Emotional Valence of "Voices" in Schizophrenia: Investigating the Role of Glutamate in Auditory Verbal Hallucinations

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Abstract

Background: Recent research seems to point towards a prominent role of glutamate in auditory verbal hallucinations in schizophrenia, with complex inter-related pathophysiological processes and mechanisms involving hyper- and hypo-activity at several fronto-temporal brain regions. However, there seems to be significant variability within and between such mechanisms, clinical responsiveness to pharmacological intervention and phenomenological aspects of hallucinations. It has been suggested that inter-individual variability in symptoms (i.e. phenomenological aspects of hallucinations) could infer differences in underlying psychopathology, however, this remains to be tested. This thesis therefore seeks to investigate emotional valence of auditory verbal hallucinations in schizophrenia and the extent to which they relate to glutamate and response to (dopaminergic) antipsychotic medications. **Methods:** This project is based on an existing dataset containing Glx (glutamate+glutamine) estimations from four fronto-temporal brain regions obtained using magnetic resonance spectroscopy, recorded from 40 schizophrenia patients with auditory verbal hallucinations. Clinical responsiveness was measured with the Positive and Negative Symptoms Scale, and emotional valence was measured using the Revised Beliefs about Voices Questionnaire. **Results:** There was significant correlations for Glx levels and emotional valence across all regions. Positive emotional valence was found positively correlated with Glx levels, whereas a negative correlation was found for negative emotional valence. There was no significant correlations between clinical responsiveness and emotional valence. **Discussion and conclusion:** Results suggest that glutamate may have an important role in the emotional valence of “voices” in schizophrenia. It can be speculated that positive and negative emotional valence of “voices” reflect sub-groups with different underlying glutamatergic psychopathology.

Keywords: auditory verbal hallucinations; schizophrenia; glutamate; spectroscopy; emotional valence

Sammendrag

Bakgrunn: Nyere forskning viser til en viktig rolle for glutamat i auditive verbale hallusinasjoner i schizofreni som involverer et komplekst patofysiologisk samspill med hyper- og hypo-aktivering i flere fronto-temporale hjerneområder. Samtidig tyder forskningen på høy grad av variabilitet innen og mellom slike mekanismer, klinisk medikament respons og fenomenologiske aspekter av hallusinasjoner. Det har blitt foreslått at interindividuell variabilitet i symptomer (dvs. fenomenologiske aspekter av hallusinasjoner) kan tyde på forskjeller i underliggende psykopatologi. Dette har dog. ikke tidligere blitt testet. Denne oppgaven sikter dermed på å undersøke hvordan og i hvilken grad den emosjonelle valensen til auditive verbale hallusinasjoner i schizofreni relaterer til glutamat og respons til (dopaminerge) antipsykotiske medikamenter. **Metode:** Oppgaven baseres på eksisterende data med estimater av Glx (glutamat+glutamin) i fire fronto-temporale hjerneområder ved magnetisk resonans spektroskopi-vektet avbildning hentet fra 40 schizofreni pasienter med auditive verbale hallusinasjoner. Klinisk respons ble målt med Positive and Negative Symptoms Scale. Emosjonell valens ble målt med Revised Beliefs about Voices Questionnaire. **Resultater:** Det ble funnet signifikante korrelasjoner mellom nivå av Glx og emosjonell valens på tvers av alle hjerneområder. Positiv emosjonell valens var positivt korrelert med nivåer av Glx, mens en negativ korrelasjon ble funnet for negativ emosjonell valens. Det ble ikke funnet signifikante korrelasjoner for medikament respons over tid og emosjonell valens. **Diskusjon og konklusjon:** Resultatene peker mot en viktig rolle for glutamat i den emosjonelle valensen til "stemmer" i schizofreni. Fra resultatene kan det spekuleres at positiv og negativ emosjonell valens i "stemmer" reflekterer sub-grupper med ulik underliggende glutamatergisk psykopatologi.

Nøkkelord: auditive verbale hallusinasjoner; schizofreni; glutamat; spektroskopi; emosjonell valens

Preface and Acknowledgements

The fields of behavioural and cognitive neuroscience sparked huge interest in me when first reading introductory books on psychological research. This interest was further facilitated by family members working as researchers and clinicians within these fields and as such, by their work, became inspired and amazed at the moldability and plasticity of the brain. After several unsuccessful inquiries to researchers and employees at the University of Bergen, a request to assist in interpreting findings from a study done as part of the ERC2-project on auditory verbal hallucinations in schizophrenia was received from post.doc. Helene Hjelmervik. As such, this master's thesis came about by a coincidence. Luckily, with reading on the topic in general and findings from the study specifically, inspiration and motivation emerged to dedicate the final two semesters of study into this subject.

However, this was undoubtedly facilitated and maintained by the expertise and helpfulness of Helene Hjelmervik in supervising throughout working on this thesis, helping to navigate among the vast literature on the relevant subjects and providing input on methodology and statistics. Together with the encouragement and insight of co-supervisors post.doc. Josef Johann Bless and prof. Kenneth Hugdahl, this input has been invaluable. For this, you have my deepest thanks.

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Literature Review

Schizophrenia from Multiple Perspectives: An Introduction

Schizophrenia is a severe mental disorder that is characterized by both positive and negative symptoms, with the former representing psychotic experiences such as hallucinations and delusions, and the latter as cognitive and behavioural abnormalities such as impaired cognitive ability, amotivation and social withdrawal (Howes, McCutcheon & Stone, 2015). It is associated with lifelong social handicaps and distress which certainly contribute to the fact that schizophrenia is recognized as one of the leading causes of adult disease burden and also one of the most costly mental disorders (Gustavsson et al., 2011; Insel, 2010). The manifestation of the disorder typically presents at early adulthood or late adolescence in the form of auditory hallucinations and paranoid delusions (Insel, 2010). It has a relatively high heritability of around 80% with a 12-fold increase in risk of development in first-degree relatives compared to the general population (Harrison & Owen, 2003). To date, the current body of knowledge on schizophrenia and its associated symptoms is derived from decades of investigations into the many aspects of the disorder, including its etiology, pathology, phenotype and treatment. From this, it is known that schizophrenia involves genetic and epigenetic factors, widespread and variable functional, structural and neurochemical brain alterations, persistent negative symptoms and episodic positive symptoms, and a relatively high treatment-response to antipsychotic medication blocking dopamine D2-receptors, respectively (Keshavan, Nasrallah & Tandon, 2011).

The pathological, etiological and clinical heterogeneity of schizophrenia presents a major challenge to the understanding and treatment of the disorder. Considering this latter aspect, although there is a relatively high treatment-response to commonly used antipsychotic (dopaminergic) medications, around 33% of the overall patient group do not respond or only have a limited response to repeated treatment with such medications (Mouchlianitis et al., 2016). Likewise, although auditory verbal hallucinations (AVH) represents one of the most salient positive symptoms involved in schizophrenia – having been reported for over 70% of patients (Hugdahl et al., 2008a) – many do not experience them, and may or may not experience hallucinations in other sensory modalities. Taken together, such heterogeneity (e.g. in medication response and AVH) has been suggested to imply the existence of various sub-types of schizophrenia – of which underlying pathophysiological factors differ (e.g. Geisler et al., 2015; Sommer, Kleijer & Hugdahl, 2018).

Because the most common antipsychotic medications primarily affect dopamine functioning, it may be that non-responders have their underlying deficits in other systems (e.g. glutamate). Likewise, because a specific symptom (e.g. AVH) may vary between patients in form and content (e.g. benevolent “voices” vs. malevolent “voices”), different underlying pathophysiological factors

may contribute to this variance. If the two examples above reflect two different sub-types of schizophrenia, a central question then concerns whether there exists a relationship between them. Given that a specific symptom (e.g. AVH sub-types) may inform about the effect of treatment (e.g. dopaminergic antipsychotic medication) on the disorder as a category, it is necessary to gain a better understanding of the neurobiological underpinnings of both the individual specific symptom and the disorder - conceptualized as the sum of the manifested symptoms (Sommer et al., 2018). Hence, by focusing on one such aspect, it may be possible to shed light on similarities and differences between sub-types, thereby contributing to the disentanglement of the heterogeneity that characterizes the disorder. To this end, this project aims to investigate the underlying neurochemical basis (i.e. glutamate vs. dopamine (medication response)) of the phenomenological aspects of a specific symptom (i.e. AVH) often associated with schizophrenia.

Auditory verbal hallucinations. In general, hallucinations refer to perceptions that do not have any corresponding sources in the external environment (Jardri, Pouchet, Pins & Thomas, 2011; Kompus, Westerhausen & Hugdahl, 2011). As such, AVHs would refer to the perception of "hearing a voice" without the presence of an actual external source. In some cases, this could be dangerous if the hallucinations take a form that is perceived as commanding the patient to commit violent behaviours (Hugdahl, 2009). Although violent behaviours are evident in a substantial proportion of patients with first-episode psychosis, severe violence directed towards others is rare (Large & Nielssen, 2011). Given the association between auditory hallucinations and other positive symptoms in schizophrenia, such as delusions (e.g. Hugdahl et al., 2008b), this risk appears increasingly relevant for this group. However, it is important to note that AVHs can also occur in healthy non-patients and does not necessarily reflect prodromal signs of schizophrenia, psychosis or other psychopathologies (Beavan, Read & Cartwright, 2011). In fact, approximately 5-10% of the non-clinical population experience AVHs at some point during their lifetime (Ćurčić-Blake et al., 2017a). Moreover, the content of AVHs can consist of positive and encouraging "comments" that is not perceived as commanding or negative by the patient (Jones, 2010). Still, there seems to be a general tendency towards the form and content of AVHs (e.g. loudness, loci, personification, and number of "voices") to be remarkably similar across patients (Daalman et al., 2011). However, contrary to non-patients who experience AVHs, psychotic patients tend to perceive AVHs with more negative emotional valence. In this study, Daalman et al., (2011) also found differences between patients and non-patients in frequency, age of onset and the amount of control subjects believed they had over their AVHs. Furthermore, what best predicted functioning was the response to AVHs by the patients, based on the perceived emotional valence of the AVHs. Thus, understanding why and how patients perceive and react to AVHs becomes highly important for successful treatment and

prevention, while also minimizing the potential of harm to the patient, other people and the larger society. Another important reason to focus on this specific symptom lies in the fact that antipsychotic medication tend to induce a rapid decline in hallucination severity (Sommer et al., 2012), making it an ideal target to investigate whether the heterogeneity of symptoms is a result of different underlying neurochemical pathologies (e.g. dopaminergic vs. glutamatergic) which, according to the same authors, may indicate sub-types of schizophrenia.

