NPY-ergic system and its contribution to PTSD. This included the contributions of the NPY-ergic system to PTSD on a molecular level, and the influence of stress and external factors on the efficacy of this system.

It can be assumed from the research cited that NPY is a central player in the game of stress regulation via the HPA-axis (see e.g. Enman et al., 2015). Moreover, NPY can be seen as the key to stress resilience, since multiple studies showed that a higher expression of NPY was associated with lower anxious feelings or behaviors (and vice versa) in both rodents and humans (Schmeltzer et al., 2016). Furthermore, there are indications that the NPY-ergic system can be influenced by lifestyle aspects and environmental factors. Multiple studies, in both rodent and human studies, point to a stress-coping response after physical activity due to epigenetic changes in NPY expression (Melas et al., 2013; Rämson et al., 2012). This might have implications for future behavioral therapies in PTSD patients in terms of adjustments in lifestyle for example. However, it must be considered that these studies do not describe a direct causality between exercise and the manifestation of epigenetic NPY changes in decreased arousal, stress, anxiety, and other symptoms of PTSD, which opens perspectives for future research.

In addition, nicotine administration in rodents has been shown to reduce NPY synthesis (Frankish et al., 1995). However, this study investigated the effects on nicotine in regard to NPY-mediated food uptake regulation. Therefore, no concise conclusion about its effects in relation to stress-coping behaviors can be drawn. Future research could potentially focus on the effect of nicotine on the resiliency potential of the NPY-ergic system in the context of animal models for PTSD (and later PTSD patients), and whether a causal link between PTSD symptoms and nicotine administration (via, e.g., smoking) could be drawn.

Other results have shown that rich environments, considered to be safe and mentally enriching for learning, social interaction, and motor activity, facilitate the development of a stress-coping response in animal models for PTSD (Varman & Rajan, 2015). A suggestion for further research is to investigate whether finding oneself in safe environments also induces anxiolytic effects in humans with PTSD. This could be promising for behavioral therapies in humans.

In summary, research has shown the potential role of the

NPY-ergic system in stress resilience, as well as evidence that this system is dysregulated in PTSD. It has been extensively shown that traumatic events can result in downregulation of NPY expression and altered function of NPY and its receptors via epigenetic changes. Also, there are indications that structural changes in NPY lead to alterations in downstream effects leading to reduced efficacy of the system, and by extension, reduced anxiolysis. A recent review of Sheerin and colleagues (2017) pointed towards the importance of investigating "ways environmental experiences (i.e. trauma exposure) impact outcomes of DNA through changes to the epigenome, and in turn confer risk for PTSD." (Sheerin, Lind, Bountress, Nugent, & Amstadter, 2017). This point is exacerbated by the fact that epigenetic changes could potentially lead to changes in mental health and could contribute to the development of psychiatric diseases. One example of an epigenetic change is DNA methylation that, besides the aforementioned acetylation, could also induce functional changes in DNA products associated with psychiatric dysfunctions in depression and suicide (Lockwood, Su, & Youssef, 2015). Trauma-related changes to the molecular structure of Y1 receptor could not be found.

It has of yet not been investigated whether trauma-induced structural changes occur in NPY and whether this structural change has, besides the downregulation of NPY expression, a direct link with increased vulnerability to develop PTSD. Existing literature show some signs in favor of this alluring hypothesis to be plausible.

Murgatroyd and colleagues (2009) modeled early life stress by separating mice from their caregiving mother and assessing stress-induced epigenetic changes in their DNA. The authors demonstrated that this traumatic event caused DNA methylation within the promoter region of the arginine vasopressin (AVP) gene (Murgatroyd et al., 2009). AVP is a neuropeptide synthesized in the hypothalamus and the limbic system and acts as a central regulator of the stress response within the HPA-axis molecular pathway as well as inducing anxiety-related behaviors as a neurotransmitter (Klengel, Pape, Binder, & Mehta, 2014). AVP acts synergistically with CRH on the release of ACTH (as discussed in part 1 of this report).

Since studies have shown that (1) the onset of a traumatic event can result in epigenetic changes and (2) certain epigenetic changes have been reported to affect anxiety-related behaviors, the assumption that expression and function of NPY can also be affected by a trauma arose. If this is true, it could be of high clinical relevance with regards to PTSD. The study of Murgatroyd and colleagues (2009) already showed this clinical relevance, since the induced phenotype after early life stress could partially be reversed by administering an AVP receptor antagonist. This highlights the potential options for treatment of anxiety disorders with NPY.

Further literature search provided some interesting insights into this hypothesis. First, research has shown that neuropeptide Y can have post-transcriptional changes by a specific protease (dipeptidyl peptidase-IV (DPPIV)) (Zhang et al., 2011). This process removes the first two amino acids from the N-terminus of NPY, thereby constructing a shorter form of the neuropeptide: NPY-3-36. "This post-translational modification leads to an altered pharmacological profile, with the affinity of the full-length form to the Y1 receptor being lost in the truncated form. It is important to note, however, that the affinity to Y2 receptors is unaltered by this modification." (Zhang et al., 2011, p. 92). The authors described that the truncated form of NPY resulted in reduced activation of Y1 receptors and no enhanced activation of Y2 receptors. These results suggest that alteration of the NPY's molecular structure can have anxiogenic effects. In addition, the study of Melas and colleagues (2013) observed that a form of NPY promoter polymorphisms in an animal model of depression, caused declined transcriptional activity of NPY (Melas et al., 2013).

It would be compelling to test whether the onset of a trauma could either (1) induce changes to the molecular structure of NPY, (2) stimulate the post- transcriptional modification to NPY by DPPIV or (3) induce an NPY promoter polymorphism, that could potentially all result in declined anxiolytic activity of the NPY-ergic system and perhaps the development of PTSD. This would add evidence to show how a traumatic event can manifest in anxious behaviors and opens new perspectives on novel treatment possibilities for PTSD patients.

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CONFLICT OF INTEREST STATEMENT

The author whose name is listed above certifies that she has NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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Sponsor of the Journal of Neuroscience & Cognition:

The roles of social play and cognitive empathy in autism spectrum disorder: A neurobiological perspective

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Autism spectrum disorder (ASD) is a broad neurodevelopmental disorder characterized by behavioural and social deficits. The characteristic social deficits in ASD remain hard to treat effectively. Social play is involved in cognitive, social and emotional development, and is known to be altered in ASD. Cognitive empathy – the ability to understand another's internal mental state - is abnormal in ASD and is thought to be involved in the social deficits in ASD patients. Therefore, altered social play and abnormal cognitive empathy may explain the core social impairments in ASD. This paper provides an overview of (1) the underlying neurobiological mechanisms of social play and cognitive empathy, (2) the interactions between social play and cognitive empathy, and the role of the mirror neuron system in this interaction, and (3) research on interventions that integrate elements of social play, cognitive empathy and the mirror neuron system. The implications of the current findings are discussed, and suggestions for future research and treatment are provided. A new treatment in which oxytocin administration is combined with interventions that aim to improve elements of social play, cognitive empathy and the mirror neuron system seems promising to improve the social skills of ASD patients in the future.

Keywords: Autism Spectrum Disorder (ASD); Social Play; Cognitive Empathy; Mirror Neuron System; Oxytocin

INTRODUCTION

Autism spectrum disorder (ASD) is a broad and heterogeneous neurodevelopmental disorder characterized by behavioural and social deficits (American Psychiatric Association, 2013). ASD patients often show repetitive and restrictive behaviours, interests or activities. Additionally, social interactions and communication are heavily impaired in ASD. For instance, patients with ASD are thought to lack social motivation (Chevallier, Kohls, Troiani, Brodkin, & Schultz, 2012) and generally seem to lack reciprocal social skills (Howlin, 1986). The social deficits in ASD are currently hard to treat (Coury, 2010). Therefore, it is valuable to understand the underlying neural mechanisms of these social deficits so that current treatments can be improved, and new treatments can be developed.

Play behaviour can be observed in a multitude of species. Graham and Burghardt (2010) report five criteria that behaviour must meet before it can be defined as play behaviour: (1) play is not functional in the context in which it appears, (2) play occurs spontaneously and is rewarding, (3) play differs from more serious behaviours, (4) play is repeatedly expressed, and (5) play only occurs when the animal is not stressed. Social play, in contrast to other forms of play, is a highly energetic behaviour between two or more conspecifics (Vanderschuren, Niesink, & van Ree, 1997; Vanderschuren & Trezza, 2014). Due to the highly energetic nature of the behaviour, it is sometimes referred to as "rough-and-tumble" play. Social play is an early form of social interaction in mammals as it peaks between weaning and puberty (Vanderschuren, Achterberg, & Trezza, 2016). Although social play has been described and investigated in humans (e.g. Fry, 2005; Logue & Harvey, 2009), neurobiological research on social play has generally investigated the behaviour in rats. This is due to the fact that neural measures in humans (e.g. EEG or fMRI) provide too much noise or are not suitable to use during such a highly energetic behaviour. Social play contains elements of social, aggressive and sexual behaviours. These behavioural elements may be exaggerated and/or may seem out of context during play-sessions (Vanderschuren et al., 2016; Vanderschuren et al., 1997). This observation spawned the idea that social play prepares one for future situations, but also to respond properly to new environments (Pellis, Pellis, & Bell, 2010; Špinka, Newberry, & Bekoff, 2001; Vanderschuren et al., 1997). As social play prepares one for the future, the development of this behaviour is important for acquiring normal cognitive, emotional and social skills. This is illustrated by the fact that rats suffer cognitive, emotional and social deficits when they are withheld from social play (Pellis, Pellis, & Bell, 2010; Vanderschuren et al., 2016). For example, play deprived rats show impaired performance on the 5-choice serial reaction time task (Baarendse, Counotte, O'Donnell, & Vanderschuren, 2013), show increased anxiety compared to controls (Lukkes, Mokin, Scholl, & Forster, 2009), and show abnormal behaviour when faced with an aggressive conspecific (Von Frijtag, Schot, van den Bos, & Spruijt, 2002). In ASD patients, social play behaviour is shown to be abnormal. Children with ASD often play alone and are less able to properly participate in more complex cognitive interactions with others (Jordan, 2003). The abnormal social play in ASD patients may occur due to their social deficits. Nevertheless, the reverse could also be true: abnormal social play may underlie the social deficits in ASD. Thus, there seems to be an association between ASD and social play, but the exact nature of this association remains unknown.

Cognitive empathy (CE) - also referred to in the literature as theory of mind, mind-reading or mentalizing - is the ability to understand the internal mental state of another individual, and to predict behaviours from this internal mental state (Blair et al., 2005). An internal mental state can contain thoughts, emotions, beliefs and feelings. Within the psychological literature, it is widely confirmed that ASD patients suffer a deficit in CE. For example, Mazza et al. (2014) used various instruments to assess the level of CE in an adolescent ASD group and a control group. The following instruments were utilized: first-order false belief tasks, advanced CE tasks (e.g. "Why did X say that?"), the reading the eyes task, the emotion attribution task and multiple questionnaires. Perhaps the most known of these instruments is the reading the eyes task (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001). During this task, participants are asked to determine the emotion of an individual based on their facial expression. However, only the eyes and closely surrounded parts of the face are presented. In this way, the ability to infer an emotional state from the eyes of another individual is assessed (Baron-Cohen et al., 2001). All instruments used in Mazza et al. (2014) showed a significant deficit of CE in ASD when compared to healthy controls. As CE is vital during social communication and social interactions, it has been theorized that a deficit in CE plays a role in the core social impairments in ASD (Tager-Flusberg, 2007).

In this paper, the roles of social play and CE in ASD will be examined. Firstly, the neurobiological mechanisms underlying social play and CE will be considered in the regular rat and human brain. Then these neurobiological mechanisms will be closely examined in ASD. Afterwards it will be discussed how social play and CE influence one another. Next, interventions intended for normalizing social play and CE in ASD will be shown. Lastly, concluding remarks, implications of the findings and recommendations for future research will be provided.

THE NEUROBIOLOGY OF SOCIAL PLAY

Since social play is a broad and complex behaviour, it is likely that multiple brain areas and neural systems underlie this type of behaviour. Although the neurobiology of social play is still not fully understood, neuropharmacological, functional and neonatal ablation studies in rats have provided insight as to how the brain realizes social play (Vanderschuren et al., 2016; Vanderschuren & Trezza, 2014). Social play behaviour has been shown to recruit a limbic corticostriatal network (Vanderschuren & Trezza, 2014). The limbic corticostriatal network can be divided into four brain regions: the frontal cortex, the striatal areas and the amygdala and habenula. The frontal cortex is involved in higher cognitive processes such as attention, working memory, planning and executive functions (Elliott, 2003). During social play these higher cognitive processes are important so that one can flexibly adapt to changing environments and properly interact with them (Vanderschuren & Trezza, 2014). Research suggests that the frontal cortex is not essential for the development of social play, but rather necessary for the proper calibration of the social repertoire of the animal (Vanderschuren & Trezza, 2014). Striatal areas (e.g. the nucleus accumbens) are known to be involved in reward processing (Arias-Carrión, Stamelou, Murillo-Rodriguez, Menéndez-González, & Pöppel, 2010). Since social play is a highly rewarding activity (Vanderschuren et al., 2016), it is not surprising that striatal areas are also heavily involved in social play. The amygdala and the habenula have different functions. Inhibition of amygdala activity has been shown to decrease social play, but only in males (Kurian, Bychowski, Forbes-Lorman, Auger, & Auger, 2008). Furthermore, the basolateral amygdala has been shown to modulate social play behaviour mainly via the endocannabinoid and noradrenaline systems (reviewed in Vanderschuren et al., 2016). The habenula reportedly attributes both positive and negative emotions to social phenomena (van Kerkhof, Damsteegt, Trezza, Voorn, & Vanderschuren, 2013). Together, these brain areas form the limbic corticostriatal network that is heavily involved in the processing of social play. Note that this is a general and relatively limited description of the neurobiology of social play. For more elaborate reviews, see Pellissier, Gandía, Laboute, Becker, & Le Merrer (2018), Vanderschuren et al. (2016), Vanderschuren, Niesink, & van Ree (1997) and Vanderschuren & Trezza (2014).

THE NEUROBIOLOGY OF CE

Since social play is abundant and easily observable in rats, more invasive methods can be used in these animals to determine the neurobiology of this behaviour. However, CE is only present in humans - and possibly other primates (Heyes, 1998) - but not in rats (Rushworth, Mars, & Sallet, 2013). Consequently, more non-invasive techniques for assessing the neurobiology of CE have been used in humans.

There is a growing consensus on which brain areas are involved in CE. CE recruits the medial prefrontal cortex (mPFC), the (right) temporoparietal junction (TPJ), the temporal poles and the superior temporal sulcus (STS; Amodio & Frith, 2006; Saxe & Baron-Cohen, 2006). CE is a complex higher order cognitive function, so it remains hard to disentangle the separate functions within the CE-network. However, social neuroscience has been able to identify distinct functions of these brain areas. First, whereas the TPJ is responsible for inference-making of other people based on transient states in a specific context, the mPFC is involved in inference-making based on more enduring and stable traits of the self and others (van Overwalle & Baetens, 2009). For instance, understanding that an individual is generally a joyful person would recruit the mPFC, while

understanding that the same individual becomes angry when losing his/her wallet would recruit the TPJ. Second, a connection between the medial temporal poles and the mPFC is thought to be involved in autobiographical memory. This connection is also involved in CE, which sparked the contemporary idea that this connection integrates autobiographical memory in the CE process (Shamay-Tsoory, 2011). Thus, this connection enables one to use autobiographical memories to better understand the internal state of another. Third, as previously mentioned, internal states may contain emotions, feelings, thoughts and beliefs. The ventromedial prefrontal cortex is recruited when more affective internal states are understood, and used to make inferences from – also known as affective CE. When cognitive internal states are understood and used to make inferences from (also referred to as non-affective CE), the dorsolateral prefrontal cortex is involved (Shamay-Tsoory, 2011). Thus, when another's emotions are understood the ventromedial prefrontal cortex is recruited, when another's thoughts are understood the dorsolateral prefrontal cortex is involved (Shamay-Tsoory, 2011). Lastly, the STS is known to respond to biological motion of the body, but also more specifically to the eye gaze of others (Hooker et al., 2003). Joint attention – sometimes referred to as shared attention - is when two individuals attend to the same stimulus. The direction of eye gaze is an important facilitator of joint attention. Joint attention can, in turn, provide important cues for predicting another's internal state (Charman et al., 2000).

THE NEUROBIOLOGY OF SOCIAL PLAY AND CE IN ASD

The neurobiology of social play and CE have now been discussed in rats and healthy humans. However, multiple neurobiological processes are altered in ASD. Since social play and CE are abnormal in ASD, the underlying neurobiological processes may also be altered. Here, the neurobiology of social play and CE in ASD are considered.

Social play has distinct neurotransmitter systems that modulate social motivation and the expression of social play (Achterberg et al., 2016). Dopaminergic and noradrenergic neurotransmission modulate social motivation and the expression of social play, respectively (Achterberg et al., 2016). Contemporary research suggests that a lack of social motivation may underlie the core symptoms of ASD (Chevallier et al., 2012). Furthermore, dopaminergic neurotransmission is thought to be abnormal in ASD (Paval, 2017) and is vital in the reward system. As mentioned previously, the reward system is recruited during social play (Vanderschuren et al., 2016). Evidence suggests that the social reward system is altered in ASD: patients with ASD experience less reward when viewing social stimuli than healthy controls (Pellissier et al., 2018). The social reward system recruits the frontal cortex and the striatal areas involved in social play. This network, including the nucleus accumbens, shows hypoactivation in ASD when receiving social rewards (Pellissier et al., 2018). Others suggest that dysfunction of the noradrenaline system underlies many symptoms of ASD (London, 2018). Since noradrenaline is important in the expression of social play, an altered noradrenaline system may contribute to altered social play in ASD. Furthermore, the mu-opioid receptor reportedly plays an important role in social reward (Pellissier et al., 2018; Vanderschuren et al., 2016). For example, *oprm1* - a gene important in the expression of mu-opioid receptors - knockout mice show seriously altered forms of social behaviour, which closely resembles that of ASD. Moreover, this gene has also been implicated in some cases of ASD in humans (Pellissier et al., 2018). Furthermore, the mu-opioid system is important in the modulation of social play behaviour (Trezza, Damsteegt, Achterberg, & Vanderschuren, 2011). This illustrates that altered mu-opioid signalling may contribute to both altered social play and the social deficits in ASD. Lastly, oxytocin is involved in social reward processing and the expression of social play. Also, plasma oxytocin levels are decreased in ASD (Modahl et al., 1998). Dölen, Darvishzadeh, Huang, and Malenka (2013) showed that oxytocin is necessary to earn social rewards gained from social interactions in rodents. Additionally, intra-septum oxytocin injections in rats reportedly reduce social play in novel contexts, but not in familiar contexts (Bredewold, Smith, Dumais, & Veenema, 2014). Only females show this effect, which may be important to consider when developing new treatments.

Although an altered social reward system activation in ASD has been reported often, one oversight is made by many authors. Patients with ASD view social stimuli differently as they focus on other aspects of the stimulus compared to healthy controls. Namely, patients with ASD tend to avoid eye contact (Madipakkam, Rothkirch, Dziobek, & Sterzer, 2017). This is thought to decrease the reward value of social stimuli in ASD patients (Senju & Johnson, 2009), which can lead to decreased social motivation in ASD. Nevertheless, whatever the exact mechanism underlying the altered social reward system activation may be, the fact remains that social stimuli are processed differently in ASD.

Furthermore, the neurobiology of CE seems to function abnormally in ASD. A fMRI study reported that the CE-network shows aberrant activation in ASD while performing the Frith-Happé animations CE-task (Kana et al., 2015). During this task, participants have to interpret the behaviours of two interacting animated shapes (a small blue square and a large red triangle). The specific brain regions that show hypoactivation in ASD when performing this task are the mPFC, angular gyrus, TPJ, STS and posterior cingulate cortex. Thus, functionally the CE-network is altered in ASD, but other evidence also suggests that the CE-network may be abnormal chemically. Oxytocin is known to be important in the CE-network (Shamay-Tsoory, 2011). As previously stated, plasma oxytocin levels are decreased in ASD (Modahl et al., 1998). Furthermore, studies have shown that intranasal oxytocin administration

can improve CE performance in ASD (Guastella et al., 2010). Potential oxytocin treatments in ASD are discussed in detail below.

In sum, the processing of social stimuli is abnormal in ASD, possibly due to an altered social reward system, which contributes to a lack of social motivation. This, in turn, can explain the impaired social play in ASD. Furthermore, the CE-network shows hypoactivation in ASD and an altered oxytocin system is likely involved.

HOW SOCIAL PLAY AND CE INTERACT

Up until this point, social play and CE have been discussed as separate and more or less independent entities. However, it would seem that social play and CE can be linked to one another. Here, the mechanisms that link social play and CE will be discussed.

The mirror neuron system (MNS) is involved in both social play and CE. The MNS is responsible for mimicry and evidence suggests that the system is abnormal in ASD (Oberman et al., 2005). However, ASD patients seem to solely show a delay in spontaneous mimicry; when patients were explicitly instructed to focus on the eyes of a social stimulus, no abnormalities were found (Oberman, Winkielman, & Ramachandran, 2009). The MNS recruits the inferior frontal gyrus (IFG) and the inferior parietal lobule (IPL; Shamay-Tsoory, 2011). However, the insula and amygdala are thought to be responsible for emotion understanding from facial expressions (Dapretto et al., 2006). The IFG, IPL, insula and amygdala all show abnormal activation in ASD when viewing social stimuli (Dapretto et al., 2006). However, Dapretto et al. (2006) only provided indirect evidence that the abnormal activation was not due to different amounts of eye contact. The study did not directly measure where the patient was fixating during the time of the experiment. This could imply that the MNS only showed abnormal activity due to altered eye fixations of ASD patients.

Simulation theory states that one mimics the bodily expressions of another individual to understand them better. This links the MNS to CE: mimicking another individual would facilitate the understanding of the other's internal state (Gallese & Goldman, 1998). However, Thioux, Gazzola, and Keysers (2008) suggest the MNS and CE-network to only activate concurrently when participants choose to deliberately reflect on the goals and intentions of viewed actions. Others suggest that the activation of the MNS is a prerequisite for the activation of the CE-network. Although the systems rarely simultaneously activate, it is possible that the MNS provides rapid input to activate the CE-network (van Overwalle & Baetens, 2009).

In social play the MNS is vital in prolonging play sessions via rapid facial mimicry (Palagi, 2018). Facial mimicry is one way to signal the social context. For instance, facial mimicry can signal the non-seriousness of play-fighting. In this way, the MNS can help signal the social context during social play, which can be used to set boundaries during a play-session (e.g. a sad face may signal when one is in pain instead of enjoying the play-session; Fry, 2005). However, in ASD, spontaneous facial mimicry is delayed (Oberman et al., 2009), which may disrupt social signalling during social play. Other studies suggest that facial mimicry enables primates to form emotional bonds (Palagi, 2018). Emotional bonds could increase social rewards during social play, and this can in turn increase social motivation for future play sessions.

Interestingly, social play also may influence the MNS in two ways. First, while mimicry is automatic, it only occurs when two conspecifics share similar goals. Whether the goals of two conspecifics are similar, can often depend on the emotional context of a situation. This has led to the belief that the goal-interpretation of facial expressions depends on past social interactions (Fischer & Hess, 2017). For example, if one suffers impairments in social play during development, they may develop an abnormal goal-interpretation of facial expressions. This, in turn, can lead to future abnormal MNS functioning. Second, social play is important in the development of neural structures. More specifically, social play has been found to promote the healthy development of the frontal cortex and the amygdala (Vanderschuren & Trezza, 2014). As previously stated, the MNS network consists of the IFG and IPL and receives input from the insula and amygdala (Dapretto et al., 2006; Shamay-Tsoory, 2011). The brain areas involved in the MNS and the brain areas that benefit from social play show great overlap. Although it may be tempting to conclude that social play seems to promote the healthy development of the MNS, caution should be taken. One should note that Dapretto et al. (2006) and Shamay-Tsoory (2011) performed their studies in different species when compared to Vanderschuren and Trezza (2014). It is important to consider whether these results can be accurately compared to one another in this manner. Furthermore, the MNS can be activated more or less right after birth, before social play is possible (Palagi, 2018). Taken together, social play is not necessary for the development of a functioning MNS but it may potentially aid the finetuning of the MNS. However, note that this is speculative and that more research is needed to identify in what way and to what extent social play can influence the MNS.

Only weak evidence suggests that social play may directly influence CE. However, there are indications for such an effect. As previously stated, social play is involved in the healthy development of the frontal cortex and the amygdala (Vanderschuren & Trezza, 2014). CE recruits parts of the frontal cortex: mainly the mPFC (Shamay-Tsoory, 2011). Abnormal social play may therefore lead to abnormal development of the CE-network. This has, however, not been directly researched and remains speculation. The mPFC is part of the social play neural circuit and of the CE-network. Yet, this does not necessarily mean that the two circuits are closely linked. mPFC activation may merely reflect the processing of the self (Amodio & Frith, 2006),

which is important in both social play and CE. This may explain the activation of this area in both networks without necessarily linking the two. All in all, the evidence for an influence of social play on CE is very limited.

As previously stated, CE is thought to be paramount in social interactions (Tager-Flusberg, 2007). This led researchers to believe that a deficit in CE may lead to the characteristic social deficits in ASD. Some studies have reported an increase of social skills when CE is trained in ASD patients – these results are however not unequivocal (intervention studies are further detailed later on pages; Bishop-Fitzpatrick, Minshew, & Eack, 2013). Naturally, social skills are core in social play. A deficiency of social signalling during social play could be due to a deficit in CE. This link has, as of yet, not been directly researched.

In this paragraph the associations between social play and CE were discussed. The MNS seems to play a vital role in coupling the two domains. This is illustrated by the fact that the MNS may influence both social play and CE (Fry, 2005; Palagi, 2018; van Overwalle & Baetens, 2009). Also, social play may facilitate the development of the MNS (Palagi, 2018). Weaker evidence suggests that social play can influence CE (Amodio & Frith, 2006; Shamay-Tsoory, 2011; Vanderschuren & Trezza, 2014), but this needs to be investigated further. Lastly, CE likely has an influence on social play as interventions targeting CE can improve the social skills of ASD patients (Bishop-Firzpatrick et al., 2013).

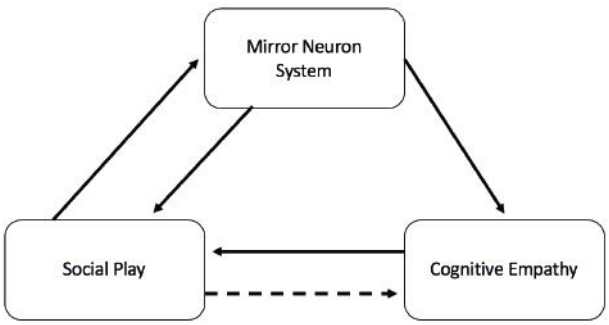

Figure 1. A schematic overview of the associations between social play, the mirror neuron system and cognitive empathy. A solid arrow implies a connection rooted in strong evidence. A dashed arrow implies a connection based on limited evidence. Five associations have been made. A) There is some encouraging evidence that suggests that social play may help to properly finetune the MNS. B) The MNS regulates social play by setting boundaries during play-sessions and forming emotional bonds in primates. In ASD, the MNS functions abnormally, which likely impairs social play. C) The MNS seems to be an important facilitator of CE as facial expressions can be important cues for the content of another's internal state. D) A deficit in CE may lead to abnormal social play but this link needs direct researching. E) There is weak evidence that social play may potentially influence CE. However, this is far from conclusive and needs to be investigated further.

HOW SOCIAL SKILLS CAN BE NORMALIZED IN ASD

The normalization of the MNS, social play and CE in ASD can potentially help alleviate the social deficits in ASD. Multiple studies have attempted to provide interventions for ASD patients with the aim of normalizing social interactions through the MNS, social play or CE. Although this is still an emerging field, a few researchers have successfully shown improvements in social skills in ASD after MNS, social play or CE interventions.