Brain abnormalities in AVHs. Several studies have shown that AVHs in schizophrenia are related to alterations in brain activity and connectivity (Jardri et al., 2011), pertaining to language, auditory and memory networks (Allen et al., 2012). Structural alterations are also evident and through the use of structural imaging techniques such as voxel-based morphometry (VBM) and region of interest (ROI) studies, an association has been shown to exist between gray matter (GM) volume reductions in temporal regions such as the superior temporal gyri (STG) and auditory hallucinations (Allen, Larøi, McGuire & Aleman, 2008; Neckelmann et al., 2006). In another quantitative meta-analysis of nine VBM studies, Modinos and colleagues in 2013 found that the GM reduction in bilateral STG observed in patients with AVH significantly correlated with symptom severity. Although such findings appear to have intuitive face value, caution should be advised when interpreting results from structural neuroimaging studies, as it is only possible to infer (i.e. speculate) functional associations from such data (Allen et al., 2012). That said, the association between GM volume reductions and auditory hallucinations is also to some extent evident in non-temporal and non-sensory regions such as the anterior and posterior cingulate cortex, insula, precuneus, cerebellum, thalamus and inferior frontal gyri (IFG), which all show significant effects of auditory hallucinations in VBM studies (Allen et al., 2008).

However, considering such studies together with evidence from functional imaging studies seem to indicate a more consistent pattern of abnormalities in auditory and language-related brain networks in schizophrenia patients that experience AVHs (Jardri et al., 2011). Activation of posterior parts of the left STG (peri-Sylvian region) is one of the most consistent findings when patients are experiencing AVHs during scanning (Allen et al., 2008; Jardri et al., 2011; Hugdahl & Sommer, 2018). These areas are collectively known as Wernicke's area (secondary auditory cortex) and represent the classic speech perception areas of the brain. Such studies of the hallucinating brain represent state-studies, as opposed to trait-studies, which is based on reports of hallucinations experienced in the past (Allen et al., 2012). The primary auditory cortex, as opposed to the secondary, has been argued to not be related to AVH emergence, but rather to phenomenological or clinical features of the AVH experience such as vividness (Allen et al., 2012). From the coordinate-based meta-analysis of Jardri and colleagues in 2011 consisting of both functional magnetic

resonance imaging (fMRI) and positron emission tomography (PET) state-studies, estimations of activation likelihoods pointed towards an increase in a more extended bilateral network encompassing fronto-temporal regions such as the anterior insula, precentral gyrus, Broca's area, STG, frontal operculum, para-hippocampal cortex, hippocampus and the inferior parietal lobule. Hence, when subjects experience AVHs, there is overall strong support for consistent activity in the bilateral STG (R-STG; L-STG) across studies. Moreover, measurements were found to be indicative of neuronal hyper-activity within both the left and right IFG (L-IFG; R-IFG) and L-STG. It should be noted however that for the IFG specifically, activity is more consistently reported to be lateralized to the left hemisphere across studies (Jardri et al., 2011). Generally, the L-STG and R-STG seem to be involved in the semantic and phonological characteristics of speech perception, and the prosodic and emotional aspects of speech processing, respectively (Allen et al., 2012). Moreover, the bilateral STG and the L-IFG have all been shown to exhibit similar patterns of activation during auditory verbal perception and AVHs (Hugdahl, 2009; Lennox, Park, Medley, Morris & Jones, 2000). In part, this is why AVHs could be considered perceptual phenomena (Hugdahl et al., 2008a). However, as will become apparent in later sections, phenomenological features of the AVH experience such as emotional valence is based more on cognitive appraisals and not perceptual processes per se. Thus, for clarification purposes, throughout this thesis emotional valence, perceived emotional valence and appraised emotional valence is used interchangeably when referring to cognitive/affective appraisals of AVHs.

As previously noted, other key brain areas implicated in AVHs in schizophrenia is the inferior frontal areas (e.g. L-IFG, R-IFG), which is part of Broca's area and is particularly important for speech production (Jardri et al., 2011; Kompus et al., 2011; Sommer et al., 2008). In this latter state-study, an association was found between negative emotional valence of AVHs and levels of activation in predominantly right hemisphere language regions such as the IFG and STG. This was not found contralaterally, which may lend support to the role of right inferior frontal areas in processing or contributing to the (negative) emotional content in AVHs. In this study they also found normal lateralization to be absent when the patterns of activity in inferior frontal areas were compared to the patterns that characterize normal language production. Reduced or impaired language lateralization with AVHs in schizophrenia is a common but important finding as it may have theoretical and clinical implications and, as such, will be dealt with in the next section.

There has also been proposed an association between alterations in limbic regions important for emotional regulation, such as the amygdala, and auditory hallucinations (Allen et al., 2008), which is supported by studies. For example, one study showed that when schizophrenia patients are instructed within a task to listen to emotional sounds, there is a reduction in the activity of the bilateral hippocampus and amygdala in patients with AVHs compared to patients without AVHs

(Kang et al., 2009). Similarly, another study showed that when emotional auditory stimuli is presented to schizophrenia patients with AVHs, there is an increase in activity of the amygdala and para-hippocampal gyrus compared to schizophrenia patients without AVHs and controls (Escartí et al., 2010). However, despite such interesting associations with limbic structures, as previously noted, a more consistent pattern of abnormalities is found in auditory and language-related brain networks, and to sum, the findings in language and speech perception and processing regions of the temporal lobe together indicate neuronal hyper-activity related to the experience of AVHs in schizophrenia.

Considering the negative symptoms of patients however, which mainly relates to impaired cognitive ability, necessitates insight into other regions of the brain. The anterior cingulate cortex (ACC) and prefrontal areas (e.g. prefrontal cortex; PFC) are critical for cognitive control, and it is thought that abnormalities in these areas contribute to AVHs (Hugdahl, 2009). This is based on the general finding of reduced activity (i.e. hypo-activity) in these areas, as measured by functional neuroimaging methods (Minzenberg, Laird, Thelen, Carter & Glahn, 2009). There is also evidence for disrupted connectivity between temporo-parietal regions important for speech perception and frontal regions important for executive functions (Allen et al., 2012). Also supporting reduced activity in frontal areas are studies utilizing proton magnetic resonance spectroscopy (^{1}H-MRS), suggesting hypofunction and high levels of glutamate in frontal regions such as the ACC (Merritt, Egerton, Kempton, Taylor & McGuire, 2016). Thus, in contrast to what is observed in temporal areas related to speech and language processing, frontal areas such as those mentioned above tend to consistently demonstrate hypo-activity related to the experience of AVHs in schizophrenia.

An important question raised regarding the observed (hyper) activity in bilateral STG concerns whether the activity originate from within the STG, and whether this is what initially triggers and drives the experience of AVHs (Ćurčić-Blake et al., 2017b). According to a two-fold model proposed by Hugdahl in 2009, AVHs in schizophrenia have their origin from neuronal hyper-activity in the aforementioned speech perception regions – driving the phenomenon in a bottom-up way. Although later sections will deal with this model in more detail, it should be mentioned that it relates to an excitatory/inhibitory imbalance between brain regions critical for language and cognitive control (Hjelmervik et al., 2019). The role of glutamate is an important one, as dysfunctions within its system have been shown to contribute to the pathogenesis of schizophrenia and associated symptoms and cognitive deficits (Egerton & Stone, 2012), and will be further considered in the following sections. To sum, decreased activity in frontal regions critical for executive control processes, together with increased activity in temporal regions critical for language processes, represent current and consistent findings within the literature and also acts as the foundation for many models seeking to explain AVHs in schizophrenia.

Deficits in language lateralization. A method of empirically testing if AVH relates to abnormalities in language lateralization and speech perception is by a dichotic listening paradigm. Here, external auditory stimuli, typically in the form of pair-wise consonant-vowel (CV) syllables, are presented simultaneously via headphones to both ears, after which the participant is required to report that which they best identified (Hugdahl, Løberg & Nygård, 2009). By comparing the relative percentage of correct reports for the syllables presented to the left and right ear across trials, deficits in left temporal lobe speech processing areas can be assessed in auditory hallucinations. Because there is a general tendency to report speech sounds presented to the right ear – the so-called right ear advantage (REA) - it is possible to infer lateralization to temporal regions in the left hemisphere (Hugdahl, 2009). This is because neuronal signals related to external speech sounds presented to the right ear directly follow to the dominant speech perception area in the left temporal lobe and therefore are perceived prior to the left ear speech sounds that go via the right hemisphere (Kimura, 2011). Failing to show REA would then be indicative of impairment or functional deficits in the left temporal lobe. Thus, demonstrated REA is expected in healthy individuals given perceptual processing of speech sounds in left temporal lobe areas and the phonological features of the presented syllables (Hugdahl et al., 2009). However, as reported by Hugdahl (2009), schizophrenic patients tend to demonstrate a reduced REA. Furthermore, this effect seems to be stronger for patients with predominantly positive versus negative symptoms, perhaps reflecting decreased capacity to process external sounds presented to the right ear as a function of AVHs competing for limited neuronal resources. In other words, the increased activation in temporal lobe speech processing areas observed in many functional imaging studies despite the absence of external stimuli during AVH, may be caused by internally and spontaneously generated abnormal neuronal activity. This activity in turn, through neuronal refractory processes, may lead to an inability to adequately respond to externally generated stimuli (Hugdahl et al., 2009). Although it is possible that auditory hallucinations experienced during testing may distract the participant and through this may skew the results in a direction indicating reduced language dominance of the left hemisphere (Ocklenburg, Westerhausen, Hirnstein & Hugdahl, 2013), a more likely explanation can be derived from the meta-analysis of Modinos and colleagues in 2013 on the neuroanatomy of AVH in schizophrenia, in that the severity of those hallucinations significantly correlate with GM volume reductions in L-STG. Two meta-analyses investigating language lateralization in schizophrenia patients in general and in schizophrenia patients with auditory hallucinations specifically, indicated that reduced language lateralization may be a weak and strong trait marker for the former and latter, respectively (Ocklenburg et al., 2013). Thus, there seems to be a clear association between schizophrenia and reduced language dominance of the left hemisphere. Moreover, this reduction is

significantly larger for schizophrenia patients that experience auditory hallucinations. Hence, degree of language lateralization in schizophrenia patients may be mediated by whether they experience auditory hallucinations. However, as noted by the authors themselves, this only applies within the context of psychosis.

Another meta-analysis of twelve neuroimaging state studies investigating AVH in schizophrenia with and without external auditory stimulation found increased activation in the right rostral PFC and left primary auditory cortex during AVH without external auditory stimulation (Kompus et al., 2011). Together with the findings from another dichotic listening study showing a reduction in REA as a function of increased frequency of auditory hallucinations, this lends support to models explaining such hallucinations as internally generated speech misrepresentations with an anchor in classic speech processing areas (Hugdahl et al., 2008b). The apparent paradox identified through the meta-analysis of Kompus and colleagues in 2011 – in that both deactivation and activation relating to AVHs in the same areas of the auditory cortex is observed depending on the presence of an external auditory stimulus – is suggested to occur as a consequence of an attentional bias towards the internally generated speech misrepresentations together with a failure of down-regulation of the default mode network and up-regulation of auditory processing networks. As a final consequence, spontaneous activation in the latter network, not elicited by an external stimulus, severely limits the processing capacity of the perceptual apparatus (Kompus et al., 2011). Finally, it appears that a central role for the reduced REA observed in many dichotic listening studies may be associated to glutamate deficits serving as a mediating factor (Hugdahl et al., 2008a). Summed, these findings present reasonable explanations for why decreased lateralization is observed in schizophrenia patients experiencing AVH, thereby having implications for models attempting to elucidate the relationship between the distinguishing features of such hallucinations in schizophrenia and their underlying pathophysiological factors.

Models of Schizophrenia and AVHs

The role of glutamate. The amino acid glutamate is highly abundant in the human brain and serve many important functions – as it is responsible for most excitatory neurotransmission (Howes et al., 2015), and also is the direct precursor to one of the most inhibitory neurotransmitters found in the brain; γ-aminobutyric acid (GABA) (Bak, Schousboe & Waagepetersen, 2006). Although it is an amino acid, glutamate can only be considered a neurotransmitter if it is properly packaged in vesicles in axon terminals (Kolb, Whishaw & Teskey, 2016, p.150). All cortical efferents together with most cortical afferents and cortico-cortical connections in the human brain are glutamatergic (Moghaddam & Javitt, 2012). Deficits in glutamatergic functioning therefore presents challenges to discovering just where and how they contribute to the symptoms seen in schizophrenia – as they are

widely distributed through cortical and subcortical regions. Glutamate has both ionotropic and metabotropic receptors, which are subdivided into groups which modulate neurotransmission both pre- and post-synaptically (Howes et al., 2015). According to the glutamate hypothesis of schizophrenia there is a dysfunction in the ionotropic receptors for glutamate that mainly contributes to the disorder. The development of symptoms observed in schizophrenia, particularly the negative symptoms such as cognitive dysfunction, is according to the glutamate hypothesis, a function of hypoactivity at one such type of ionotropic receptor; namely at N-methyl-D-aspartate (NMDA)-type receptors (NMDAR) leading to an imbalance between excitatory and inhibitory signals in the cortex, especially in the dorsolateral PFC, through its effect on GABA interneurons (Coughlin et al., 2015). However, before further considering the glutamate hypothesis in more detail, lets first consider how and by what inter-related processes glutamatergic neurotransmission takes place.

Starting from the packaged vesicles containing glutamate located in axon terminals, when triggered by an action potential it is released into the synaptic cleft and from there mainly binds to NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors on the post-synaptic membrane (Kolb et al., 2016, p. 501). One function of AMPA receptors is to mediate the response to glutamate when it is released from the vesicles embedded in the pre-synaptic membrane because NMDAR channels are normally blocked by magnesium ions. Thus, weak electrical stimulation only activate AMPA receptors, whereas stronger stimulation sufficiently depolarize the post-synaptic membrane over its threshold for responding, which causes displacement of the magnesium ions and subsequently opens gated ion channels on NMDAR, allowing the influx of calcium ions (Kolb et al., 2016, p. 501). Note that this is not to say that other receptors, sites and processes are not involved. For example, glutamate will not be able to open channels in NMDAR units without the presence of glycine at its modulatory site (Coyle, Tsai & Goff, 2003). Coincidentally, the general process described above is also what underlies many aspects of memory and learning by long term potentiation (Kolb et al., 2016, p. 198). However, although the exact mechanisms are not fully known, it also contributes to the highly neurotoxic effects of glutamate (Plitman et al., 2014). Coined as early as in 1969 by Olney, the term excito-toxicity refers to this process - and is thought to occur through excessive NMDAR stimulation, as the calcium influx through specific genes, activated by second messengers, lead to apoptosis (Kolb et al., 2016, p. 198). Glutamate-mediated excito-toxicity has been hypothesized to contribute to the neuroanatomical abnormalities often observed in schizophrenia such as GM reductions and cortical thinning (Plitman et al., 2014). The neurotoxic effects of glutamate are well-established by early animal studies (e.g. Olney, 1969) and it is generally widely accepted by most current scientific fields that excessive stimulation by glutamate exert neurotoxic effects leading to apoptosis (Plitman

et al., 2014). Thus, in order to avoid these detrimental effects, critical mechanisms constantly need to maintain glutamatergic homeostasis within these systems. This is thought to occur through a complex glutamate/GABA-glutamine cycle (Bak et al., 2006). In essence, glutamate released into a synapse is subsequently taken up by surrounding astrocytes and synthesized into glutamine – the main metabolite of glutamate (Plitman et al., 2014) - by a specific enzyme, after which glutamine is returned to the neuron wherein it is re-transformed into glutamate by a process of phosphate-activated glutaminase. Similarly, GABA is taken up by surrounding astrocytes (and presynaptic terminal) and through several metabolic steps is converted to, among others, glutamate, which then follows similar mechanisms as the above mentioned glutamate-glutamine cycle, but differs by finally converting the glutamate in the GABA-neuron to GABA via glutamate decarboxylase (Bak et al., 2006). Thus, although the above description of the glutamate/GABA-glutamine cycle is highly simplified and lack neuro-molecular specificity, it serves as an example of the complex inter-related processes involved in the role of glutamate and excitatory/inhibitory neurotransmission.

Returning to the glutamate hypothesis, the role of glutamate is furthermore and originally supported by evidence showing that even low doses of NMDAR antagonists, such as ketamine, tend to produce the kind of cognitive dysfunctions common in schizophrenia, such as memory and attentional problems, when administered to healthy non-patients (Coyle et al., 2003; Insel, 2010). In contrast to positive symptoms, which may be induced and exacerbated by administration of amphetamines, cognitive impairments and negative symptoms seen in schizophrenia are more commonly and strongly affected by administration of dissociative anesthetics such as phencyclidine (PCP) and ketamine (Coyle et al., 2003). However, that is not to say that positive symptoms such as hallucinations is not affected, as there is clear evidence for an exacerbation of such symptoms when administered to patients with schizophrenia as well as for inducing such symptoms in healthy non-patients (Lahti, Weiler, Tamara, Parwani & Tamminga, 2001). These agents are use-dependant and non-competitive antagonists that act by binding to sites within the voltage-dependant channels of NMDAR units (Coyle et al., 2003). The observation of induced and exacerbated symptoms in response to such agents were made several decades ago and resulted in the postulation of the glutamate hypothesis of schizophrenia (Merritt, McGuire & Egerton, 2013). However, the findings and observations that neurophysiological alterations associated with schizophrenia, along with symptoms and cognitive deficits, can be induced by NMDAR units does not provide any account for how they arise in the first place and how they should be treated to obtain the best possible outcome (Moghaddam & Javitt, 2012). For example, given the vast amount of established empirical evidence for NMDAR dysfunction in schizophrenia it would seem only logical to aim the target of treatment to restoring function at NMDAR units themselves. However, as there are more uncertainties related to causes of NMDAR dysfunction and to the processes and mechanisms that

leads from this dysfunction to cognitive impairments and psychosis, it may not be reasonable to focus directly on correcting or normalizing function at such units. Modulation of the glycine modulatory site on NMDAR also have been reported to enhance some cognitive symptoms involved in schizophrenia, further lending support to the role of glutamate in the disorder (Insel, 2010). As previously mentioned, the glycine modulatory site on NMDAR units must be occupied in order for glutamate to be able to open the channels (Coyle et al., 2003). GlyT1 is a glycine transporter that is exclusively expressed in astrocytes. Similarly, some transporters for glutamate (e.g. excitatory amino acid transporter; EAAT1 and EAAT2), which serve significant neuroprotective functions relating to the excito-toxicity of glutamate, are also expressed exclusively in astrocytes. According to the authors, astroglia therefore serve an important role in modulating glutamatergic neurotransmission (Coyle et al., 2003). In other words, optimal NMDAR functioning is dependent upon optimal regulation of glycine availability at the modulatory sites. Furthermore, the same authors argue that in some patients with schizophrenia the observed NMDAR hypofunction may be accounted for by increased levels of endogenous antagonists (e.g. kynurenic acid) at the glycine modulatory site on NMDAR units or at other metabotropic (e.g. mGluR3) glutamate binding sites. This may again have clinical implications for the development of pharmacological agents that act on these sites, which again highlight the need for a better understanding of the glutamatergic system in general and in schizophrenia and AVHs specifically. Finally, some recent studies have shown that the severity of AVHs is associated with levels of glutamate in L-STG, L-IFG and ACC (Ćurčić-Blake et al., 2017b; Hjelmervik et al., 2019; Hugdahl et al., 2015). These studies will be further considered in following sections.

Estimating levels of glutamate *in vivo* with ^{1}H-MRS. ^{1}H-MRS is an imaging technique that can be used to estimate and quantify the relative amounts of different metabolites in different parts of the brain in vivo, utilizing the magnetic properties of hydrogen protons (van der Graaf, 2010). This is possible because the resonant frequency of the hydrogen proton is affected by the environment to which it is attached, allowing the identification of different molecules based on their inherent magnetic properties. That is, nuclei within molecules have different chemical shifts, which means that they absorb energy at different magnetic frequencies, allowing discrimination between metabolites based on the difference between their inherent magnetic properties and the magnetic field introduced by the scanner (Juchem & Rothman, 2014, p.5). In vivo, such chemical shifts are expressed as parts per million (ppm.) and are usually internally referenced to standards such as N-acetylaspartate (NAA). Thus, the signal represents the difference in energy between nuclei and the induced magnetic field. In this way, based on different chemical compositions, the observed signal from the metabolites results from the spectral localization between water and lipid peaks in the

MRS spectrum, in which the signal decrease as a function of its localization closer to the latter compounds (Hugdahl et al., 2015). The development and refinement of this technique has allowed a unique approach and provided valuable insight into the neurochemistry involved in many disorders. In developing and refining MRS techniques, future clinicians and researchers may be able to assess treatment response to novel and putative (glutamatergic) medications based on studies demonstrating changes in glutamatergic indices post-treatment (Poels et al., 2014). In other words, MRS may in the future help demonstrate to what extent experimental pharmacological (glutamatergic) agents actually engage the glutamate system and thereby consequently aid in identifying sub-groups of patients that have a relatively large beneficial effect of those agents. Furthermore, given the link between genetics and glutamatergic neurotransmission in schizophrenia, the authors argue that MRS as a method could be used to link glutamatergic dysfunction to changes in specific genetic risk factors. In return, this could help elucidate the large heterogeneity that characterizes the disorder, associated symptoms, clinical responsiveness and results from functional and neurochemical MRS studies.