As briefly mentioned in the previous paragraph, studies have successfully improved social skills in ASD patients when training CE. Typical CE training consists of 16 weekly sessions lasting approximately 90 minutes each. During these sessions, five to six children are initially trained in precursors to CE (e.g. imitation and properly listening to another) and the training gradually progresses to complex and difficult CE training (e.g. "Where does Mary think that John thinks he will find his toy?"; Begeer et al., 2011; Steerneman, Jackson, Pelzer, & Muris, 1996). Bishop-Fitzpatrick et al. (2013) reported that most, but not all, CE interventions show improvements in social skills in ASD. However, others report that these improvements are not generalizable to the 'real world', as the patients likely improve on the specific CE-task but not CE in general (Begeer et al., 2011). Moreover, ASD patients generally tend to view the world very systematically, which limits these patients to make uncertain assumptions needed to generalize skills over to novel situations (Golan & Baron-Cohen, 2006). Others suggest that CE interventions in ASD may not improve social skills independently, but may prove fruitful when part of a broader socio-cognitive intervention (Kimhi, 2014). This seems plausible, as the construct of CE is complex and consists of multiple social and cognitive components that work together. Thus, CE interventions may be an important part of future interventions, but the focus should be on developing broader socio-cognitive interventions instead of solely focusing on CE.

Social play interventions in ASD have reported encouraging results. Sherratt (2002) underlines the importance of social aspects of play in an everyday environment where real play is experienced. This is in sharp contrast with highly digitalized forms of social play interventions (Sherratt, 2002). Some studies have reported no effects of social play interventions, but that could be caused by more artificial environments and forms of play (Jordan, 2003). Integrated peer group interventions aim to improve social skills through promoting play in small groups of children. These groups consist of up to five children of which one or two are ASD patients and the rest are normal/typically developing. Integrated peer group interventions generally consist of 16 weekly 30-minute sessions (Zercher, Hunt, Schuler, & Webster, 2001). One integrated peer group intervention study encouraged ASD patients to establish joint attention with other members from the group, which improved social skills (Zercher et al., 2001). Moreover,

parents of the children with ASD reported that the improved social skills were generalizable to 'real world' situations (Zercher et al., 2001). Kok, Kong and Bernard-Opitz (2002) report that interventions using social play in ASD should be more structured when social play is relatively heavily impaired, and that interventions should be more facilitatory when social play is relatively spared. This illustrates the heterogeneity of ASD. It naturally follows that different forms of ASD may benefit most from different interventions. It is, however, important to note that most studies using social play interventions have a low number of participants. This may limit the generalizability of the results presented above.

The MNS influences both social play and CE. It is also an essential system in achieving successful social communication. As previously mentioned, the MNS is not impaired in ASD per se, but the spontaneous activation of the system does seem abnormal (Oberman et al., 2005, 2009). This is likely due to the characteristic lack of eye contact made by ASD patients. Interventions that can increase the amount of eye contact made in ASD patients could potentially promote the healthy development of social skills. Oxytocin is known to be involved in emotion and face processing (Guastella et al., 2010). Moreover, oxytocin increases the amount of eye contact made by ASD patients in a naturalistic environment (Auyeung et al., 2015). Also, oxytocin seems to have limited short-term side effects, but little is known about potential long-term side effects (MacDonald et al., 2011). Oxytocin treatment, in combination with a potential intervention that aims to normalize the MNS, seems like an encouraging method to improve social skills in ASD. But as Young and Barrett (2015) notes, more research is needed to determine the clinical relevance of oxytocin. Furthermore, caution should be taken when interpreting these results due to methodological and statistical problems in intranasal oxytocin studies (Walum, Waldman, & Young, 2016). For instance, intranasal oxytocin studies have almost exclusively tested male ASD patients (Preti et al., 2014). Nevertheless, as seen in figure 1, normalizing the MNS may also improve social play and CE. This can, in turn, contribute to further development of social skills in ASD.

Taken together, a few conclusions can be made. The overarching problem of intervention studies in ASD is that the field is still emerging. This means that more research is needed to investigate this seemingly potential fruitful area. Currently, broad socio-cognitive interventions and social play studies should be further investigated. It should also be investigated whether these different interventions can potentially be combined to further the improvement of social skills in ASD. Maybe even more promising are the interventions that target the MNS. These MNS interventions, combined with oxytocin treatment seem like attractive ways to treat the social deficits in ASD, but have their limitations.

FUTURE PERSPECTIVES

This paper has discussed the neurobiology of social play and CE both in healthy individuals and in ASD. Ample evidence suggests that the neurobiology of social play and CE are altered in ASD patients (e.g. Chevallier et al., 2012; Kana et al., 2015; Pellissier et al., 2018; Shamay-Tsoory, 2011). Furthermore, the interactions between social play, CE, and the MNS have been described (see figure 1). Potential interventions for the characteristic social deficits in ASD have also been examined. It is important to consider how the reviewed literature can help guide future research and future treatment of the social deficits of ASD patients.

Intervention treatments are currently not researched enough, but the available results seem promising. Future research should aim to further develop broad socio-cognitive interventions that integrate both aspects of social play and CE; as these promote the development of social skills. Moreover, interventions that target the MNS should be researched further. If the MNS can be properly utilized in ASD, the social skills of the patients can be increased. Also, the interventions should be specialized for specific forms of ASD, as ASD is a very heterogeneous disorder. The specialization of these interventions can help to improve treatment in the future.

Pharmacological agents can potentially aid the treatment of the social deficits in ASD. Dopamine, noradrenaline, mu-opioid and oxytocin are the most encouraging targets (London, 2018; Paval, 2017; Pellissier et al., 2018; Young & Barrett, 2015). Nevertheless, more research needs to be done before these pharmacological agents can be used in clinical practice. One should consider the long-term effects and side effects of potential new pharmacological treatments. Special caution should be taken for opioids, as opioids are known to be addictive and have side effects. Currently, oxytocin is the most researched target and the results seem promising (Preti et al., 2014; Young & Barrett, 2015). Moreover, oxytocin influences both social play and CE, which in turn can further stimulate the development of social skills (Bredewold et al., 2014; Guastella et al., 2010). However, note the methodological and statistical limitations of intranasally administered oxytocin studies (Walum et al., 2016). Future research should explore the dopamine, noradrenaline and mu-opioid systems as targets, but should consider the potential serious side effects. More importantly, the potential long-term side effects and efficacy of oxytocin should be researched in a clinical setting while accounting for potential methodological and statistical problems. Also, as oxytocin seems to have a sex-specific effect on social play in rats (Bredewold et al., 2014) and since intranasal oxytocin studies have almost exclusively tested males, gender should be taken into account (Preti et al., 2014). Another interesting area that is not yet explored greatly, is the combination of pharmacological agents with interventions. An example of such a treatment is the combination of the aforementioned MNS intervention with an oxytocin treatment. This combination seems promising, but more research is needed to properly determine the

relevance of such a treatment.

In conclusion, research should focus on the implementation of pharmacological agents and interventions into the treatment of ASD. The heterogeneity of ASD should always be taken into account when designing treatments, as different forms of ASD may benefit from different treatments. Broad socio-cognitive interventions that incorporate social play and CE could help alleviate the social deficits of ASD patients. However, a treatment in which oxytocin administration and MNS interventions are combined, seems like one of the most promising ways to promote the development of social skills in ASD patients.

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CONFLICT OF INTEREST STATEMENT

The author certifies no conflict of interest in the subject matter and/or material discussed in this manuscript.

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The fight for new biomarkers in Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA) is characterized by a spectrum of phenotypes classified into four different subtypes, ranging from SMA I to SMA IV. Patients with SMA show progressive anterior horn cell (AHC) loss, leading to muscle atrophy and weakness. SMA is caused by a homogenous deletion of the SMN1 gene. However, the SMN2 gene, which is a homolog of SMN1, is present with a variable amount of copy numbers in our genome. Both SMN genes produce SMN protein. However, exon seven is excluded in SMN2, resulting in a non-functional SMN protein. In general, earlier treatment is linked to slower disease progression. Current treatments used in the clinic are oligonucleotides that affect the splicing of SMN2, and gene therapy which focuses on reintroduction of SMN1. Biomarkers can help in the diagnosis, prognosis, treatment, and monitoring of disease progress. Thus, finding new biomarkers is crucial to diagnose patients as early as possible. Biomarkers could play a vital role in the screening process of newborns, which would allow detection of SMA immediately after birth. In addition, multiple different newborn screening approaches may be combined and thereby allow the screening for multiple motor neuron degenerative diseases simultaneously. Furthermore, biomarkers can help us to determine what is the most optimal therapeutic window when treatment would seem to yield most promising results. Thus, biomarkers are the crucial last step in SMA research. Together, biomarkers in combination with multiple therapies, may be the optimal solution for an integrated therapy, in which biomarkers could help to keep track of treatment efficiency. This holds the promise to further improve outcomes for SMA patients.

Keywords: Biomarkers; Spinal Muscular Atrophy (SMA); SMN1; SMN 2; Diagnosis; Treatment

INTRODUCTION

Spinal muscular atrophy (SMA) is a recessive autosomal motor disorder which is characterised by muscle weakness and atrophy. Normally, SMA develops early in life and leads to severe motor problems and even death due to respiratory failure. SMA is one of the most prevalent rare diseases, affecting between 6000 – 10 000 newborn children each year (SMA Foundation, 2019). These numbers are comparable with other motor degenerative diseases like multiple sclerosis (MS) and Amyotrophic lateral sclerosis (ALS), with around 1 in 11 000 individuals being diagnosed (Sugarman et al., 2012). The spectrum for SMA severity is broad and the disease onset ranges from infancy to adulthood. This diversity makes it hard to study SMA pathogenesis and eventually find possible therapies or medicines for SMA (Sugarman et al., 2012).

SMA causes loss of anterior horn cells (AHCs) which leads to muscle atrophy and motoric weakness. The disease is caused by a homogenous deletion of the SMN1 gene. The SMN1 gene is present in the DNA, together with a nearly identical gene SMN2 which both code for the SMN protein. However, alternative splicing of SMN2 creates a non-functional protein. Hence, the SMN1 gene is the only one responsible for SMN protein (Lefebvre et al., 1995). How the absence of SMN protein, which is expressed in multiple cells in the body, causes defects, specifically in the anterior horn cells, is still unknown. Current therapies in the SMA field are focussing on enhancing SMN2 splicing and thereby creating more functional SMN protein (Arnold et al., 2016). To treat patients with SMA, the disease needs to be understood better and treatments need to be validated. Biomarkers can help in this validation and thereby lead to better diagnosis, treatment and monitoring for SMA. Currently, there are some biomarkers for SMA diagnosis and treatment present. However, these biomarkers are not well suited for progress monitoring and are sometimes not well suited for the broad spectrum present in SMA (Arnold et al., 2016; Kolb et al., 2016; Bonati et al., 2017). Furthermore, the ongoing developments in SMA research and progression for treatments in SMA, both cause a strong demand for novel or better biomarkers. This strong demand for new biomarkers in SMA research purposes the following research question: What is the current state of development in terms of knowledge about biomarkers, and how can biomarkers help in SMA diagnosis and treatment?

In general, a biomarker is defined as an indicator which is measurable and signals some biological state or condition ("Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework", 2001). This indicates that a biomarker is a substance which can be used to denote the presence of a certain state or condition. A substance which shows the presence of a mutated protein in a disease is an example of a possible biomarker. Biomarkers in general are used for examining normal biological or pathological processes. In addition, pharmacological effects on these processes can be examined. Nearly all scientific fields make use of biomarkers ("Biomarkers and surrogate endpoints:

Preferred definitions and conceptual framework", 2001). In SMA research biomarkers may help in diagnosis, treatment and monitoring of the progress of the disease. Biomarkers are evaluated upon the following aspects: sensitivity, specificity, robustness, accuracy and reproducibility. The higher the score on these aspects, the more suitable the biomarker is ("Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework", 2001).

In this review, the current state in SMA research is reviewed. In addition, the way in which biomarkers can help or have an effect in this research field is discussed. First of all, the wide variety of the SMA spectrum is discussed. Biomarkers may be able to help in spectrum identification which creates more possibilities for treatment and diagnosis. SMA pathogenesis is illustrated and possible pathways and key players which may be implicated in SMA are highlighted. This creates opportunities for biomarkers which can help in diagnosis and monitoring of disease progression. The biomarkers that are currently known are reviewed, and possible improvements are put forward. Finally, possible treatment methods, which are being used or researched at present, are described, and some therapeutic insights are provided.

Novel biomarkers may play a role in this by helping to create novel opportunities for the evaluation of treatment efficiency. Finding new and improved biomarkers may create individual treatment possibilities for SMA patients. Additionally, these biomarkers could make it possible to achieve better individual predictions and prognosis. Furthermore, biomarkers might create opportunities for screening of newborns, which would allow earlier diagnosis which is crucial for treatment possibilities. The earlier treatment is initiated, the less damage would be present in the anterior horn cells, which would result in less muscle weakness. As discussed in this paper, novel therapies for SMA are appearing and being developed, which enormously increases the need for new biomarkers to assist the advancement. Biomarkers can help in diagnosis of SMA, treatment of SMA and disease monitoring. Finding new biomarkers may eventually lead to new insights in disease pathogenesis and thereby identify novel pathways or networks which are included in SMA pathogenesis.

SMA AS A BROAD SPECTRUM

SMA is defined as a spectrum of different types of diseases. A common symptom, which is present in all types of SMA, is the degeneration of the AHCs, present in the spinal cord. Due to the loss of these motor neurons, muscle atrophy and weakness are induced (Sugarman et al., 2012). This spectrum of SMA types makes it hard to classify patients to a specific phenotype. Therefore, clinical subgroups are created to be able to handle the broad spectrum of the disease better (Table 1). Subgroups are established based upon phenotype estimation. In general, for each subtype of SMA, the rule applies that an earlier age of onset is associated with a poorer prognosis.

Type I SMA is the most severe form of SMA. Patients with type I SMA have a very early onset and mostly SMA is already present at birth. Moreover, infants who are diagnosed with type I SMA will never be able to sit or roll. Death is mostly due to respiratory failure and normally a patient with type I SMA will die within the first two years of life. In contrast, type II SMA is associated with a later onset than type I. Most type II SMA patients are diagnosed during childhood. Moreover, these patients will be able to sit independently during life. However, they will never be able to walk independently. The age of survival for type II SMA patients lies around twenty years old. Type III SMA is characterized by a normal life span and is associated with a very late diagnosis. Most of the type III SMA patients can walk independently at some point in their life. However, loss of ambulation still occurs in fifty percent of the patients. The effect of ambulation in type III SMA is associated with the age of onset. As the general rule states, type III SMA patients with a late diagnosis have a higher possibility to walk independently later in life. On the other hand, early prognosis enhances the chance for loss of ambulation significantly. The last subgroup SMA type IV is very similar to SMA type III. In general, only the onset of the disease differs between those two types (Munsat & Davies, 1992; Farrar et al., 2013).