There are, however, some caveats to the method that is important to be aware of. First, it can not differentiate between extra- and intracellular components and thus may reflect the extracellular levels of metabolites, intracellular levels, or both (Howes et al., 2015). Hence, it becomes difficult, if not impossible, to discern whether observed metabolite levels are due to increased post-synaptic signalling or pre-synaptic firing (Ćurčić-Blake et al., 2017b). Second, the estimates are not specific to neuronal levels of the metabolite (Merritt et al., 2013). As such, it is not possible to infer altered neurotransmission of a given metabolite such as glutamate based on changes in concentration, because the observed change could be masked by other unrelated metabolic processes. Third, when using scanners with a standard available field-strength of 3 Tesla, it becomes difficult to accurately differentiate between the relative levels of glutamate and glutamine because their metabolite peaks in the spectrum may overlap (Rae, 2014). In order to avoid this problem, a composite variable (Glx) is created, reflecting the summed glutamate and glutamine levels. The rationale for this is often based on two principal findings. First, synaptic glutamate is taken up by astrocytes and is then converted into glutamine (Bak et al., 2006). Glutamine could therefore be viewed as a marker for glutamatergic activity. The second rationale is based on the findings of several studies that have shown that glutamate and glutamine are highly correlated in vitro in healthy tissue (Rae, 2014). Although the Glx (glutamate+glutamine) composite variable is typically what is measured in MRS investigations, for clarification purposes it should be noted that, given all of the above, within and throughout this thesis “glutamate” and “Glx” will be used interchangeably to refer to Glx when discussing results from MRS studies in general and from the analyses of this thesis specifically.

Finally, an important consideration that must be accounted for when using MRS to measure

levels of glutamate, specifically in the ACC (Srinivasan et al., 2006), is that they may vary as a function of the relative amounts of white matter (WM) and GM within the voxel to be measured. In a more general sense, this could also be argued to be well as important in other brain areas, given that glutamate is highly concentrated intracellularly, whereas extracellularly, the concentration is comparatively low (Rae, 2014). Hence, there is more glutamate located in GM than in WM, which is supported by studies finding almost twice the amount (Bustillo et al., 2011). Such differences between concentrations in WM and GM are evident for most metabolites, although the direction and magnitude varies as a function of different sources of error, such as partial volume effects, different water concentration and relaxation times between different tissues (i.e. GM, WM, cerebrospinal fluid; CSF), as well as differences in metabolite relaxation times between tissues (Gasparovic et al., 2006). Given that anatomical differences exist in GM of the ACC between schizophrenia patients and healthy controls (Fornito, Yücel, Dean, Wood & Pantelis, 2009), in order to reduce the chance of confounding the measured glutamate levels by inter-individual comparisons, it is important to test for such differences within the selected voxel (Falkenberg et al., 2014). However, such inter-individual differences can be compensated for, thereby enabling a more precise Glx measure, by adjusting or scaling the Glx estimate to the amount of GM and WM within the voxel using an internal water reference (Gasparovic et al., 2006). As noted by the same authors, in contrast to using external concentration references, internal water references have the advantage of avoiding potential sources of error related to the former, such as coil loading and radiofrequency homogeneity, because the signals from water and metabolites are obtained in a similar manner and from the same voxel. However, its reliability is contingent upon accurate measures of volume fractions of GM, WM and CSF, as well as partial volume effects, because the concentration reference consists of the combined signal from the GM-WM fraction of the total water (wherein detectable metabolites are located – i.e. CSF contain no observable metabolites), whereas the combined GM-WM-CSF fractions (each weighted by different relaxation times) give rise to the observed water signal (Gasparovic et al., 2006). GM fraction (i.e. voxel proportion of GM/GM+WM) composed together with partial volume corrected concentrations of metabolites (e.g. Glx) in a scatterplot with corresponding regression lines, enable the comparison of the Glx concentration values to “pure” GM and WM values, thereby allowing detection of GM and WM group differences with excellent sensitivity (Bustillo et al., 2011). However, “pure” GM metabolite values may be biased by segmentation errors of, for instance, CSF fraction (Gasparovic et al., 2006) – providing an argument for also using voxel selection approaches (Bustillo et al., 2011). That is, due to variability in CSF fraction estimates by different image segmentation approaches accounting for partial volume effects, variability is also reflected in metabolite level estimates, especially in GM (Gasparovic et al., 2006). That said, accounting for all of the above, MRS as a method provides

a non-invasive sensitive measure and unique approach to neurochemical investigations in vivo that is arguably not matched by other currently available methods.

Glutamate as a mediating variable. Returning to the meta-analysis of Merritt et al., (2016), they found high levels of glutamate in medial-frontal areas such as ACC in individuals at high risk for schizophrenia but not in patients with chronic schizophrenia or in patients with a first-episode psychosis. In addition, high levels of glutamate were found in medial-temporal areas in patients with chronic schizophrenia but not in patients with first-episode psychosis or in individuals at high risk for schizophrenia. Other consistent findings related to levels of glutamate in AVH is generally lower levels in temporal and frontal areas important for speech perception, and there also seems to be a positive correlation between degree of hallucinations and levels of glutamate in these areas (Ćurčić-Blake et al., 2017b; Hjelmervik et al., 2019; Hugdahl et al., 2015).

The study by Hugdahl and colleagues in 2015, by using ^{1}H-MRS, was the first study to investigate the association between levels of glutamate in language regions of the brain and AVHs in schizophrenia. Here, data was collected from four voxels, two from the bilateral inferior frontal lobe and two from the bilateral superior posterior temporal lobe. In line with the idea of disrupted glutamate functioning in AVHs in schizophrenia, they found that schizophrenic patients as a group had significantly reduced glutamate levels compared to controls in the aforementioned brain regions. They also found that patients, when divided into sub-groups of high vs. low symptom load, had more glutamate in said brain regions if their symptoms were of higher load. It should be noted that this was only found for positive symptoms, as no significant correlation was found for a single or total sum of negative symptoms. According to the authors, this could point towards a specific glutamate-GABA deficit underlying AVHs in schizophrenia; namely that glutamate hyper-activation in cortical regions is not kept in check by corresponding GABA release to inhibit this activity. As such, glutamate could act as a mediating transmitter for AVHs in schizophrenia.

Expanding on these findings, Ćurčić-Blake et al., (2017b) sought to investigate the relationship between AVHs and levels of glutamate in the dorsolateral PFC. With this study they also attempted to overcome some of the limitations to the study of Hugdahl et al., (2015), which mainly pertains to a small sample size. In addition to these differences between the studies, the latter did not only consider schizophrenia but also included other psychotic disorders, defined these groups according to the absence or presence of lifetime AVHs vs. symptom severity the previous week, and also had a shorter duration of illness (8,4 years vs. 12,25 years). Supporting the findings of glutamate as a mediating factor in AVHs, this study found higher levels of glutamate in patients with lifetime AVHs than patients without lifetime AVHs. They did not, however, find a significant correlation between the severity of AVHs and levels of glutamate, which the authors suggest may be

attributed to methodological differences between the two studies. Finally, the study by Hjelmervik et al., (2019), by investigating whether AVH severity is associated with Glx-GABA imbalances, also lends support to the mediating role of glutamate. Similarly to the two former studies, this study used ^{1}H-MRS procedures to measure Glx (and GABA) in brain regions previously associated with AVHs in schizophrenia, using a sample of 77 schizophrenia patients and 77 controls. Here, voxels were placed in bilateral STG, L-IFG and ACC. The results showed a significant positive correlation between Glx and AVH severity in L-STG. A significant negative correlation was also found for Glx and AVH severity in ACC. Compared to low-hallucinating patients as a group, decreased levels of Glx in ACC together with increased levels in L-STG was found for the high-hallucinating group. For both groups however, overall Glx levels were not found to be significantly different in patients compared to controls. Levels of Glx for the control group was in between that of low- and high-hallucinating patient groups. For GABA in all regions and Glx in L-IFG and R-STG, no significant results were found. Thus, with regard to the L-STG, the results seem to converge and extend upon the findings of Hugdahl et al., (2015), although with a larger sample. If the finding of increased levels of glutamate in L-STG with AVH severity in the Hjelmervik et al., (2019) study translate to neuronal hyper-activity (which was not measured), this has theoretical implications: Given that patients did not have overall higher Glx than controls, the positive correlation between Glx in L-STG and AVH severity (i.e. neuronal hyper-activity) could possibly reflect an compensatory effect wherein severely reduced glutamate levels increase and approximate “normal” levels as a function of AVH severity (Hjelmervik et al., 2019). Alternatively, if it instead translates to neuronal hypo-activity, then this might reflect the downstream consequences of glutamatergic excito-toxicity (Plitman et al., 2014). However, it is not clear whether measured Glx estimates in the patient group is enough for excito-toxicity to occur. In any case, considering these findings together arguably seems to support the idea of an existing relationship between AVHs and glutamate in schizophrenia. Why and how they are related, however, is less clear-cut and warrants further investigation. Whether also phenomenological qualities, such as emotional valence, can predict levels of glutamate in AVHs remains to be tested. As noted by Hjelmervik et al., (2019), the fact that a significant positive correlation was found exclusively for L-STG, which accord with fMRI studies demonstrating L-STG activations in patients with AVH (Jardri et al., 2011; Kompus et al., 2011), provide support for language- and speech-related lateralization processes. However, one might still expect to find a significant correlation between levels of Glx and emotional valence of AVHs in schizophrenia in R-STG, given that this area is associated with the prosodic and emotional aspects of speech processing (Allen et al., 2012) and also appear to increase in activity in response to negative emotional valence of AVHs (Sommer et al., 2008). Based on such findings together with the fact that none of the above studies (i.e. Ćurčić-Blake et al., 2017b; Hjelmervik et al., 2019;

Hugdahl et al., 2015) explicitly investigated these aspects, phenomenological qualities such as the perceived emotional valence of AVHs in schizophrenia may very well be associated with significant Glx increases or decreases in R-STG.

Excitation/Inhibition model of AVHs. Based on findings from several important studies comes a model that explains AVH in schizophrenia as a combination of an inability of frontal regions to suppress input from temporal areas involved in speech perception (Hugdahl, 2009). Here, AVH is generated as a bottom-up phenomenon originating from STG. Through activation of parietal areas, this leads to attention being involuntarily oriented towards the "voices". That parietal areas such as the bilateral inferior parietal lobes are involved in sustaining and regulating attention in several modalities, such as for instance the auditory domain, is generally well-known (Falkenberg et al., 2014). During cognitive control processes there has also been established a functional association between the inferior parietal lobes and other areas important for control in the fronto-parietal system such as the ACC (Vincent, Kahn, Snyder, Raichle & Buckner, 2008). Findings from the meta-analysis of Minzenberg et al., (2009), supported by the results of the 2014 study by Falkenberg and colleagues, showed that during conditions when demands for cognitive control are high, schizophrenia patients do engage inferior parietal areas and ACC, albeit to a lesser extent than healthy controls. This latter point demonstrates an important finding in schizophrenia and AVH research and lays the foundation for many models seeking to explain the pathogenesis of AVHs in schizophrenia, including the two-fold model proposed by Hugdahl in 2009, as there is reduced functional connectivity in frontal-parietal-temporal areas during language processing in patients with AVH (Ćurčić-Blake et al., 2013). Moreover, it seems attention is affected by the phenomenological qualities of AVHs, as studies have shown that, for example, perceived malevolence in AVHs to a significant degree predict lower levels of attention than AVHs perceived as benevolent (Kråkvik, Stiles & Hugdahl, 2013).

This aside and returning to the excitation/inhibition model, because of the hypofunction of ACC and prefrontal areas, these frontal areas are not able to exert their top-down executive control – thereby failing to adequately suppress the activity generated by temporal regions (Hugdahl, 2009). Because glutamate levels increase as a function of decreased input to inhibitory GABA neurons from dysfunctional NMDAR units, it has been proposed that high levels of glutamate over time exert neurotoxic effects, thereby contributing to structural changes that ultimately leads to decreased glutamate levels (Plitman et al., 2014). This might explain some of the findings from the meta-analysis of Merritt et al., (2016), as they did not find high levels of glutamate in medial-frontal areas of patients with chronic schizophrenia. However, this does not explain why it was found high levels of glutamate in medial-temporal areas of the same patient group. Likewise, although perhaps

due to methodological technicalities concerning sampling and defined groups, it does not explain the findings of the Ćurčić-Blake et al., (2017b) study – as it was found higher levels of glutamate in patients with vs. without lifetime AVHs. Considering the findings of the Hugdahl et al., (2015) study, which consisted exclusively of schizophrenia patients with a longer duration of illness, glutamatergic excito-toxicity might still provide the link between why patients in this study showed decreased glutamate levels compared to controls.

Summed, there seems to be converging evidence from several sources for the excitation/inhibition model of AVHs in schizophrenia, providing a bridge across multiple levels of explanation. Among others, cognitive processes are explained in terms of attentional deficits, which contributes to AVH emergence via involuntary attention towards the “voices”. However, the attentional deficits do not spontaneously emerge from nothing. Rather, underlying brain correlates, in turn caused by cellular changes by relative excitatory/inhibitory neurotransmission, contribute to such attentional deficits. For instance, with disease progression, abnormal structural changes might occur in response to the excito-toxic effects of excess glutamate, thereby decreasing the degree to which frontal areas such as the ACC is able to inhibit the activity from temporal areas such as the STG – which then ultimately leads to AVHs through an increasing inability to voluntary direct attention. However, although such reductionistic reasoning inevitably exceeds molecular levels and into the realm of quantum mechanics and beyond, integrating explanations at multiple levels nevertheless facilitates coherence across fields and prevents theories and models from becoming isolated from the phenomenon they are attempting to explain (Hugdahl & Sommer, 2018).

The proposed two-fold model presents some attractive explanations for the cascading mechanisms that leads to the experience of AVHs. However, it still does not provide any definitive answer to the question of AVHs neuronal origin. The observed (hyper) activity of STG may very well be the preceding trigger for AVHs, but it could also reflect altered activity elsewhere in a secondary fashion. Some authors (i.e. Jardri et al., 2011) have argued that a preceding trigger for the activations of language-related regions leading to AVHs may be oscillations in the activity of the para-hippocampal cortex, as some studies have shown activation of this area during AVHs versus deactivation prior to AVH emergence (Diederen et al., 2010). In essence, what they suggest is that deficits in verbal memory systems (i.e. para-hippocampal cortex) could trigger the occurrence of AVHs in schizophrenia through inadequate relay of information to secondary auditory cortex areas from the hippocampus. Both explanations on the origin of AVHs are plausible but may also fall into a major caveat relating to state-studies which concerns how results may be interpreted, as activated regions only inferentially can be related to the generation of AVHs – highlighting especially the speculative roles of non-language regions such as the cerebellum or hippocampus (Allen et al., 2012). As much research on this subject utilize imaging methods that provide relatively course

temporal resolution (e.g. fMRI), better understanding of the neuronal origin that triggers the cascading reactions that leads to AVHs may be achieved by combined and converging methods (e.g. fMRI/EEG) and generally more instances of replication studies. Finally, although much information on the role of glutamate and excitatory/inhibitory neurotransmission have been presented thus far in this thesis, much is still not clear regarding how this relates to phenomenological qualities of the AVH experience. However, considering the results from studies such as, for instance, that of Kråkvik et al., (2013) in the context of an integrated levels of explanation approach does seem to provide some clue as to how phenomenological qualities may relate to levels of glutamate. Before further investigating the role of glutamate in relation to the emotional valence of AVHs, it is necessary to first consider the role of another prominent neurotransmitter involved in AVHs in schizophrenia, namely dopamine.

The role of dopamine. The earliest theories attempting to explain the underlying neurobiological factors contributing to schizophrenia also highlights dopamine as a key component in the disorder. Evidence for its involvement originally came from the observation that administration of amphetamines led to increased extracellular levels of dopamine and tended to induce psychotic-like symptoms similar to those observed in schizophrenic patients, in healthy participants (Howes et al., 2015). Support for the involvement of dopamine in schizophrenia and psychosis also came from the fact that dopamine receptors are blocked by antipsychotics, which is a characteristic of all currently licenced antipsychotics, indicating that clinical response is dependent upon its functional manipulation (Howes et al., 2009a). However, it is important to note that symptom improvement is limited to positive symptoms, as there is little, if any, improvement to be made on negative and cognitive symptoms using dopaminergic treatment (Keshavan et al., 2011). Moreover, although AVH is one of the most responsive symptoms to antipsychotic medication, this does not mean that the response is equally similar across patients, as there is relatively large inter-individual variability (Johnsen, Hugdahl, Fusar-Poli, Kroken & Kompus, 2013). The early discovery that the clinical effectiveness of antipsychotics was related to their affinity for dopamine (e.g. D2) receptors (Seeman, Lee, Chau-Wong & Wong, 1976), subsequently led to the hypothesis that schizophrenia was the result of abnormal density of dopamine receptors (Howes et al., 2009a). However, as this hypothesis lack evidence from more recent single photon emission computed tomography (SPECT) and PET studies, D2 receptor densities most likely is not the key to understanding schizophrenia and hallucinations (Howes et al., 2012; Howes et al., 2009a). Although blocking D2 receptors may contribute to the clinical effectiveness of antipsychotics, it is not the only mechanism for antipsychotic activity, as the locus for the major dopaminergic abnormality is upstream from D2 receptor dysfunction (Howes et al., 2012), and antipsychotic agents furthermore

demonstrate great heterogeneity concerning their neuro-pharmacodynamics, even within their “typical” and “atypical” classifications (Gardner, Baldessarini & Waraich, 2005). For instance, Clozapine binds to D2 receptors but also acts at D1, D3 and D4 receptors and also affects receptors for serotonin, histamine, acetylcholine and others (Gardner et al., 2005). Thus, although dopamine is clearly involved in this process, there is no single mechanism of action common for all antipsychotic agents that alleviates schizophrenia and its associated symptoms. Rather, it is more likely that antipsychotics exert their effects by complex neuro-pharmacodynamic interactions involving down- and upstream effects among several neurochemical processes. Of the currently available antipsychotic medications, none directly target the glutamate system (Falkenberg et al., 2014). What is important with regard to this and all of the above is that, irrespective of whether patients have underlying deficits in dopaminergic systems, when psychoeducation and psychotherapy fail in alleviating hallucinations in patients, the majority of patients are offered antipsychotic (dopaminergic) medications for their symptoms (Sommer et al., 2018). As noted by the authors, psychotherapy and psychoeducation can be helpful for hallucinating patients, as it targets and alleviates the associated distress. In contrast, treatment with pharmacological agents can only alleviate symptoms if the corresponding and underlying brain mechanisms causing the symptoms are present. If they are not, then such treatment is likely to do the patient more harm than good. This latter point represents a real cause for concern, as systems involved in dopamine signalling has been shown to be normal in many patients with schizophrenia (Sommer et al., 2018). For instance, an important [18F]DOPA PET study investigating treatment-resistance in schizophrenia found, as the first of its kind, evidence for a lack of effectiveness of first-line (dopaminergic) antipsychotics when patients displayed frequent hallucinations coupled with normal dopamine synthesis capacity (Kim et al., 2017). The authors take their findings to suggest the usefulness of dopamine synthesis capacity as a biomarker to predict how patients will respond to treatment and note that if differential responses between patients are observed to first-line antipsychotics then this may imply different underlying neurobiology.

In essence, the dopamine hypothesis states that there are abnormally high levels of presynaptic dopamine in several brain regions. A meta-analysis of 44 studies incorporating nuclear imaging techniques, such as SPECT and PET, on findings of dopamine dysfunction in schizophrenia found that this mainly relates to presynaptic hyperdopaminergia in the striatum – which affects baseline synaptic dopamine levels, the synthesis capacity of dopamine and its release (Howes et al., 2012). Howes and colleagues (2015) suggest that sub-cortical dopamine dysfunction might be the culprit of both negative symptoms such as cognitive impairment and of positive symptoms in schizophrenia. The authors argue that antipsychotic medication that acts on D2 and D3 receptors fail to target these deficits and that controlling presynaptic dopamine synthesis and release

capacity should be the main focus of future drug development (Howes et al., 2012). An interesting association between dopamine and glutamate comes from early studies investigating the cascading pathways of glutamatergic neurotransmission induced by administration of ketamine. Together they indicate that the manifestation of positive symptoms in schizophrenia could be secondary to abnormalities in NMDAR (hypo)function. For example, one study indicated increased dopamine release in ventral striatum and frontal cortex to administration of ketamine (NMDA antagonist), which resulted in behaviours consistent with active psychosis (Moghaddam, Adams, Verma & Daly, 1997). Another study with similar results showed that, by using PET imaging and healthy participants, sub-anesthetic doses of ketamine triggered increased release of striatal dopamine (Kegeles et al., 2000). In this study, the finding that disrupting the activity at NMDAR units by pharmacological means (ketamine) leads to a two-fold increase of amphetamine-induced dopamine release in dopaminergic midbrain areas, suggests that glutamatergic projections from prefrontal regions regulate the activity of midbrain dopaminergic neurons via NMDAR units. While these studies show modulation of dopaminergic activity by glutamatergic processes, there is also evidence to support modulation of glutamatergic activity by dopaminergic processes. For example, in a study of Underhill et al., (2014) they showed that, by administration of amphetamine, an increase in internalization of EAAT3 (glutamate transporter sub-type found in dopamine neurons) potentiate excitatory synaptic responses in midbrain dopamine neurons. From findings such as these it becomes evident that there exists a complex relationship between dopamine and glutamate in contributing to the positive symptoms of schizophrenia. Another study using [18F]DOPA PET-imaging investigated dopaminergic functioning in auditory hallucinations in non-patients (Howes et al., 2013). Note that an upside to using such a sample is that the results may be interpreted without the possibly confounding variables of medication and other symptoms (Allen et al., 2012) – which is not limited to PET investigations. For example, in fMRI investigations, a review of the effects of antipsychotic medications used in schizophrenia showed that abolishment or normalization of the blood-oxygen-level dependent (BOLD) signal tends to occur as a function of increased/longer exposure to the drugs (Abbott, Jaramillo, Wilcox & Hamilton, 2013). Thus, in imaging studies, the inclusion of patients who have used antipsychotic medications over longer periods of time may confound the results by masking between-group differences (Falkenberg et al., 2014). Returning to the 18F-DOPA PET study by Howes et al., (2013), between this sample and controls they found no significant difference between striatal dopamine synthesis capacity and sub-clinical psychotic symptom severity, which points toward a non-significant role of dopamine synthesis capacity in non-patient auditory hallucinators. Hence, it may be that the association between deficits in dopamine synthesis capacity and AVHs is specific to psychotic and/or schizophrenia patients.