The progression of the disease varies between patients. Moreover, a slow rate of progression is seen after the age of fifteen for the milder phenotypes of SMA (Mercuri et al., 2016). This slow progression in disease development creates a problem for researching less severe SMA types. Especially developmental studies for SMA III are hard to perform due to this phenomenon. It is currently not known why this slower progression is established in less severe SMA types and thus constitutes an active field of research. Furthermore, this slower progression makes it hard to detect changes in progression of the disease. Therefore, it is hard to see if a possible treatment, which blocks the progression of the disease, has any effect or not.

Motor unit estimation (MUNE), which estimates the number of motor units in a muscle, and maximum compound motor action potential amplitude (CMAP), which idealizes the summation of a group of almost simultaneous action potentials from several muscle fibers in the same area, are often used to estimate denervation in humans with SMA. CMAP defines the total electrophysiological output from a group of muscles. Moreover, studies show that CMAP and MUNE correlate with age, function, SMA type and SMN2 copy number. This indicates that CMAP and MUNE might be possible methods for diagnosing the appropriate SMA type for each patient. Additionally, CMAP levels remain stable during the slow progression period in the less severe SMA types, while MUNE score still decreases. This suggests that the slow progression period is due to physical growth of SMA patients. Finally, it is suggested that the motor neurons in the types of SMA that manifest later in life are

Table 1. Overview of the different clinically established subtypes for SMA. The estimated age of onset, maximal motor usages, abilities and features and final prognosis are shown for each subtype (supplemented with (Munsat & Davies, 1992, Farrar et al., 2013)).

Type	Age of onset	Maximal motor usages	Abilities and features	prognosis
SMA I	< 1 year	None	Unable to sit or roll	Death within 2 years
SMA II	6 to 18 months	Sitting	Unable to walk independently	Survival into adulthood
SMA III	Childhood	Walking	May lose ability to walk	Normal life span
SMA IV	Adulthood	walking	May lose ability to walk	Normal life span

preserved in early life stages (Swoboda et al., 2005).

THE SMN GENE PLAYS A KEY ROLE IN THE DEVELOPMENT OF SMA

SMA is present as a spectrum of different phenotypes and differs in severity between the different SMA types that are present. These different types of SMA are caused by the same genetic mutation. The mutation is present in the 5q13 region of our genome. In this region there are two genes present for the SMN protein. A telomeric form known as SMN1 and a centromeric form SMN2. Both genes code for the SMN protein and are expressed in all cell types of our body. Studies in which the cause of SMA is studied stated that a homogenous deletion of the SMN1 gene is responsible for SMA development. This means that in SMA patients only the SMN2 gene and transcript is present. Transcription of SMN1 results in a 1.7 kb transcript which after RNA editing and translation creates survival motor neuron protein (SMN protein) (Figure 1). This protein consists of 294 amino acids and has a weight of 32 kDa. Both genes are actively expressed. However, only SMN1 has the ability to create functional SMN protein. That is why a homogenous mutation of SMN1 has such an enormous effect while SMN2 is still present. SMN2 transcription and translation result in a truncated SMN protein. This protein is non-functional and will be degraded in the proteasome. This difference in outcome is mediated by a substitution mutation, T to C mutation. Due to this, SMN2 is alternatively spliced and exon seven is excluded from the mRNA. This exclusion in SMN2 results in a final truncated protein which is non-functional. However, this alternative splicing does not account for all transcribed RNA from SMN 2 and in fifteen percent of the SMN2 transcripts the translation results in a functionally normal SMN protein (Figure 1) (Lefebvre et al., 1995). This demonstrates that SMN2 is not completely useless, because in fifteen percent of the cases SMN2 results in a functional SMN protein. This suggests that SMN2 in SMA patients can rescue the phenotype a little bit. Thus, SMA patients still have some amount of SMN2 present in their cells, even when they have a homogenous mutation of the SMN1 gene. Moreover, the SMN2 gene varies in copy number in the genome between individuals (Lefebvre et al., 1997). It is suggested that SMN2 copy number can lead to different severities which are observed for SMA. Indeed, studies have found that SMN2 copy number negatively correlates with the severity of the phenotype (Lefebvre et al., 1997). The loss of SMN1 and the resulted reduction in SMN protein somehow causes loss of the AHCs in the spinal cord. The mechanisms and underlying pathways are still unknown and more research is needed to solve this.

Furthermore, SMN2 gene copy number is not the only factor which influences the severity of the disease. There are cases of SMA known in which the copy number did not correlate with the severity of the phenotypes. An example is a mutation present in the SMN2 gene which creates a new splicing enhancer site (ESE). This newly created ESE results in better splicing of the SMN2 gene and no exclusion of exon seven. Consequently, the SMN2 gene creates one hundred percent functional SMN protein and no phenotype of SMA is observed while SMN1 is still homogeneously deleted (Prior et al., 2009). Furthermore, the severity of SMA is linked to a number of genetic modifiers. Upregulation of modifier proteins and sequence variations may have a big effect on SMA severity. Plastin 3 is a positive modifier for SMN2. Plastin 3 is responsible for a less severe phenotype as expected. Moreover, introduction of Plastin 3 in animal models rescues the normal phenotype (Oprea et al., 2008). This indicates that genetic modifiers like Plastin 3 are possible treatment candidates and may give insights in pathways and underlying mechanisms involved in SMA pathogenesis. Thus, SMN2 copy number alone is a possible candidate to determine SMA severity. However, it may not be sufficient because there may be other factors that influence the phenotype of the patient. This is particularly valid for familial cases because an individual may be a carrier of severe SMA genetically, while phenotypically they may not exhibit any symptoms or impairment, induced by the homogenous deletion of SMN1.

THE SMN PROTEIN IS IMPLICATED IN MULTIPLE FUNCTIONS, WHICH MAY EXPLAIN THE OBSERVED EFFECTS IN SMA

SMA studies revealed that depletion of SMN during development causes loss of AHCs in the spinal cord. Furthermore, reduction of large diameter axons in the ventral roots and reduction in myofibers in the muscles

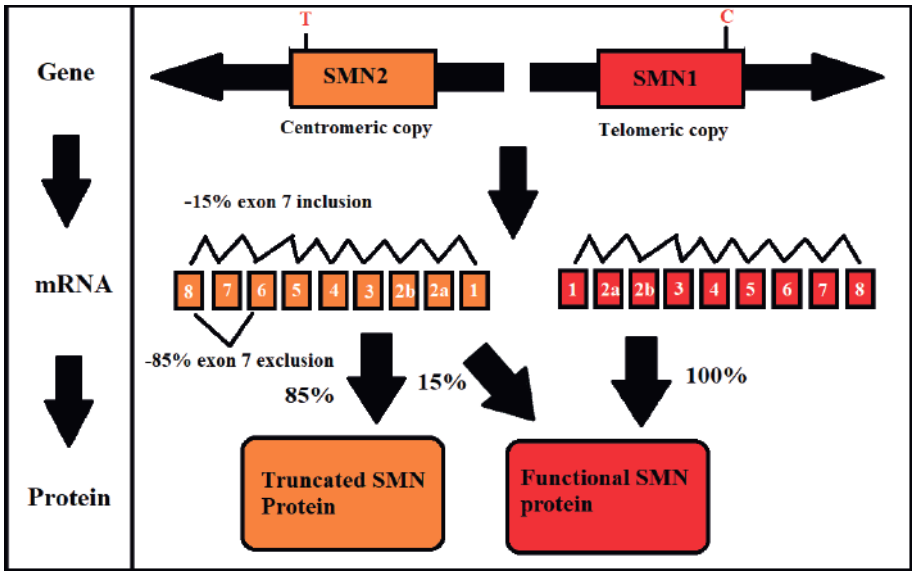

Figure 1. General overview of the SMN gene splicing in SMA, in which the mutation from T to C is shown in the genes. All SMA patients have a homogeneous deletion of the SMN1 gene which makes them fully dependent on their SMN2 gene. Eighty-five percent of the time, due to the mutation, exon 7 in the SMN2 gene is excluded, which is shown in the figure, and this results in only fifteen percent functional SMN protein in SMA patients which is indicated with the arrows.

are also observed. This implies that SMN protein is crucial for motor neuron survival and function (Crawford & Pardo, 1996). The observed muscle weakness which is found in SMA patients is related to the axonal degradation and disruption of neuromuscular junctions (NMJ). The disruption of the NMJ is one of the earliest pathological signs which finally causes axonal degeneration and/or AHC death. Additionally, less neurotransmitter vesicles are found for the NMJ in SMA. This disturbance in vesicular signalling results in retarded maturation of the post-synaptic NMJ terminals and myofibers. These effects are the underlying mechanisms which are responsible for the observed muscle weakness (Kong et al., 2009). Thus, SMA patients have degeneration of AHCs and weaker or loss of NMJ. How can the SMN protein be responsible for such a variety of different effects?

First of all, SMN protein is present in the nucleus as well as in the cytoplasm of the cell. It forms a complex with seven additional proteins which are Gemin 2-8. Together, this complex is known as the SMN complex. This SMN complex has several functions, most of them are involved in RNA processing. Moreover, the most essential role for the SMN complex is assembly of small nuclear ribonucleoproteins (snRNPs). These snRNPs are important players for the spliceosome, which is important for pre-mRNA editing in the form of splicing (Kolb, Battle, & Dreyfuss, 2007). Without a good performing spliceosome, the cell is not able to perform proper splicing of pre-mRNA which influences the gene expression and cell survival. This indicates that cells without SMN present, will not be able to survive. That is why there is no obtained genotype which lacks both SMN1 and SMN2 together (Lefebvre et al., 1995). Contradictory, based on the effects of SMN protein absence, it should have been expected to have an effect in all the cells of our body. However, SMN is rather actively expressed in all cells of the body and only affects the AHCs in the spinal cord (Crawford & Pardo, 1996). This posits a question: how can SMN absence only have such a devastating effect in motor neurons and not in other cell types present in our body (Kolb et al., 2007)? One possible hypothesis which could explain the difference between different cell types, is that the SMN protein has different functions in these distinct cell types. In neurons, SMN is known to have an axonal transporter function for proteins which are specific for motor neuron survival (Burghes & Beattie, 2009). This may account for the different phenotypes which are observed for motor neurons and other cell types in our body. Another possible hypothesis is that SMN depletion affects the RNA splicing of proteins in particular, which are essential for motor neuron function and survival (Burghes & Beattie, 2009). This could lead to a possible explanation for the observed difference. The only remaining challenge is to find such alternative RNAs.

Finally, it has been documented that the SMN complex is important for specific proteins which are involved in the NMJ and motor-sensory synapses. The absence of SMN protein alone is insufficient to induce mRNA abnormalities, which would cause problems with proteins important for these synapses. Moreover, the functions of these genes are important for maintenance of the synapse and synaptogenesis of NMJ. This may explain why homogeneous loss of SMN1 can have such an effect on the NMJ. Additionally, some genes which are affected by this altered spliceosome show splicing disturbances which indicates that splicing of specific genes is disrupted and that splicing is important for the stability of the motor

neurons. For instance, C1q mRNA is enhanced in motor neurons due to the homogeneous deleted SMN1 gene. This mRNA is responsible for synaptic pruning and thus a huge factor for the loss of NMJ in SMA (Zhang et al., 2013). These kinds of factors should be further investigated to know why and how they are enhanced in these affected motor neurons. These factors may also form possible biomarker possibilities for detecting SMA in a very early stage. Thus, the whole transcriptome of the AHCs differ due to the absence of SMN1. These together with other, still unknown, factors are most likely to cause the observed loss in synapses, motor degeneration and finally muscular atrophy.

Having outlined the role of SMN, possible diagnostic methods and screening approaches could be introduced. Nowadays, many different techniques are used for diagnosis of SMA which may find possible biomarkers for detection. Baseline screening for biomarkers revealed CMAP and EIM to be both able to distinguish between different cohorts. This suggests the possible ability for these techniques to form sustainable biomarkers in SMA diagnosis. However, CMAP only correlates with motor functioning in mildly affected patients, which indicates that CMAP diagnosis efficiency depends on the severity and phenotype of the patients. Therefore, CMAP is not an ideal biomarker to apply, because it is not suitable for all types of SMA. Additionally, EIM is not a very reliable biomarker for diagnosis of SMA. The high frequency reactance slope in the EIM measurements can distinguish SMA from healthy controls. However, very young individuals show different impedance spectral characteristics, which make it hard to diagnose them. Therefore, EIM could be a well-suited biomarker for diagnosis if this difference is further investigated and eventually solved (Kolb et al., 2016). Analysis of serums between healthy controls and SMA patients reveal some analytes which are different. Most analytes which were found to differ between SMA and control were less present in the SMA patients. Contradictory, YKL-40 and myoglobin were the only two analytes which have been found to be enhanced in SMA in comparison with healthy controls (Kolb et al., 2016). Hence, this kind of analytical screenings may be crucial to find possible factors which can give insight in SMA pathology. Analytes like YKL-40 and myoglobin may reveal underlying mechanisms which are affected in SMA and could be crucial biomarkers for SMA diagnosis.

Another possible method which could be useful for diagnosis of SMA is quantitative magnetic resonance imaging (qMRI). Data show that a reduction takes place in tissue mass and density in muscles of SMA patients. Moreover, the fat to muscle ratio is significantly higher in SMA patients as compared to healthy controls. This effect can be explained due to the fact that tight muscles are infiltrated by fat tissue in SMA patients (Bonati et al., 2017). Furthermore, multipeak-fat fraction is the only MRI-based marker which can be used to detect the progress of SMA.

Finally, motoric test screenings are used to diagnose SMA patients. MFM and 6MWT are such tests and should allow detecting the severity of SMA. MFM consist out of 32 task items that allow evaluation of physical functioning in three dimensions: standing and transfer, axial/proximal motor function and distal motor function. 6MWT measures the walking ability of patients. Patients must walk as fast as possible in this task. In general, the lower the score for these two tests, the more severe the type of SMA. Conventionally, these tasks are well suited for diagnosis of SMA based on phenotype. However, in a test period of 52 weeks, no significant results were found for the progression of the disease. This demonstrates that motoric tests are well suited for diagnosis and severity estimation of SMA, but they cannot serve as proper biomarkers for disease progression (Bonati et al., 2017). These motoric tests (MFM and 6MWT) are currently used as screening methods, allowing detection of SMA as quickly as possible in older children. In addition, screening of babies immediately after birth (Newborn screening) is the most suitable method for even earlier detection and treatment. Short amplicon melt profiling is a possible method for newborn screening. In this method primers are used in combination with PCR to amplify a homogenous region of SMN1 and SMN2. Melt profiling allows detecting the presence of SMN1 and furthermore immediately estimates the copy number of SMN2. This method can thereby diagnose SMA and tell something about the severity of the disease simultaneously (Dobrowolski et al., 2012). Related to this method is newborn blood spot screening (NBS). In this method, a dried blood spot is screened for absence of the SMN1 gene. Real-time PCR is used for this, which analyses the DNA composition of the cells present in the bloodspot. SMN1 homogenous deletions can thereby be detected and this allows very early SMA diagnosis. Additionally, it has been shown that this method also works for other disorders like severe combined immunodeficiency (SCID) (Taylor et al., 2015). Hence, several newborn screenings can be used for diagnosis of SMA as early as possible. Techniques like NBS may be used for screening of multiple disorders at the same time which makes it a perfect technique for biomarker detection. Moreover, these techniques form sustainable biomarkers for SMA detection.

POTENTIAL TREATMENTS FOR SMA WITH RECENT CLINICAL APPLICATION

In general, the progression of SMA correlates with the amount of damage at the NMJ. This damage reflects the amount of restoration, which is potentially possible with treatment (Lutz et al., 2011). Therefore, it is widely accepted that earlier treatment holds promise for less severe SMA phenotypes, and yields better results. However, treatments are just being developed and still not every SMA patient can be helped by the currently available treatments. All possible treatments for SMA are divided in four subtypes: SMN1 recovery, SMN2 splicing reduction, neuroprotection and lastly improvement of muscle strength and function

(Figure 2) (Farrar et al., 2017).

Preventing the exclusion of exon seven in SMN2 is a possible treatment method for SMA patients. SMN2 will translate into a normal functional SMN protein, when exon seven is not excluded from SMN2 mRNA. This leads to more functional SMN protein present in the motor neurons and may cause a rescue of the healthy phenotype. A possible method for preventing exon seven exclusion is the introduction of oligonucleotides. An example for such a drug is ASO-10-27. This component is known to actively interact with the splicing of SMN2, which results in a restoration of SMN expression in the motor neurons. Introduction of this drug is done by a combination of intracerebroventricular (ICV) and subcutaneous injections (SC). Patients need to be systematically treated and this leads to a dose dependent effect of survival rate (Hua et al., 2011). However, it is found that treatment is only effective in a certain time window. Treatments introduced later were still functional but up to a certain extent, while extremely late treatments did not show any effect at all (Hua et al., 2011). This may explain the variety in current treatment results obtained in the clinic. This suggests that treatment is only successful if it is administered in a certain time window. Another effective compound which is currently used in the clinic is Nurinersen. This compound is also an antisense oligonucleotide which increases the production of SMN protein. This drug is well tolerated and does not cause any side effects in humans (Haché et al., 2016). Other genetic modifiers like Plastin 3 which was previously discussed also form possible treatment methods. Introducing Plastin 3 in animal models rescues normal phenotype (Oprea et al., 2008), which indicates that these kinds of modifiers may also be used in the clinic for treatment of SMA patients (Figure 2). Reintroducing SMN1 expression in animal models of SMA has an optimal effect only for a certain time window. This suggests that this previously described therapeutic window also accounts for these kinds of techniques. Introduction of SMN1 post symptomatic in the therapeutic window is sufficient enough for rescuing the phenotype almost completely. As for SMN2 splicing modifiers, the effect of re-introducing SMN1 declines as the disorder progresses. Contradictory, animals treated in a later stage still receive benefits from restoring SMN1. However, they will never rescue their symptoms to control level (Lutz et al., 2011). Currently, gene therapy is also applied in humans at a very young age (< 9). This gene therapy works with administration of an adeno associated virus (AAV) which carries the SMN1 gene. Patients who undergo treatment display less symptoms, suggesting that the therapy is working. However, for some patients, no desired effects are exhibited, which may be due to the therapeutic window (Figure 2) (Sheridan, 2018). Reintroducing SMN1 and the possible drugs which enhance production of stable SMN2 mRNA, are all treatments which are studied and used for acute SMA types. However, slower progressing SMA types, as well as the types in which onset begins later in life, are not studied that exhaustively. Therefore, the therapeutic window, as described above, may not be applicable for these types of SMA. In this regard, SMN-C3 is a splicing modifier which enhances the production of SMN protein by inhibiting the exclusion of exon seven. This splicing modifier also enhances phenotype in a dose-dependent manner in animal studies. Introducing this compound in a later stage of SMA still has beneficial effects. This result shows that treatment in later stages of SMA types still have beneficial effects on symptom treatment. It is hypothesized that the therapeutic window for these types of SMA is much broader and not as short as the therapeutic window in the more severe types of SMA. This may explain why treatment is still effective in these slow developing SMA types. Biomarkers can help in defining these therapeutic regions. Additionally, they can help with estimating the optimal treatment possibilities for patients suffering with slower progressing SMA types, like type II and III (Feng et al., 2016).

Possible biomarkers which can be used for analysing treatment success and disease progression are CMAP and MUNE. Both techniques are currently used for the estimation of treatment success. These techniques allow a functional assessment of the motor unit pool which is supplying a muscle or group of muscles. Denervation will cause decreased CMAP and MUNE scores. This indicates that these techniques are suitable for detecting treatment efficacy. In addition, EIM is another technique that can be used for this purpose, as it can determine muscle health (Arnold et al., 2016). These electrophysiological techniques suit very well for detecting treatment efficacy and thereby form perfect treatment biomarkers. Furthermore, clinical studies using gene therapy and SMN2 splicing modifiers in order to enhance SMN protein expression, support the notion that electrophysiological biomarkers are needed for treatment stratification, treatment response determination, as well as definition of therapeutic windows (Arnold et al., 2016).

Thus, modifiers for SMN2 splicing and techniques to reintroduce SMN1 are currently used in the clinic. However, these therapies should consider the therapeutic window, as described above, and keep it in mind when applying a given treatment to a patient. Biomarkers can help with the detection and monitoring of this therapeutic window. In addition, they can detect progression of the disease and effectiveness of the treatment. This will allow patients with SMA to be screened and treated individually which is the best possible way for treatment of SMA. Finally, SMA biomarkers are crucial for monitoring treatment effectiveness. Finding good biomarkers can thereby lead to improved treatment results.

DISCUSSION

SMA exists as a broad spectrum which is subdivided into clinical groups to aid diagnosis and treatment possibilities (Sugarman et al., 2012). The described subgroups in this

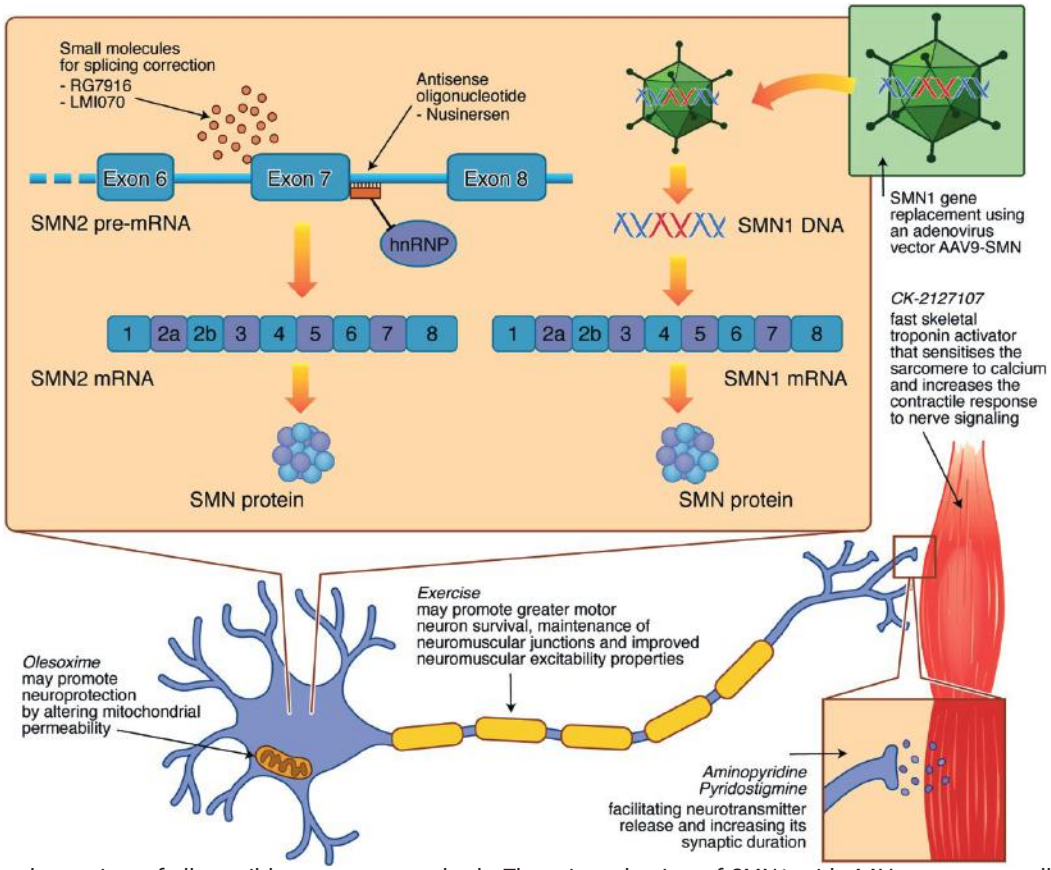

Figure 2. General overview of all possible treatment methods. The reintroduction of SMN1 with AAV vectors as well as the SMN2 splicing modifiers are shown. Furthermore, some other treatment possibilities which focus on another point in SMA are shown (Farrar et al., 2017).

review are not that clearly separated. This may indicate that the border between different subgroups is not that clear at all and this can give problems in the diagnosis of a patient with SMA. Moreover, the phenotype, which is observed in SMA, is progressive and more symptoms appear the longer the disease is present. That is why it is very hard to suggest a possible subtype of SMA based on the current information. However, it is suggested that efforts should be tailored towards personalised diagnosis. This would only be possible if there are sufficient identified biomarkers that can pinpoint with high sensitivity and reliability which type of SMA is the most likely case. How SMN defects cause such a diverse spectrum of diseases is still elusive. It is proposed that SMN2 copy number plays a role. However, there might be other undiscovered factors that may explain this difference. These factors may also be beneficial biomarkers for clinical application. The latter indicates the importance of research into the spectrum of SMA. The slow disease progression which characterises SMA type III and IV is also not that beneficial for further research. This is due to the fact that, with slow disease progression, it gets increasingly more difficult to monitor changes with the available techniques at present. What is more, neurons still show degeneration despite the slow rate of progression (Mercuri et al., 2016). However, most likely the body compensates for this loss. Biomarkers could help in research as well as the monitoring of this slow progression period. Thus, understanding the variability in SMA with respect to age of onset, type and ambulation, will assist in the development of proper scales for diagnosis. This makes it possible to monitor meaningful changes that cannot be measured without the use of biomarkers.

SMN1 is homogeneously deleted in SMA patients which makes SMN2 the only source for SMN protein in SMA patients (Lefebvre et al., 1995). This shows the importance of SMN protein in the survival of motor neurons, because a lower concentration of SMA causes motor neuron degeneration. Many diagnoses are examining SMN2 copy number to estimate the severity of the disease. However, as shown in this review, many patients show mutations, like the one described in SMN2, which may account in another observed phenotype as predicted by the copy number (Lefebvre et al., 1997). This indicates the importance for perfect biomarkers which may help in the diagnosis and prognosis of SMA and even further can give crucial insights in the severity of the disease. As discussed, earlier treatment is crucial for better results in survivability and rescue of the healthy phenotype (Lutz et al., 2011). This shows the importance of good newborn screening which should be present in standard health care upon birth. First steps are made into this by the introduction of NBS and short amplicon melt profiling (Taylor et al., 2015, Dobrowolski

et al., 2012). These techniques can be used to examine newborn on SMA characteristics and thereby lead to an early diagnosis and treatment. Newly discovered biomarkers can play a crucial role in newborn screenings. Moreover, newly discovered biomarkers which are present in an even earlier period of the disease and are clearly detectable, allow these techniques to screen for them immediately following birth, and thereby associate them with the correct disease type. Nowadays, it is hard to separate different motor neuron degenerative diseases from each other. Diseases like ALS, SMA and MS are hard to separate, because they all affect motor neurons and these phenotypes are very similar. NBS is a possible solution for this problem. Furthermore, another motoric disorder (Duchenne muscular dystrophy (DMD)) can already be detected, by using a biomarker for DMD in NBS analysis (Taylor et al., 2015). This indicates the potential benefit by using NBS to screen new-borns for multiple biomarkers, which are all unique for their related motoric disease. In this way treatment can start immediately after birth which gives huge benefits to the patient's prognosis. Thus, diagnosis is mostly performed by examination of the symptoms with motoric tests and excluding all the other conditions which lead to the same symptoms. So, one is looking for similarities, rather than cellular signs that may signal the presence of the disease. Earlier treatment is also important due to the progressive damage which is induced by the disease. Moreover, the longer SMA is present, the more motor neurons will degenerate and this will lead to more muscle atrophy and more obtained symptoms (Lutz et al., 2011). Treatment rescues these dying neurons. However, there is no remedy that can be undertaken, if the neurons are already degenerated. It is implied that the currently available treatments like oligonucleotide introduction or gene therapy both rescue motoric neurons form degeneration, and in turn, prevent disease progression (Haché et al., 2016; Oprea et al., 2008; Sheridan, 2018; Lutz et al., 2011). A possible hypothesis is that motor neurons, due to a lack of sufficient SMN protein, enter a kind of resting state in which they are not responding and would eventually die due to the lack of activity. This may indicate why the treatments, which are currently used, are so efficient with their results. If these cells become active due to the reintroduction of sufficient SMN protein they will form NMJ again and prevent muscle atrophy and further progression in SMA pathogenesis. However, nothing is known about the long-term effects on these patients. SMN protein is especially crucial in the development based upon the huge problems which exist in early types of SMA (type I & II) (Munsat & Davies, 1992; Farrar et al., 2013). This may suggest that SMN later in life is not that crucial anymore. However, treatment cancellation, with the currently available medication, reintroduces symptoms very quickly (Sheridan, 2018). This indicates that whole-life drug administration is necessary, and that other mechanisms beyond SMN may be affected. Finally, there seems to be a therapeutic window for treatment efficiency in SMA patients (Haché et al., 2016; Oprea et al., 2008; Sheridan, 2018; Lutz et al., 2011). This indicates that treatment is not equally effective for each patient. Better understanding of these windows and monitoring of these windows allows for better treatment possibilities and may lead to even better treatment successes. It is hypothesized that this therapeutic window is even more beneficial if SMA patients are treated prenatally. The NMJ in SMA may, for example, play a role in the early pathogenesis and finally the axonal degeneration, synaptic disconnection and eventually AHC death. This effect is already observable before birth and may form a perfect prenatal biomarker (Kong et al., 2009). Furthermore, it is stated that treatment may have even better results if it is applied before birth (Arnold et al., 2016). However, this raises an ethical question of whether to allow preborn screening and treatment at such an early stage.