Dysregulated release of large amounts of dopamine in several brain regions (e.g. striatum)

without any appropriate triggering stimuli could, according to the dopamine hypothesis, lead to misattribution of salience to an external unrelated source, based on temporal association (Howes et al., 2015). This lends support to models explaining AVHs as a result of inability to self-monitor inner speech, and could help explain the development of positive symptoms observed in schizophrenia (Allen, Aleman & McGuire, 2007). According to these models, an inability to adequately monitor one’s own thoughts and/or memories leads to the patient misattributing these to an external and unrelated source. If AVHs arise as a function of inner speech being misattributed to an external and unrelated source, Tracy and Shergill (2013) pose the question of why they then almost always consist of speech patterns and acoustic properties different from the hallucinating person. That is, why are AVHs typically expressed in third-person dialogue? These authors further speculate that this may have something to do with deficits in processing prosodic components of speech in a top-down fashion, as prosody have non-lexical properties such as emotion and intonation and thus may not only involve deficits in specific speech areas in the brain. Similarly, it has been proposed that speech activity associated with emotional valence within right hemisphere language regions leads patients to erroneously attribute this to an external source as a function of desynchronization within these areas (Ćurčić-Blake et al., 2013). In addition, early work by Allen et al., (2004) showed an increased likelihood of misattributing self-generated inner speech when the prosodic nature of that speech was of higher emotional valence. However, taken together this can not account for why some, but not all, patients respond to antipsychotic medication blocking D2-receptors (Sommer et al., 2018).

A plausible explanation could be that there exist sub-types of schizophrenia, each with its own set of underlying neurochemical pathologies. Such an explanation might also account for findings pertaining to the relationship between dopaminergic dysfunction and negative symptoms in schizophrenia - showing that the well-established link between dysfunctions in frontal areas and cognitive impairment in schizophrenia might be mediated by increased striatal dopamine dysfunction (Bertolino et al., 2000; Howes et al., 2009b; Meyer-Lindenberg et al., 2002). Sommer et al., (2018) further argue that through a better understanding of the phenomenological aspects of hallucinations specific to the patient, it may be possible to infer which specific neuro-physical pathology that contributed to the hallucinations and, as such, may guide subsequent treatment in a way that is tailored to the individual patient.

Assessing the Phenomenological Qualities of AVHs in Schizophrenia

Emotional valence construct. It is important to be aware that emotions represent very wide and complex phenomena that can be understood as discrete categorical entities (e.g. anger, happiness, etc.) or as dimensions such as valence and arousal. Although often used interchangeably

within the literature, emotional arousal refers to the levels of activity generated in the autonomic nervous system by an eliciting event which range from low to high, whereas emotional valence is defined along a continuum from negative to positive and refers to the levels of subjective pleasantness generated by an eliciting event (Bestelmeyer, Kotz & Belin, 2017). There is also an assumed independence between emotional arousal and valence in many neuroimaging studies, which the aforementioned cited study claims to be unwarranted. This is based on their finding of overlapping activity between emotional arousal and valence in a distributed brain network consisting of bilateral STG, insula, amygdala, precuneus, caudate, inferior and mid- frontal cortex, as well as medial orbito- and superior frontal cortex, which resulted through combining whole brain analyses with behavioural tasks on a representative sample of normal sound perceivers (i.e. non-patient and non-AVH). Hence, for emotional valence and emotional arousal there is a common involvement of both classic brain areas associated with emotion (e.g. orbitofrontal cortex, bilateral insula and amygdala) and modality-specific brain areas associated with auditory processing (e.g. bilateral STG). Furthermore, Bestelmeyer et al., (2017) found increased BOLD signal with extremely positive or negative valence ratings compared to more neutral valence ratings in bilateral PFC, mid- and anterior cingulum, hippocampi and para-hippocampi, but also in bilateral temporal voice areas (e.g. STG). This suggests that it does not matter whether the emotional valence is positive or negative; as long as there is an increase from neutral to an extreme value this will reflect in increased brain activity in aforementioned areas such as the bilateral STG. Taken together, these findings indicate that emotional arousal and valence might not be as independent as previously thought and as such, what can be inferred from functional neuroimaging studies on schizophrenia and AVH must take into account that what is being measured might in fact reflect overlap between these constructs. However, as noted by Bestelmeyer et al., (2017), this overlap in activity does not exclude the possibility that single neurons within regions might differentially be dedicated to the processing of one specific affective dimension.

PANSS. The Positive and Negative Syndrome Scale (PANSS), originally developed by Kay, Fiszbein and Opler (1987), and particularly the P3 item of the scale, is often used to quantify the frequency and severity of AVHs in schizophrenia (Hugdahl & Sommer, 2018). The PANSS contains a total of 30 items that the participants, through a clinical interview, must rate using a seven-point scale (ranging from 1 – absence, to 7 – extreme). Total items are typically aggregated into three sub-divisions; 7 measuring positive symptoms, 7 negative symptoms and 16 general psychopathology symptoms. Together these provide the grounds for assessing positive, negative and general psychopathological symptoms experienced the previous week. It is important to be aware of a potentially major drawback to the use of this scale when attempting to measure AVHs, as the P3

item is not specific to AVHs, nor any other specific sensory modality. The results of studies investigating AVHs using a small sample size, wherein, for instance, most patients only report hallucinations in the tactile or visual modalities on the P3 item, consequently may lead to the rejection of the null hypothesis when in fact it is true (i.e. Type I error). Indeed, this represents a real cause for concern for the validity of many studies, as AVHs in schizophrenia in about half of cases are accompanied by hallucinations in other sensory modalities (Sommer et al., 2012). In fact, some of the reasons why there are relatively large inconsistencies across ^{1}H-MRS studies investigating some aspect of the hallucination experience in schizophrenia, may be attributed to different symptom profiles (Merritt et al., 2016) – which partly could be the result of measuring hallucinations with the P3 item of the PANSS.

BAVQ-R. In order to obtain reliable insight into the phenotypical characteristics of AVHs, it is important to also make use of instruments that, as opposed to commonly used measures such as the PANSS, measure hallucinations with more than a few questions – which do not arguably provide enough information to probe phenotypes (Sommer et al., 2018). As a substitute, or preferentially addition, the revised “Beliefs About Voices Questionnaire” (BAVQ-R), developed by Chadwick, Lees and Birchwood in 2000, could be used for this purpose. Before considering the specifics of this questionnaire, it is important to provide some context from its original development and theoretical foundations, as this has implications for the emotional valence construct and how it is operationalized in this thesis. In an early and influential paper by Chadwick and Birchwood (1994), a cognitive model was applied to the understanding of AVHs in patients with drug-resistant schizophrenia. The cognitive model applied to AVHs considers them involuntary and internally generated thoughts that are misattributed to an external source (van der Gaag, Hageman & Birchwood, 2003). This work since laid the foundation for the development of the original BAVQ. Through this paper and with this cognitive approach, they showed that the way in which the identity, meaning or intention of AVHs was cognitively appraised by the patients changed the way in which their behavioural, affective and cognitive responses varied (Chadwick & Birchwood, 1994). That is, it was not the contents of- but rather their beliefs about “voices” that influenced the degree to which they were considered as benevolent or malevolent. Subsequently, AVHs believed to be benevolent were engaged, whereas those believed to be malevolent were associated with fear and resistance.

Thus, the results seem to indicate that affective responses (e.g. fear) to AVHs is closely linked with their behavioural response (e.g. resistance), which ultimately is based on their cognitive appraisals or beliefs (e.g. malevolence). Hence, it is not the inherent negative or positive content of AVHs that influence responses but rather the ascribed beliefs about malevolent or benevolent intent

(Chadwick & Birchwood, 1994). For example, a patient could experience AVHs with strong negative content directed towards him/herself but at the same time believe that this ultimately will make him/her stronger or better – i.e. negative emotional content of AVHs with a positive, beneficial or benevolent intent. However, this is not confirmed in other studies. For example, a study by Close and Garety (1998) found no incongruence between the contents of AVHs and the beliefs about them held by patients, which they suggest is a function of an inherent feature of the AVH experience to elicit negative affect in the patient despite the content being benevolent. Thus, there still are difficulties in ascertaining the relative contribution of beliefs about AVHs intent and their content to affective responses. In an attempt to disentangle this relative contribution of belief and content to distress, van der Gaag et al., (2003) applied more rigorous testing procedures and barrages of self-report measures and interviews to a large sample of patients, analysing the results using multivariate analyses of variance. From this, they found that it is the patient's cognitive appraisal of the AVHs meaning and purpose (i.e. intent) that is associated with distress, and not their contents. In concordance with results from previous studies, the results also confirmed that AVHs that are believed to be malevolent are resisted and not engaged, whereas the inverse relationship was found for benevolence. Moreover, beliefs about malevolent intent was clearly associated with both depression and anxiety.

If beliefs about AVHs mediate the affective, behavioural and cognitive responses to them, this may have important clinical implications, especially for patients that do not adequately respond to antipsychotic medications, as changing those beliefs may change the associated distress. This was also what was found in a second study in the same paper by Chadwick and Birchwood (1994), along with the unexpected finding of reduced AVH occurrence experienced by both patient groups – having beliefs and construing their AVHs as benevolent versus malevolent – in response to cognitive treatment aimed at challenging and decreasing those beliefs. However, as pointed out by the authors, this may not necessarily be universally beneficial, as malevolent and benevolent beliefs might rely on and be maintained through different psychological processes. As such, given the association between malevolent beliefs and negative emotional responses on the one hand and benevolent beliefs and positive emotional responses on the other, decreasing the occurrence of AVHs may only be desirable in the former patient group, as for the latter this could lead to unexpected (secondary) consequences, for example, as a reduction in self-esteem. Putting aside this speculative argument however and focusing on the possible implications from the results from the van der Gaag et al., (2003) study, the distress associated with AVHs could be reduced, despite its high negative content, by changing the patients underlying beliefs about their “voices”. Hence, the focus of cognitive and cognitive behavioural therapy should not be on changing or reducing the negative content of the AVHs but rather on changing their appraisal of meaning, purpose and intent.

From this, and in sum, there seems to be large support for the applicability of the cognitive model to AVHs.

Returning to the BAVQ-R, in essence, there is a main distinction between the assumptions and the reactions to the AVHs. The assumptions are further divided into the perceived degree of benevolence (BEN), malevolence (MAL) and/or omnipotence (OMN) of the AVHs. The reactions are divided into the degree to which the person feels compelled to engage and cooperate with the AVHs (ENG), versus the degree to which they resist this (RES). Generally, as shown above, there is often assumed to be a positive correlation between the factors BEN and ENG, and between the factors MAL and RES, which is supported by studies (e.g. Chadwick & Birchwood, 1994; Sayer, Ritter & Gournay, 2000; van der Gaag et al., 2003). This makes intuitive sense, also within an evolutionary framework, as it would be a logical and adaptive reaction to avoid what is perceived as harmful and to approach what could benefit the individual. Furthermore, it can also be inferred a positive correlation between OMN and ENG, based on studies (e.g. Braham, Trower & Birchwood, 2004) showing that AVHs perceived as more powerful than the individual are more likely to be complied with than those perceived as less powerful. In the study, and with forming the revised BAVQ, Chadwick et al., (2000) found a positive and negative correlation to both anxiety and depression for RES and ENG, respectively. They also found that OMN and MAL separately had positive correlations with both depression and anxiety, suggesting that they both are related to negative emotional valence. Perhaps to some degree this might reflect the later finding by a large-scale confirmatory and exploratory factor analysis, showing a strong positive correlation (.83) between the MAL and OMN items of the scale, suggesting that they to a large extent load onto a common factor (Strauss et al., 2018). However, putting this aside and considering the BAVQ-R as is, the overarching goal is to obtain a measure of thoughts, feelings and behaviour related to the auditory hallucinations in schizophrenia (Chadwick et al., 2000). Through the use of this scale, themes and contents of AVHs may be uniquely associated with brain and neuropsychological correlates (Hugdahl et al., 2009). As a final note on the BAVQ-R (and PANSS), it is important to be aware that the results from this only reflect how the respondents experienced and reacted to the hallucinations the previous week. As will become evident in the following sections, the specific aim of this project deviates from its general aim in at least one important aspect, as the postulated hypotheses (H1-H2) refer to the more constrained concept of “emotional valence”. Considering the aforementioned associations between factors as beliefs of intent of AVHs on one hand and items in the BAVQ-R on the other, given the assumed positive correlation between MAL-RES and BEN-ENG, negative emotional valence could be operationalized as measured MAL-RES values above mean values. Likewise, positive emotional valence could be operationalized as measured BEN-ENG values above mean.

Current Project: Aims and Hypotheses

In sum, previous MRS studies suggest associations between AVH severity and glutamate in language and prefrontal regions (Ćurčić-Blake et al., 2017b; Hjelmervik et al., 2019; Hugdahl et al., 2015). In addition to subcortical regions, the same regions have been shown to be involved in the processing of emotional valence of real voices (Bestelmeyer et al., 2017; Lennox et al., 2000; Sommer et al., 2008). However, the perceived or appraised emotional contents and reactions to AVHs have not previously been tested with regards to the underlying neurochemical mechanisms and involved neurotransmitters. Sommer et al., (2018) have posed the question as to whether the phenomenological experience of AVH can predict the underlying psychopathology – that is, dysfunctions of glutamatergic and dopaminergic systems. The current study therefore investigates whether there are associations between emotional valence of AVH and glutamate on one hand, and emotional valence and response to dopaminergic antipsychotic medications on the other (as an indirect measure of dysfunction in dopaminergic transmission). The research questions were investigated by using an already existing dataset that measured Glx in bilateral STG, L-IFG and ACC using ^{1}H-MRS; emotional valence of "voices" using BAVQ-R; and response to antipsychotic (dopaminergic) medications as symptom development over time by the P3 item of the PANSS. Although the aforementioned data will act as the foundation of this thesis, it will also be necessary to look at other relevant studies in order to facilitate comparisons and discussion across results. Furthermore, given the relatively large inconsistencies reported in the literature and the fact that no studies have linked such phenomenological aspects of AVHs to underlying pathophysiological mechanisms (Sommer et al., 2018), the approach of this thesis will be of an exploratory nature. Thus, the working hypotheses are two-tailed and as such, do not infer any specific direction among variables. Still, it is presumed that more positive valence implies less negative valence of "voices", and therefore these two measures are expected to be inversely related to the dependent variables (glutamate and clinical response to dopaminergic medications). This in mind, the following two hypotheses reflect the aim of this thesis:

H1: The emotional valence of AVHs in schizophrenia is associated with levels of glutamate in brain regions previously associated with auditory hallucinations (L-STG, R-STG, L-IFG, ACC).
H2: The clinical response to antipsychotic (dopaminergic) medications will vary as a function of the emotional valence of AVHs in schizophrenia.

Methods

Participants

In the current study, estimates of regional levels of Glx were collected from 40 patients in R-STG, L-STG, L-IFG and ACC (see Figure 1) using a point-resolved spectroscopy sequence (PRESS) in a 3 Tesla GE-SignaHDx MRI scanner. The mean age of the sample was 26,50 years with a standard deviation (SD) of 8,50 and consisted of 27 males and 13 females. The patients were diagnosed with schizophrenia according to the ICD-10 diagnostic manual (World Health Organization, 1992; Norwegian translation; https://ehelse.no/kodeverk/kodeverket-icd-10-og-icd-11). The mean length between being diagnosed and age of inclusion in the study (i.e. mean sickness duration) was approximately 4,10 years (SD 7). Scores for the positive, negative and general psychopathology symptoms, as measured through the PANSS across all patients, revealed means of 18 (SD 4,70), 17 (SD 4,70) and 36 (SD 8,50), respectively. Most patients were right-handed (N=34) – the remaining being left-handed (N=3) or ambidextrous (N=2). Some patients (N=19) had in addition to their primary diagnosis of schizophrenia also been diagnosed with a secondary diagnosis, while some (N=8) also had tertiary diagnoses. In addition to using antipsychotic medications, some also used antidepressant medications (N=4), mood stabilizers (N=2), anticholinergic (N=1) and benzodiazepines (N=5). Furthermore, one patient used antidepressant medication and mood stabilizers, and another used antidepressant medication and benzodiazepines. The study was approved by the Regional Committee for Medical Research Ethics at the University of Bergen (REK no. 2010-3387) and conducted according to the Declaration of Helsinki. The participants had received oral as well as written information about the study before signing a written consent form.

Instruments

MRS. Subsequent analysis of the obtained MRS data was done using LCModel version 6.3-1J, whereby a base set of 15 metabolites were estimated (Alanine, Aspartate, Creatine, γ-aminobutyric acid, Glucose, Glutamine, Glutamate, Glycerophosphorylcholine, Phosphorylcholine, Lactate, myo-inositol, N-acetylaspartate, N-acetylaspartylglutamate, scyllo-inositol and Taurine). The LCModel provides automatic quantification of in vivo proton MR spectra by analysing it as a linear combination of a complete model spectra of a basis set of metabolite solutions obtained in vitro, thereby minimizing bias caused by having too few parameters and artifacts caused by too many parameters (both causing errors in estimates) by selecting the most consistent baseline and smoothest lineshape between them (Provencher, 1993). With regard to this project and its main hypotheses, only the combined measure of glutamate and glutamine (Glx) is relevant for further

analyses. Adjustment of GM/WM within selected voxels was done by scaling the metabolite estimates to an internal water reference and subsequently deriving tissue content from within the voxels from T1-weighted structural image using Statistical Parametric Mapping (SPM8) segmentation tool software (www.fil.ion.ucl.ac.uk/spm). Two different protocols for scanning participants were used, in which the main difference was a change of head coil, but also regions scanned (L-STG, R-STG and ACC vs. L-STG and L-IFG) and number of participants (25 vs. 15). Data were missing for two patients in the R-STG and for one patient in the ACC. For more detailed information about the procedures used and obtained spectra, see Hjelmervik et al., (2019).

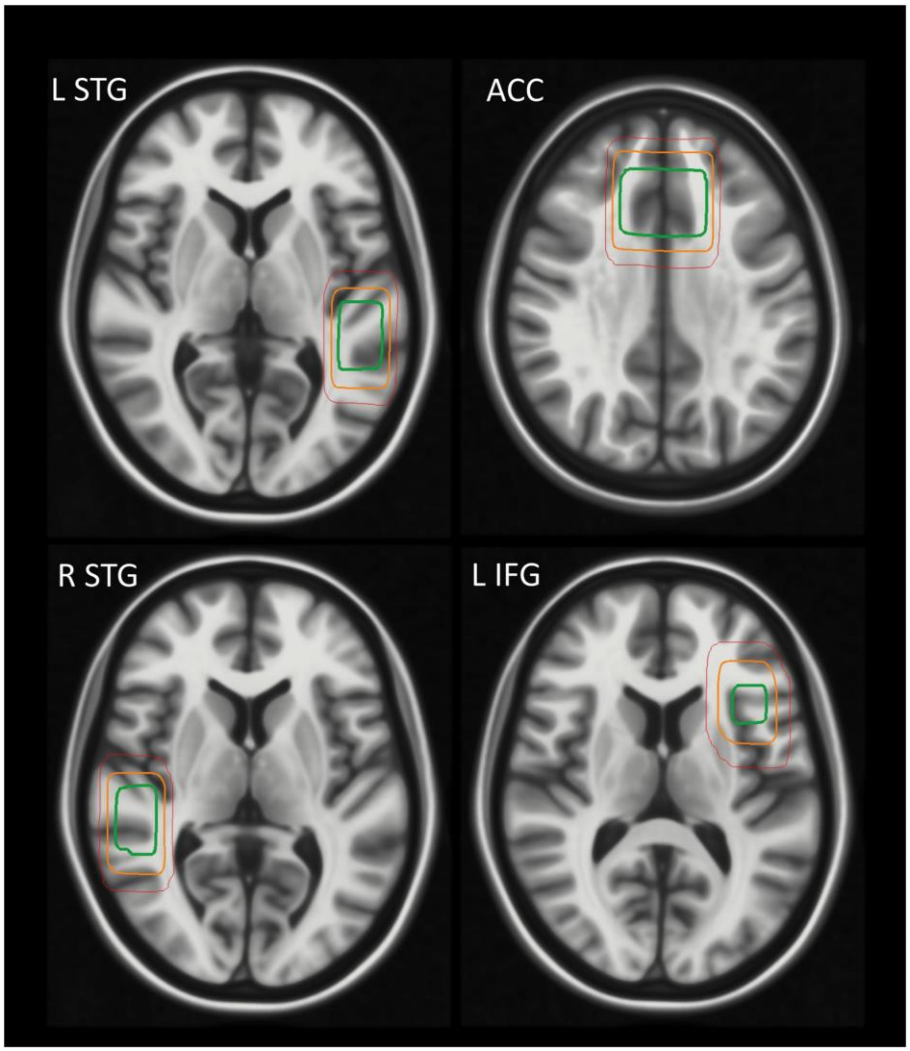

Figure 1. Voxel placement in L-STG, ACC, R-STG and L-IFG shown in horizontal views. The orange (mid) box represents placement for a single subject, whereas the red (outer) and green (inner) indicate 95% and 65% confidence regions across the entire group, respectively. L-STG, left superior temporal gyrus; ACC, anterior cingulate cortex; R-STG, right superior temporal gyrus; L-IFG, left inferior frontal gyrus. Reprinted from "Intra-Regional Glu-GABA vs Inter-Regional Glu-Glu Imbalance: A 1H-MRS Study of the Neurochemistry of Auditory Verbal Hallucinations in Schizophrenia" by H. Hjelmervik et al., 2019, *Schizophrenia Bulletin, 46*(3), p. 636. Copyright 2019, Authors. Reprinted with permission.