SMN is expressed in all cell types in our body, yet it only exerts considerable effects in motor neurons. A possible hypothesis is that this difference between cells is due to a distinct function of SMN in various cell types. SMN may have a specialised transporter function for certain proteins which is essential for motor neuron survival. Furthermore, it is hypothesized that RNA splicing of proteins that are important for motor neuron function, is affected by this SMN depletion (Burghes & Beattie, 2009). This demonstrates that the function of SMN is still far from understood, calling for further investigation. Moreover, the lack of knowledge in SMN depletion effects on the different cell types can give a problem in the currently used treatments. It may be possible that currently treated patients show effects in other cell types, which are affected by the depletion of SMN, in a later stage of life. This means that good monitoring of these treated patients is crucial in order to address any side effects as quickly as possible, and improve treatment even further.

At large, the SMN protein serves as a maintenance factor and/or element which is required for the elaboration of the adult neuromuscular synapses. This protein may be crucial for neurodevelopment. However, it is highly likely that there are other unknown factors which also play a role in the degeneration of motor neurons. Discovering these underlying factors may form perfect biomarkers for all described processes. Finally, to find new biomarkers, which can be helpful in all of the above-mentioned processes, a baseline search may be performed between healthy controls and SMA patients. Moreover, new biomarkers will be found when there are more results present about the pathways which determine the severity of SMA. These biomarker screens should be performed in an unbiased fashion in a way that would allow for more possible new candidate biomarkers. These may reveal other pathways and mechanisms beyond SMA. Currently, biomarker screens for SMA are already underway. They are focused on proteomics and metabolomics (Finkel et al., 2012). This field of research reveals a whole list of potential candidate biomarkers in the class of proteins and metabolites. Focussing on the therapeutic window, in this regard, may help in terms of finding the most suitable possible biomarkers for treatment. These biomarkers, in combination with multiple treatments addressing other symptomatic aspects of SMA, may create integrated therapies. The latter might be the

most optimal solution for treatment of SMA.

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CONFLICT OF INTEREST STATEMENT

The author certifies no conflict of interest in the subject matter and/or material discussed in this manuscript.

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Diagnosing psychiatric disorders: How can machine learning help?

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Dr. Hugo Schnack is a physicist and Assistant Professor who develops mathematical models to understand the relationships between the human brain and behavior in health and disease. Central to his research is understanding how (image) data represents information and how knowledge of data quality can be used to perform optimal analyses. More recently, the focus of his research line is on developing individual prediction models based on MRI brain image data and clinical data from psychiatric patients, with special attention to interpretable models.

Psychiatric disorders are diagnosed based on DSM classifications. However, these diagnoses are artificial constructs and determined subjectively via interviews (Figure 1). If we could find biomarkers for the disorders, diagnoses could be made in an objective way. Moreover, biomarkers may point to the biological mechanisms of the disorders, the knowledge of which, in turn, could lead to improved treatment, early recognition, or even prevention of these disorders.

Psychiatric disorders are not the result of, e.g., a single genetic defect or focal brain abnormality. Instead, they are the result of the interaction of many (small) effects. Thus, rather than investigating all possible defects one by one, analyzing the whole genome or brain structure in relation to the disorder would be much more powerful. Such multivariate pattern recognition analyses can be performed by machine learning algorithms. By training these algorithms on a labeled dataset, one can discover patterns in the input data (genome, MRI brain images) that are related to some output (the diagnosis) (Figure 1). The resulting prediction model can be used to make predictions in new patients. Testing a model in new, preferably independent, samples is a key property of (good) machine learning studies (Nieuwenhuis et al, 2012; Dwyer et al, 2018). In addition, many machine learning algorithms produce interpretable models, indicating which input features ('biomarkers') are relevant for the prediction.

Robust neuroimaging based diagnostic models have been created this way, but with modest accuracy (Schnack and Kahn, 2016). Multi-modal, multi-scale modeling may improve this (Figure 1). However, the heterogeneity of psychiatric disorders calls for innovative approaches that go beyond modeling single relationships between biology and diagnosis (Schnack, 2019). The modest reliability of diagnoses (Regier et al, 2013) poses another problem since these 'expert labels' serve as gold standard to train our models. How can we expect our models to perform well if the reference is unreliable? Avoiding expert labels is one solution: in unsupervised learning, an algorithm clusters subjects based on the input data. Now a new problem arises: How meaningful are these biology-related clusters? One way to 'validate' them is to see if they are related to

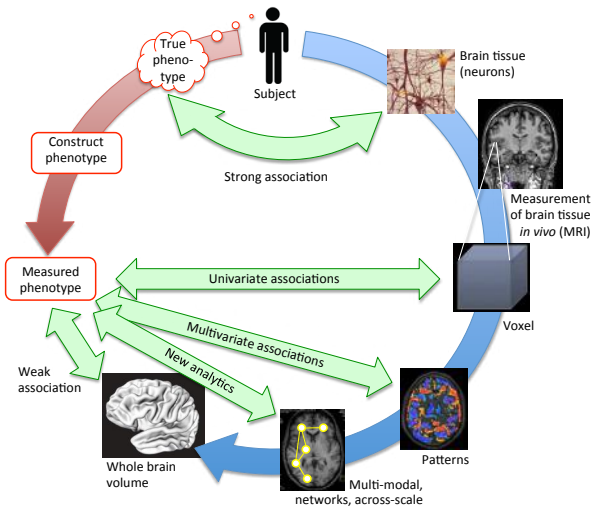

Figure 1. Long-distance modeling in psychiatric neuroimaging. Figure adapted from Schnack (2019).

other subject properties (Clementz et al, 2016). Another way to bypass the unreliable gold standard is to abandon diagnostic categories and try to predict continuous disease dimensions (e.g., symptom severity; see also the RDoC initiative (Insel et al. 2010)). Finally, leaving diagnosis to the psychiatrist, machine learning can complement the work by making prognostic models, e.g., predicting treatment responses, so that the right treatment for an individual patient can be chosen as early as possible.

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PhD Experience

Louisa Linders, MSc

PhD student at the Brain Center UMC Utrecht

What characterizes my path in science so far, is the way I made choices. As I was not sure whether I wanted to do a PhD, I took my time and I was always on the lookout for what my next step could be. I have always been interested in how diet and nutrition affect the brain and the way we feel. This led me to do a Bachelor's in Psychobiology at the University of Amsterdam. I completed my Bachelor's with an internship at TNO, where I studied the effects of a plastic stabilizer (found in e.g. plastic food packaging) on brain development. I really liked the end of the internship, when all the data came together and I had to search for explanations, but the work itself had been a bit boring at times. Was I fit for life as a researcher, wasn't I too impatient? I took a year off after my Bachelor's to think about what I wanted to do.

"Was I fit for life as a researcher, wasn't I too impatient?"

I decided to do the Master 'Basic and applied neuroscience' at the UvA (now called Physiology of Synapses and Networks). I did my second nine-month master internship in Bordeaux at the Laboratory of Nutrition and Integrated Neuroscience (NutrINeuro). Here I discovered how well research could fit me. The subject, technique, and supervisor were great and I could not imagine being bored by this kind of work.

When I returned to Amsterdam, I needed some time to adjust. I had just finished this amazing experience and again I did not know what I should do next. Although research was a lot of fun, I was in doubt whether I wanted to commit to it for 4 years. So, while I figured out what kind of job I wanted, I started baking sourdough bread in a baking café. At first, this was great; I was doing something completely different and I learned how to bake bread. But after a while, I started to miss science and the way you are challenged by it. Maybe a PhD was something for me after all?

I asked around in my network, emailing previous supervisors and scientists I met in Bordeaux, looking for researchers working with electrophysiology and eating behavior. Because if I had figured anything out from my previous experiences, it was that if I was to do a PhD, it would be one with the right subject and the right people. What I would recommend is not to do a PhD because you think it is the logical next step, but to do it because you want to.

I heard about Frank Meye, who works on neural circuits of reward-seeking in the context of aversive events. I sent him an email and got lucky; he was about to hire a PhD student in his group. I went for an interview and now, 2 years later, I am really happy to be working on dopamine circuit plasticity and stress eating. The lab is great, we discuss everything together and help each other out. It is very rewarding when you get good results or you think about the bigger context of your research. A PhD is also hard work, you are responsible for your own project and it can be frustrating if things do not go the way you anticipated. Days go by and I am sure that within no time my four years will be up, and the time will come for yet another decision.

"What I would recommend is not to do a PhD because you think it is the logical next step, but to do it because you want to"

Inbound

"My favourite benefit of being abroad is the opportunity to reinvent yourself entirely"

NAME
Leon Frajmund

HOST INSTITUTION
Utrecht University, Utrecht, The Netherlands

Leon is a first-year Neuroscience and Cognition Master student who came to study here after finishing his high school and university in the USA. We asked him to share his reflections on his experiences abroad, and what it was like to move from the USA to the Netherlands.

Some people go to study abroad; they leave for a semester to do an internship at a foreign lab or, if they're feeling audacious, they enroll at a foreign university and get a degree, spending one to six years studying abroad. Myself, I didn't so much go study abroad as just move out of my country. Ever since leaving Brazil at age 13, I've lived eight years in the United States, moved to The Netherlands to do a two-year Master, and am planning on studying Medicine in either Portugal or France. Suffice to say that the label "international student" has become as core a part of my identity as anything else.

Living abroad brings many unique opportunities. You get to meet new people and become deeply immersed in their language, culture, cuisine, and worldviews. You get the chance to do things you never thought you would be doing; for example, I got to try deep-fried Coca Cola in the US (which I regret) and raw herring in the Netherlands (which I deeply regret). And you get to learn more about society and humanity as a whole and how nothing that you took for granted back home is necessarily as universal as it may have originally seemed. Basically, everything you've read on the "Go Study Abroad!" pamphlet is true; however, there are some extra benefits of studying abroad which aren't mentioned… Like the bond you instantaneously have with all other international students. Or how you can always use the fact that you are from somewhere else as a conversation topic (I've lost count of how many times I've had a Dutch person give me an impromptu Dutch lesson or talk to me about stamppot). And you can always use the excuse that you are a "foreigner" whenever you screw up ("Oh! Is that the way you guys do it? It's different where I'm from!"). But my favourite benefit of being abroad is the opportunity to reinvent yourself entirely. Back at home, you have a history and a way of being that people are used to; they remember how you are "supposed" to be like. When you leave the country, nobody knows who you are or what you are like, giving you a literal blank slate upon which to reconstruct yourself as you wish. You get the chance to move on, unhindered by who you were, to become who you want to be.

Of course, living abroad is not without its difficulties. The bureaucracy can be a nightmare if you are unlucky. Academically, you might feel unsure of what is expected of you, and you may miss opportunities or struggle with your work as a result. Socially, you will often feel like you're two steps behind everyone else; you don't know the jokes, the social mores, the specific ways people interact with one another. Sometimes you don't even know the language. If you aren't careful, it can be easy to succumb to the feeling of isolation and loneliness. But it is also easy to overcome every single one of these challenges. Seek out people! Put yourself out there! Talk to others, don't be afraid of asking for help, be open to learning new things and having new experiences, and I assure you, you will have the best time of your life.

Outbound

"I hate you, but I love you"

A short story about my experience in New York City

NAME
Welmoed van Zuiden, MSc

HOST INSTITUTION
Mount Sinai Hospital, New York City, The United States of America

Welmoed has finished her master's in Neuroscience and Cognition last year. During her master's, she decided to do her minor research project at Mount Sinai Hospital, New York City. We asked her to share her reflections on her experience abroad, and what it was like to move from the Netherlands to the USA for half a year.

Just over a year ago I flew across the Atlantic to my new home for the next 6 months. I was going to the Big Apple, the concrete jungle, the city of dreams: New York! When I first left the subway and entered the rainy, grey streets of Manhattan, I thought to myself: is this supposed to feel like home now? Because yes, it really is a big city, and it can be hard to find your own way. But this also made the experience exciting and at the end of it, I felt proud of myself to know my way in the neighborhood.

During my first walk through Central Park, I saw the tall black building of the Mount Sinai hospital tower over me. This is where I would spend my days investigating microglial changes in schizophrenia in the lab of Dr. Lot de Witte. If I wanted to, I could go to an interesting talk every day of the week and get free cookies or pizza. The Mount Sinai hospital is located in the Upper East Side (xoxo, Gossip girl…) right next to Central Park. This meant lunch breaks in the park and stunning views from the rooftop, even though technically nobody is allowed up there… Mount Sinai arranged a room for me just a few blocks from the hospital, so I could walk to work. This is a luxury in New York City: many people travel to Manhattan by subway, because the borough is crazy expensive to live in. My room was 'only' 975 dollars a month, an amount you normally pay for a tiny room deep down in Brooklyn. Food and drinks are just as pricey: one bell pepper is over 2 dollars and a 7-dollar beer is considered cheap. Finding scholarships and thinking about your budget beforehand is, therefore, a must when going to New York.

New Yorkers often say about their city: "I hate you, but I love you", and over time I learned what this means. The travelling, crowdedness, and cost of living are definitely part of the 'I hate you'. But there is also much to love about New York City. One of them is the diversity and dynamics. All sorts of people from all over the world come to the city, often temporarily like me. This made me feel more at home: I wasn't some stranger amidst the locals, but one of many world citizens trying to find their way. I was also lucky to find friends in a similar situation. The first week after I arrived, I joined an open evening at the New York Urban volleyball association and formed a team with girls from the US, Italy and even Venezuela. One of them was French and had also just started her Master's internship. We became good friends and together we met more French students with whom we did many fun activities. It's a cliché thing to say, but the people really make the experience. Especially in big places like New York, it's easy to feel lonely in the big crowd. So if you're still going abroad: get out there, join a sports club or some kind of social group, and make some new friends!

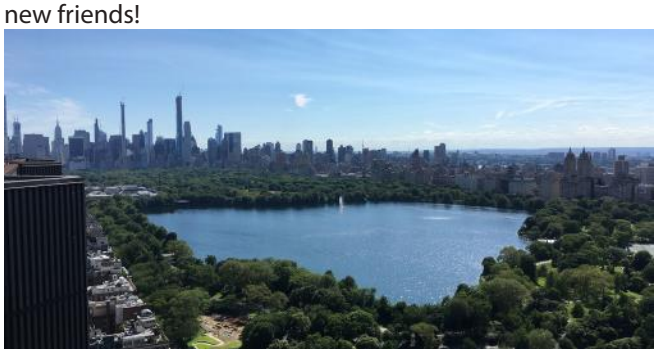

Figure: View towards downtown Manhattan from the rooftop of the Mount Sinai Hospital.

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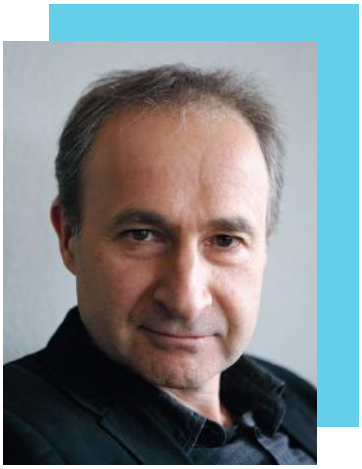
Interview: Prof. dr. Jim van Os



Jim van Os is a Professor of Psychiatry and Chairman of the Division of Neuroscience at Utrecht University Medical Center. He finds it important to develop innovative care to better assist people with mental disorders. One of his core values is the need for co-creation, that is: the need to closely work with patients to develop language, concepts and practice, including research, that are acceptable and helpful to patients in the painful process of recovery from mental suffering.

Who or what inspired you in your vision of psychiatry?

I think an important person was Robin Murray. He is a professor of psychiatry at King's College in London and probably the most cited psychiatrist worldwide. He was my teacher during my psychiatric training at the Institute of Psychiatry in London, where he directed a large department of clinicians and researchers. Those were very interesting days; there was tremendous optimism that we would soon find genetic variation and neuroimaging paradigms, paving the way to advanced treatments for severe mental illness, particularly schizophrenia. There was a lot of speculation and feverish blood sampling happening around this time.

At the same time, we were working as part of the mental health services in the city of London. It was a hectic place for psychiatric training, but also an exciting and rewarding time. Robin said: "I want you to train in epidemiology, because the quality of the research in psychiatry is so poor in terms of underlying methodology and quantitative analysis." So, he sent me on the path of psychiatric epidemiology and I trained in the London School of Hygiene and Tropical Medicine, as part of a personal MRC fellowship.

I developed a keen population perspective and a very critical methodological attitude. The London School was really the Ivory tower of how you should do research, it resembled a sort of open science platform in its earliest stage. I learned the embedded tendencies in research, resulting in bias, underpowered samples, and non-replicability. I also learned to not be overly concerned with reforming your own hypothesis and having an outward scope to (possibly) disconfirm what you are thinking.

Robin was very interested if not to say obsessed with the 'obstetric complications' theory or seeing mental illness as an outcome of events during pregnancy and childbirth. That was the dominant environmental hypothesis of schizophrenia at the time. Then we saw the rise of the schizophrenia molecular genetic discovery efforts, first through simple genetic association studies. There was hope for discovery of genetic variants explaining liability for schizophrenia. The fascinating thing, however, is that now, 30 years later, the neuroscientific approach to schizophrenia has shown that we have not been able to have clinical impact. We have been unable to unravel the mystery of psychosis and the hypothesis that schizophrenia is a brain-based disease. It remains a hypothesis. So that is the fascinating thing about mental illnesses, they are all variations of normal mentation: we all have low moods, anxiety and pre-psychotic states. Mental illness is clearly an exaggeration of these states, just like high blood pressure is an excessive state of blood pressure, and diabetes is an excessive state of glucose tolerance. However, we have not been able to pinpoint the clear contributing factors to mental illnesses, although we have lots of associations.

What is your opinion on the current DSM-V?

People are now saying that the dimensional representation is better for the future of research and clinical practice. Phenotypes clearly are not discrete entities. So just like blood pressure and glucose tolerance can merge into hypertension and diabetes respectively, mental illnesses can be considered as exaggerations of the population phenotype. There is a threshold, above which a person likely will develop need for care. The threshold, and the sort of resilience that moderates it, can be different for each person. In the categorical model of, for example depression, the problem is that there is more that sets persons with the same diagnosis of depression apart than that makes them similar. So, a more dimensional representation of mental illnesses would be a better model.

Some people are going even further and say: "You should not be diagnosing in the first place". The best way to help people in mental health care is to say: "Who are you? Where do you want to go with your life?" Then the diagnosis should be the objectives that people want to accomplish in life. In other words, the diagnosis is not focused on illness, but rather on healthy goals in life, and then you help them reach these goals. You would say: "If you have mental problems, that keep you from accomplishing your dreams, we are going to help you reach them!" The more you diagnose it as an illness, the more the diagnosed are drawn into an identification with illness resulting in medicalization of their mental variation, resulting in a strong patient identity and dependence on medical care in ways you do not want. You want to promote resilience.

The success with which DSM-V, and its illness-based perception of mental variation, was promoted is drawing more and more people into an illness-based identity. Our own success has now led to medicalizing too many people. Neuroscience therefore should study mental variation across different dimensions, not using illness-based language. We should embrace the complexity of mental states, rather than reduce them to apparently simple, but not really helpful diagnostic categories. We now have scientific tools that can help in embracing complexity, for example we make networks of symptoms that dynamically evolve over time and create a more agnostic model to work with.

How would you address a person-centred diagnosis and treatment?

A person-centred treatment would start with trying to understand what has happened to the patient and how he formulates his own mental vulnerability. Then, explore together with him where he wants to go in life, what he wants to achieve and, finally, what sort of help he needs to get there, given the presence of mental problems. Many mental illnesses and vulnerabilities often do not simply go away. Therefore, helping people with mental problems is more like helping them to cope with their experience, such as hearing voices. After treatment they still hear the voices, but the voices have less power over them, and they learn how to negotiate with the voices. So, tailoring the treatment much more to the patient is good clinical practice. 'Personalized' treatment in the sense of applying genetic stratification of diagnosis, treatment or prognosis currently is not possible in psychiatry. And I think this will be very difficult anyway because, for example, there are thousands of minor genetic risk variants that contribute to the liability of mental disorders. It is unlikely that these will identify subgroups with a high likelihood ratio.

Do you think that there is a problem with our current Dutch psychiatric health system?

What is happening in mental health care is very important. The current general thinking in society is that the whole notion of health care is unsustainable. We are spending more and more money on health care, close to 17% of the GBP in the USA and around 11% in the Netherlands. However, what we are seeing is that, paradoxically, health care does not contribute much health at the population level. In other words, fighting illness at the back end is much less effective for population health than fighting illness risk factors and promoting resilience at the front end. This latter effort is called public health. For example, treating lung cancer in people who already have the disease of course if useful but a much better way to fight lung cancer is to reduce smoking levels in the healthy population. The equivalent in psychiatry would be not to spend all your resources on treating mental illness, but to also invest in reducing risk factors like a toxic society where young people experience too much pressure and focus on things like success as a 'choice'.

In many countries, the mental health care capacity is not enough to provide all the care that people ask for. For instance, in the Netherlands, 20% of the population meet the criteria for a mental illness diagnosis, which is a lot; mental health care, however, only has the capacity to cater for 7% percent, at most. Therefore, you need to have a way of keeping the mental health service for 7%, for the most severe mental problems and have other solutions for people with mild anxiety, mild addiction, mild depression etc. That can be done, for example, via online communities of having local low-cost mindfulness centres.

"It is good, as a neuroscientist, to have a feeling of what the wider implications of neuroscience research are"

There is also a science of health services, which is "How do you organize health services for complex things like mental disorders?". Health science, health services science and economic science (which is about how do you pay for it? what sort of model should you have to make it affordable for society?), clinical science and neuroscience, all working together to have an organized response to mental suffering in society. It is problematic because you are either a clinical scientist or neuroscientist or economic scientist or health services researcher, but nobody is all four things. It is good, as a neuroscientist, to have a feeling of what the wider implications of neuroscience research are.

How would you envision the future of psychiatry as a field?

I think psychiatry and psychology are useful, in the sense that we have two therapies: psychotherapy and medications; that's all we have! That's okay, they both work interchangeably and moderately well. We need experts who are able to relate to people with mental illnesses, who can talk to patients who have psychosis, suicidality or an addiction. These experts also need to be more scientific in the sense of having a scientific interpretation of the phenotype and the causes (which we do not know much about). They should get rid of pseudo-science like DSM5 diagnoses. We need experts by experience who can help other people with mental suffering and we need integrated research, in the sense that clinical scientists, neuroscientists, health service researches and economists try to figure out models of mental illness, and how to organize treatments in the field that are cost-effective and affordable. Thus, multidisciplinarity is important for mental disorders and their treatment. Neuroscientists in the lab need to have an idea of what it is to be a patient, and how difficult it is to organize mental health care for patients. Also, the mental disorder research in neuroscience should be based on why some people respond to biological treatments and others not. In other words, we need experimental neuroscience around treatment effects. I do not think that research comparing 100 patients and 100 controls in an MRI scanner is going to bring us anything. What will be useful, however, is sampling 100 patients, 50 of whom displayed a good response to antipsychotics and 50 who did not show any response; what are neuroscientific markers differentiating these two groups? This type of experimental medicine approach can be very useful, I think, also in terms of differentiating prognostic groups, response to psychotherapy and psychotogenic drug responses.

What I also find very interesting is the area of neurostimulation in psychiatry. Not deep brain stimulation where you must operatively insert electrodes, which is much too messy and dangerous, but other sorts of stimulations, like high intensity focus ultrasound (HIFU) and Magnetothermal stimulation. With HIFU you can influence the permeability of the blood brain barrier with ultrasound. Those sorts of things are interesting, as it allows you to temporarily open the blood brain barrier and administer a substance. Targeted neurostimulation would be helpful, because medications are really too primitive: giving an antipsychotic to a person is like breaking the table with a huge hammer to kill a fly. Much more subtle ways of locally influencing and impacting the brain in subregions would be much better, helping an individual finding the right area for stimulation. There is a lot of work that needs to be done there, but it will be done of course.

What else can help? The experiments we do with psychedelics are very interesting. The idea that somebody with depression is given a psychedelic just once, following which he/she makes a trip. In the trip, they may see their diseased father or mother, saying things like: "Come out of your suicidality, I gave birth to you to live longer, you can't kill yourself". They wake up and say: "I feel reinvigorated, my life has a new meaning to me". This is potentially a very interesting way of dealing with depression. We in fact create a chemically induced transcendent experience that helps escape a suicidal loop – similar to the loops you observe in addiction, anxiety, obsessions or hearing voices – you chemically break them out of it, they get an insight that helps put the experience in perspective, thus suffering less.

We should keep an open mind on this. What we do a lot in psychiatry is changing people's perspectives. A perspective is seeing a future, being able to look past your suffering to see a future world. This can be done chemically, creating a transcendent experience, but it can also occur in psychotherapy. In psychotherapy, the therapist can create very strong interpersonal experiences and get the same result. People come out of the session and say: "Wow, what I just experienced from psychotherapy gave me an insight, this dramatic disclosure of mine makes me feel able to change my perspective". Maybe one day we can construct a psychedelic 'gun' with HIFU or some other form of localizable neurostimulation, and we 'shoot' the person, thus giving them an experience that can help them break out of a toxic mental loop. Of course, we would need to find the right target for each person. I think we will see less chronic biological treatments but rather a focus on applying a treatment just once or twice to bring people in altered states of consciousness, helping them literally break away from the mental loop they are stuck in. Sounds like a neuroscientific research hypothesis: we may not understand the loops, but we strongly suspect they are there.

Interview: Prof. dr. Hilleke Hulshoff Pol

Hilleke Hulshoff Pol is a full Professor of neuroscience, in particular of psychiatric disorders, at the University Medical Center Utrecht. She is head of the Brain Plasticity group at the Department of Psychiatry. Her research focuses on structural and functional brain plasticity throughout life in health and in psychiatric disease, and the influences of genes and environment on brain plasticity. For this purpose she studies individuals and their (twin) family members, using magnetic resonance imaging at ultra-high field.

Who or what inspired you in your vision of psychiatry?

I would say there are a few people who have had an influence on my vision as a researcher. Of those, I want to mention two that have been particularly inspiring to me. Neuropharmacologist Professor Jan van Ree, who was my Ph.D. supervisor, inspired me to look at psychiatry in a very original way, to always try to contribute to science and in doing so, improve the lives of patients. Also, my supervisor as a researcher, psychiatrist Professor René Kahn, has been very inspiring. Not only because he stands for his patients and for the improvement of the entire clinical process, but also because he taught me to think big, he has always given very good advice and has provided a fruitful environment to conduct research in psychiatry.

What does the line of research on structural and functional connectivity tell us about the different classifications of psychiatric disorders?

Structural and functional connectivity refers to the brain being highly connected through fibers linking anatomically distant regions in functional networks. The idea that altered connections are present in some psychiatric disorders has been around for quite some time. There has been, for example, a hypothesis for psychosis about diminished connectivity or dysconnectivity. But also, hypo-connectivity and corresponding changes to connectivity have been suggested to exist in anxiety and depression. So, different conditions can show altered connectivity of the brain, with differentiation and overlap between diagnostic boundaries. This is now being confirmed by structural and functional brain imaging on connectivity using magnetic resonance imaging (MRI).

I am currently working with a Master's student where we are investigating whether, and if so, to which extent, altered functional connections can be an indicator for genetic liability and environmental risk in schizophrenia, by measuring resting-state fMRI in a discordant twin cohort. What is special about this cohort is that whilst one twin suffers from schizophrenia, the other one does not. We already found that structural brain alterations can partly be explained by the increased genetic risk for the disorder. We are now looking at what leads to a risk of psychosis, whilst a sibling has resilience against it? And whether there are indicators for genetic and environmental risk and resilience in psychosis in resting-state fMRI data. These are the types of questions that need to be answered relating to connectivity.

How would you implement your findings on connectivity in the new DSM-VI, and do you think it is possible to use connectivity markers in the diagnostic process?

It is still too early to implement connectivity findings in the clinical field. I think it could inform both state and trait findings, and thereby aid DSM-VI. We and others are working hard to transfer the research findings into clinical practice. Looking at more individual changes, the longitudinal alterations can be very informative, in particular; in the Netherlands, research has a strong history in conducting such longitudinal investigations. It allows for assessment of individual trajectories in development and ageing that can inform precision medicine, including precision psychiatry. Taking the individual into account, structural and functional connectivity measures could thereby inform treatment choices in the future.

How can we overcome the barriers between research findings and implementation into clinical practice?

The best way to overcome barriers is to provide measures and show that they help the diagnostic process and treatment options. I believe that through Precision Psychiatry and a more prominent focus on developing mathematical models and using artificial intelligence techniques, such as machine learning, deep learning, and unsupervised learning, we can overcome these barriers. Combining multiple promising markers most likely provides the best way forward.

How would you envision the future of psychiatry as a field?

I believe the potential of Precision Psychiatry is key here. I envision it to be more based on individual optimisation, where a patient with early stage symptoms can be treated according to their specific needs. There is a discussion on what the future diagnostic criteria should be based on. This could mean a system that is more symptom-based, rather than diagnostic-based. Ultimately, what is of greatest importance is that psychiatrists and other caregivers, and patients themselves, are optimally informed to deliver the best treatment options for each individual.

Oliver's perspective on Autism Spectrum Disorder (ASD)

Oliver* is a 14-year-old boy who has been diagnosed with Autism Spectrum Disorder. He answered some questions for us that provide insight into his unique experience with autism.

Do you feel like the Dutch healthcare system is helping people with autism?

I am 14 years of age and I have no idea what the Dutch healthcare system really does for people with autism. I do know that it took a lot of time before I was diagnosed and it took even longer to get help after that. I know that it took a long time for my parents to get the right health care organizations that could offer the right professional help for me (for example an autism coach, but also help with my school). I have always learned to copy other people's behavior, which makes me blend in more, which in turn makes it harder for people to recognize my autism.

If you think that there are misconceptions about autism, what would you like people to know about autism?

I have given some lectures before (for universities) about autism and almost every time my message to them was that every person with autism is different. You have movies like 'Rain Man' or series like 'Atypical' that basically shape the view of autism. Because of this "biased" view of autism, people were shocked, when I told them I had autism, because they didn't expect it or sometimes didn't even believe it. This makes it hard for me to tell people about my autism, but also makes it hard to get help, because of this misconception.

Is there something you would like to tell us (young researchers) that you want to know about autism?

I would like to know what the cause of autism is, and why there are so many different "versions" of autism. I feel like if you know the cause of autism, finding a better way to help it, or maybe even cure it, would happen a lot faster. This would also help people (and maybe even me) understand me and other people with autism better.

How do you interpret the world differently?

I have two examples of this. Example no. 1:

I had to go to the supermarket to buy some stuff for my parents for dinner. I had a good plan in my head of what I needed to do, what I had to get and where to get it. So, I stood there, looking for what I needed to buy because there were different variations of what I needed to buy. A few examples of names for the variations were "carnival", or "festival". I had no idea what I needed to do, and I was stressing out because of this and someone I knew kept walking past me, and eventually asked if I was looking for something. I said no because I had no idea how to ask this as it would sound absolutely ridiculous. After some time, I went to a different employee to ask where I can find it (hoping I could buy myself some time to think) but of course, he pointed me to the exact same location. I called my parents, but they just told me I needed to choose for myself. I was getting really frustrated and pretty desperate too, to be honest, so I just grabbed some and left (with paying of course).

Example no. 2:
I remember opening the email with the questions for this interview. The first question made absolutely no sense to me, so the Autism basically took over. So here is an explanation from what happened in my point of view: Because of how I read the first question, I was starting to get really chaotic in my head. I basically put my phone down and started to do something else. I didn't read the rest of the questions or the explanation, which said that I can also think of a question for myself. I also felt like these questions were a little too closed, meaning I could only write a few sentences.
Some of the people in my area didn't experience this the same way as I did. I think for me (a person with autism), it would be more useful to get multiple questions with some follow-up questions, because I don't think of these follow-up questions for myself. That's also why I suggested adding the question 'How do you interpret the world differently?'. In that way, I can provide better insights into how I interpret the world."

"Every person with autism is different"

** Oliver's real name has been changed for privacy reasons.*

3rd Utrecht Brain Conference 2019

Utrecht, The Netherlands
December 10th, 2019

Oxana Garritsen

December 10th 2019. Not even two weeks into my internship, and I was already invited to my first real neuroscience conference: The Utrecht Brain Conference 2019! Excited! Because this conference is organized by the Utrecht Brain Centre, all interns and employees of the department of Translational Neuroscience gathered early in the morning in the coulis of the University library at Utrecht Science Park. After the check-in and a delightful cup of coffee, we were all ready to start with the first talks in the Boothzaal of the library.

The day started with two introductory talks of Prof. dr. Elly Hol and dr. Tom Snijders, the ones responsible for organizing this day full of neuroscience-related talks. It became clear that the day consisted of two main topics. The bigger part of the morning mainly focused on the development of new techniques within the field of neuroscience to facilitate research into brain diseases. Delegates from different companies and laboratories were present to introduce other scientists to new methods, including organoids, microfluid chambers for drug screening and organs-on-a-chip. Looking back on this day, I really enjoyed the morning programme, since I am always intrigued by innovation in research, and new techniques, which we can incorporate into our own research practices in order to reduce, as an example, the number of animals used in our experiments. Besides, it allowed researchers from the industry and academia to meet with the aim to foster possible collaborations, and unite the Neuroscience community in Utrecht.

During the remainder of the day, we welcomed the two Keynote lecturers and several other speakers, who all dealt with the second main topic of today: research into glioblastoma. As a student who is not really into the field of neuro-oncology, I gathered a lot of knowledge on the fundamental processes underlying glioma, as well as possible treatments and interventions.

The highlights of the day for me personally were the poster presentations of the PhD students during the long lunch break. This gave me, an intern completely new to the field, the opportunity to talk with researchers, speakers and other people from the audience in an informal setting while exchanging thoughts and insights in our field. Since there were people present from both academia and industry, I was able to join several discussions on the differences between the two worlds, and how these worlds meet in order to answer questions that are of great importance nowadays. The combination of multiple fields together at one conference leads to the benefit of obtaining a broader view and gaining new insights, which made it all together very interesting. And last but not least, the poster presentations were a great source of inspiration for our own posters we have to make for our very own Mind the Brain Symposium in May 2020!

All in all, the Utrecht Brain Conference was an informative and inspiring way to get an overview of current neuroscientific research performed within Utrecht University and its associated partners. Moreover, because of its relatively small scale, this conference could be seen as a perfect first introduction to a conference, of which probably a lot will follow.

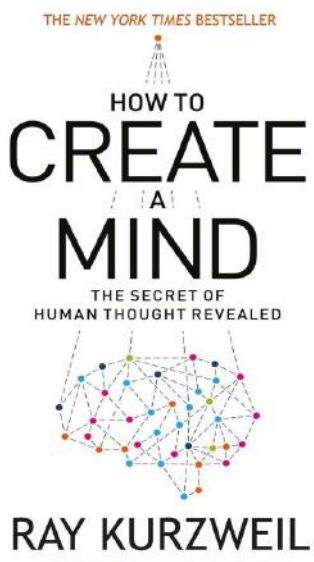
How to create a mind

Ray Kurzweil

Sara-Zohra Arrouf

What can an engineer teach a neuroscientist? Maybe how to create a mind. "Artificial intelligence will reach human levels by around 2029", as said by Ray Kurzweil. The author of *The age of spiritual machines* brings with *How to create a mind* a plan to reverse-engineer the human mind and takes our neuroscience knowledge to the next level: creating a mind. The number of laboratories dedicated to the brain increased tremendously over the years and more precise theories of how utterly specific parts of the human brain can produce utterly specific behaviors were generated, mainly in sterile and empty environments of laboratories. Ray Kurzweil offers a fresh new point of view with his Pattern Recognition Theory of Mind, which argues that pattern recognizers are the core structures of the neocortex and the human thoughts. The book is a journey with thought experiments, mathematical models of pattern recognizers, development of similarities between the most famous artificial intelligences over the near history and our daily way of thinking. Finally, it offers predictions about the wonders hidden in the future of computational

"It offers predictions about the wonders hidden in the future of computational neuroscience"



Ray Kurzweil tells us his experience on how to mimic brain's abilities, such as recognizing unsegmented natural speech, and how these computations have given birth to artificial intelligence available in all smartphones over the world. Based on their similar performances, he also underlines the necessary similarities between mathematics used in AI and firing patterns of cortical regions.

Even though some neuroscience aspects described could seem a little shallow (a neuroscience enthusiast would probably want him to go deeper in his reasoning and an expert will probably find many ways to argue against a global model), Ray Kurzweil has proposed his unifying theory in a brilliant way. His theory will provide you with fresh insights about studying the brain and *How to create a mind* will probably make you think longer about how this little smartphone in your pocket can unveil the key to a new approach on how to understand a mind.

Interested? Read it yourself: How to create a mind, Ray Kurzweil. ISBN: 978-0670025299

Behind the Scenes

Mind the Brain Committee 2020

Solée Pop

The organisation of the symposium was a challenging but rewarding task. Each committee member had her own responsibilities during the preparation and we updated each other during weekly meetings. We started in November 2019 and one of the first things we had to decide on was the theme of the symposium. Through several brainstorming sessions and discussions about our ideas, we selected the best-fitting-theme for both tracks of the Neuroscience and Cognition Master: 'Eat Your Brain Out'. The theme focuses on the interaction between food, microbiota and the brain, and their impact on mental wellbeing and brain disease.

There was a lot involved in organising the symposium: booking the venue and catering, preparing the program, inviting keynote speakers, arranging workshops, contacting students, collecting sponsors, making a website, promoting the symposium and revealing the theme. Unfortunately, due to the COVID-19 pandemic, we were forced to change the physical format of the event. Nonetheless, we became very creative and organised an online version. So a new aspect became part of the organisation - how to make a live online symposium happen?

We still think that it is useful to have a poster session in real life. This event will take place in September 2020, during the

Fundamentals of Neuroscience course of the new Neuroscience and Cognition Master students.

Although we faced some obstacles during the organisation of this scientific event, we learned to work together as a team, we broadened our network and, in the end, it was very rewarding and a lot of fun!

On behalf of the Mind the Brain committee 2020,

Solée Pop
Sponsoring/Promotion

Committee members:

Emma Eeltink – *Chair*
Annabel Timmers – *Secretary*
Sofie van Logtestijn – *Treasurer*
Simone Visser – *Logistics*
Anouschka van Dijk – *Logistics/Webmaster*
Jeske Hoogeboom – *Promotion*
Sara-Zohra Arrouf – *Sponsoring/Webmaster*
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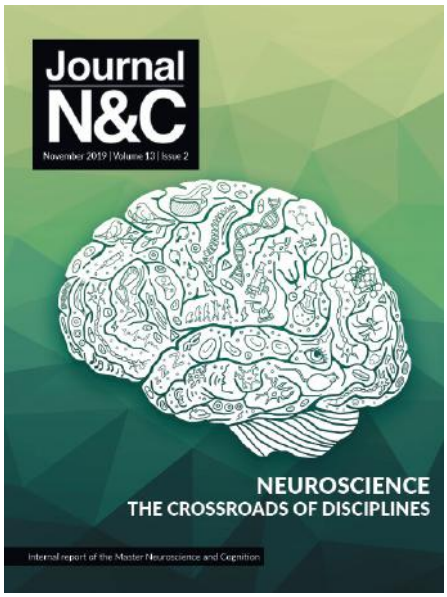
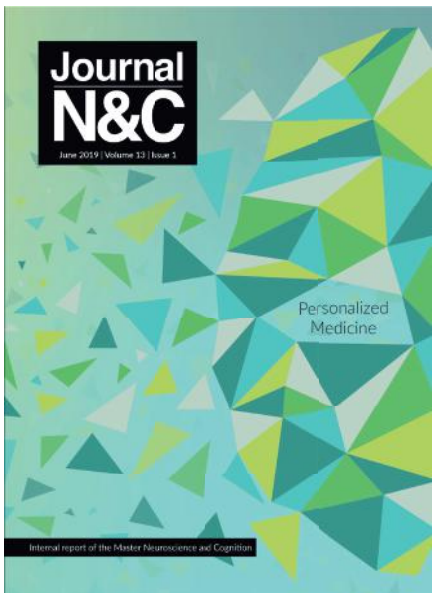
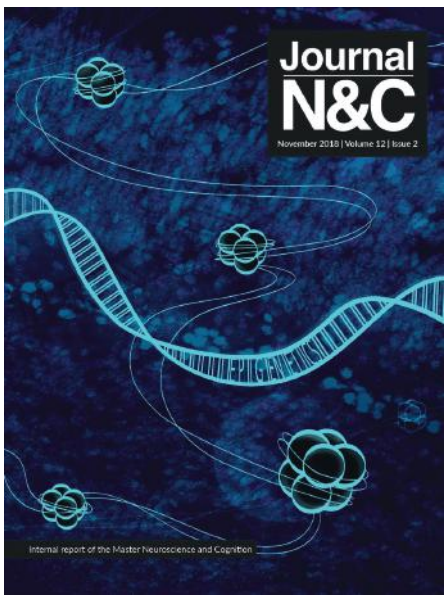
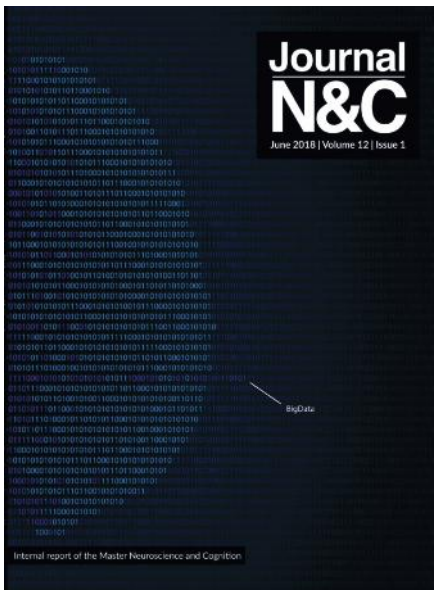
** Oliver's real name has been changed for privacy reasons.*

Contribute to the next issue of the Journal of N&C!









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