BAVQ-R. Widen (2000) translated BAVQ-R into Norwegian, and it is this edition that will be relevant for the purposes of this project. This questionnaire presents several statements relating to hallucinations and the respondents then make use of a four-point Likert scale to rate how much or how little they agree with each statement (ranging from 0 – disagree, to 3 – strongly agree). The results are then summed for each category and thus make up a score in a total of five categories, as noted in previous sections of this thesis (RES, ENG, MAL, BEN, OMN).

Statistical Analysis

First analysis. Because the general aim of this project in part was to investigate whether there is a linear relationship between thoughts, emotions and behaviour related to AVHs in schizophrenia and regional levels of Glx, statistical analyses must take into account levels of Glx across all relevant regions as a function of scores on the BAVQ-R sub-categories. Glx met normal distribution criteria for dependent variables and parametrical tests could therefore be carried out. Thus, in the first analysis, in order to account for Glx levels across regions as a function of scores on the sub-variables in the questionnaire, statistical analysis of the obtained data was done using Linear mixed modelling in SPSS software (https://www.ibm.com/analytics/spss-statistics-software). Here, a multivariate regression model was set up using region (L-STG, R-STG, L-IFG, ACC) as a repeated and fixed factor with BAVQ-R sub-variables (MAL, BEN, OMN, ENG, RES) as regressor. This yielded a total of five models, each testing BAVQ-R sub-variables to levels of Glx across regions. The default correlation matrix option ‘Diagonal’ was used for all five models.

Control analyses. In order to rule out whether the result of the first analyses reflects a change in protocol, it was necessary to include some control measure for this. This was done by checking for a significant difference between effect sizes of BAVQ-R sub-variables against Glx for data collected with the two respective coils/protocols. Differences in the standardized beta weights between protocols for each BAVQ-R sub-variable were estimated by calculating the overlap between their corresponding 95% confidence intervals. As an example, for the BAVQ-R sub-variable “MAL”, differences between protocols were estimated by first calculating 50% of the average of the overlapping confidence intervals (.26) and then adding this to the first protocols' beta weight lower bound estimate (-.63). This yields a score of “-.38”. Because the upper bound estimate (.41) of the second protocols' beta weight exceed this value, the difference between the standardized beta weights of protocol one and protocol two was considered not to be statistically significant. This was found for all BAVQ-R sub-variables (p>.05). Thus, the results from the first analyses are not likely due to differences between protocols. Another possible confounding factor might be that the results to some degree reflect variations in hallucination severity between patients. For instance, it

could be that patients who perceive negative emotional valence also have more severe/frequent hallucinations. Thus, hallucination severity could serve as a mediating variable between levels of glutamate and perceived emotional valence of AVHs. In order to ascertain that the observed effects are due to emotional valence rather than degree of hallucination severity, similar procedures as those used to control for protocols were performed. Here, the P3 item variable from the PANSS (range of scores from 1-6) was used to create a new variable that splits hallucination severity into high (4-6) and low (1-3) values using a median split. This new variable was then entered as a filter in a similar manner to those described for protocols before subsequently running the analyses and comparing results. From this, no statistically significant results were found (p>.05), indicating that the results from the original analyses are not due to the degree of hallucination severity as measured with the P3 item.

Second analysis. To test whether there is an association between the perceived emotional valence of AVHs and clinical responsiveness to antipsychotic medication, a bivariate correlational analysis was conducted. In order to measure the potential- and degree of symptom improvement over time, the P3 item of the PANSS was used for this purpose. However, since patients were tested for improvement in symptoms/medication response at a total of seven different time-points (between one week and nine months; i.e. degree of response from visit 1-2, from 1-3, from 1-4, etc.), a new variable had to be created prior to running the analysis. In this variable, the means of each patient was calculated across all available time-points, yielding the mean score in degree of medication response/non-response for each patient. Also prior to running the analysis, the five BAVQ-R sub-variables were set up as illustrative graphs and then visually inspected for normality distribution. From this it was concluded that none of the variables were normally distributed. Because this violates the assumptions of Pearson product moment correlations, the non-parametric Spearman's rank correlation test was chosen. Hence, when entering the five BAVQ-R sub-variables together with the new mean medication response variable into the analysis, rank coefficient (rho) were selected over Pearson.

Results

First Analysis

The results showed a significant main effect of BAVQ-R sub-variable RES on levels of Glx across all regions (F(1,59.36) = 7,50, p=.01). The results for RES are shown in Figure 2.

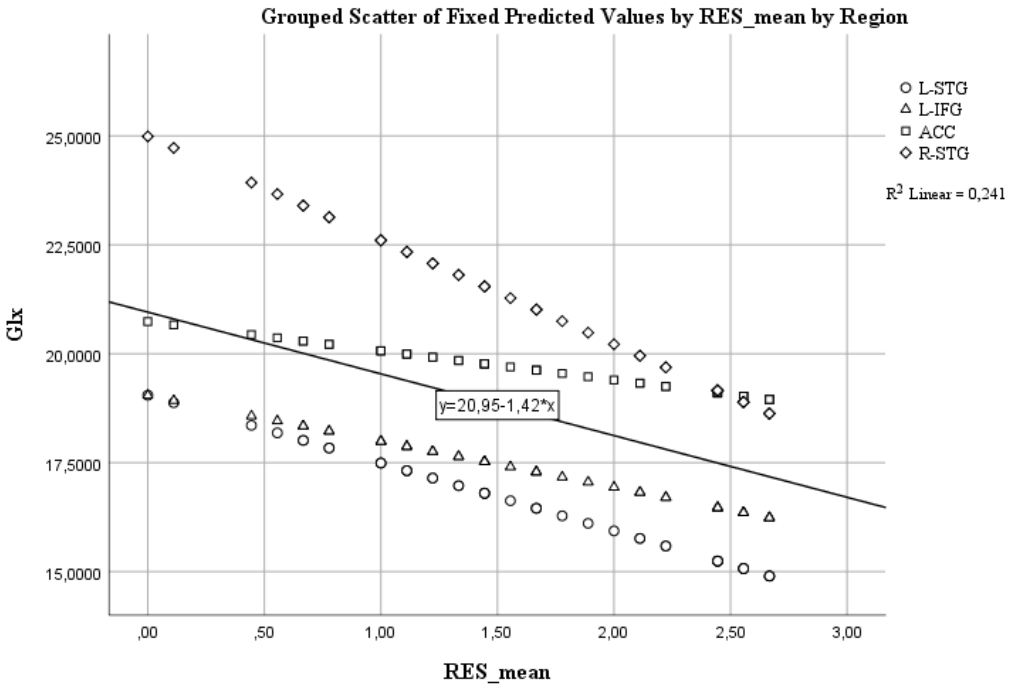

Figure 2. Scatter plot demonstrating differences in Glx levels (Institutional units) across regions for mean values of BAVQ-R sub-variable RES. The correlation was negative, such that increased resistance to AVHs is associated with decreased levels of Glx across regions. Note that fixed predicted, not raw, values are used (*y*-axis).

There was also a significant effect for both MAL ($F(1,52.46) = 4,15$, $p=.05$) and BEN ($F(1,34.23) = 4,27$, $p=.05$) sub-variables. The results for MAL and BEN are shown in Figure 3 and Figure 4, respectively.

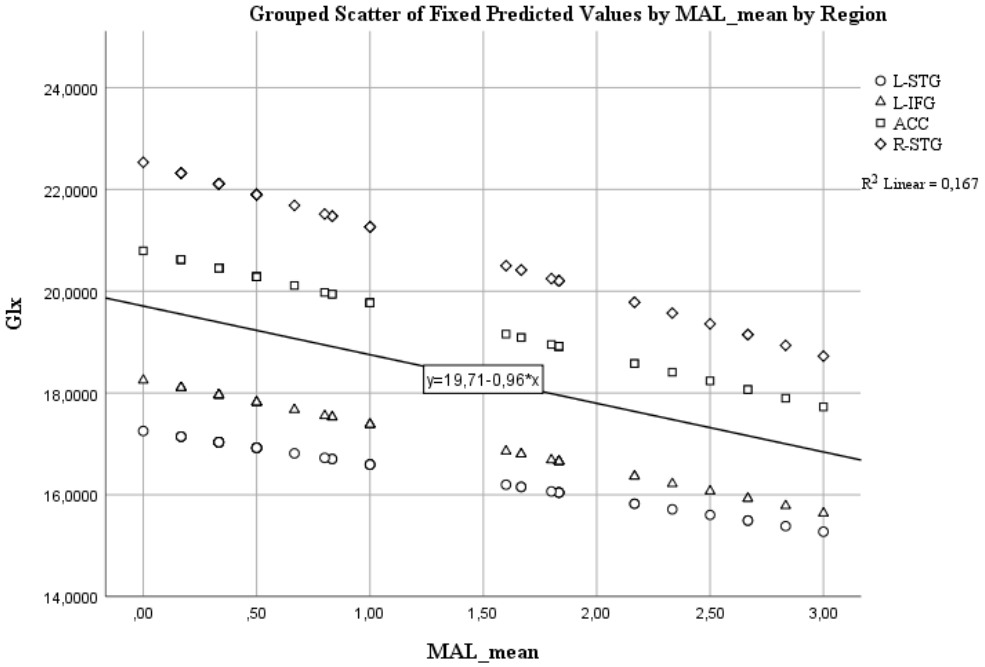

Figure 3. Scatter plot demonstrating differences in Glx levels (Institutional units) across regions for mean values of BAVQ-R sub-variable MAL. The correlation was negative, such that increased malevolence of AVHs is associated with decreased levels of Glx across regions. Note that fixed predicted, not raw, values are used (*y*-axis).

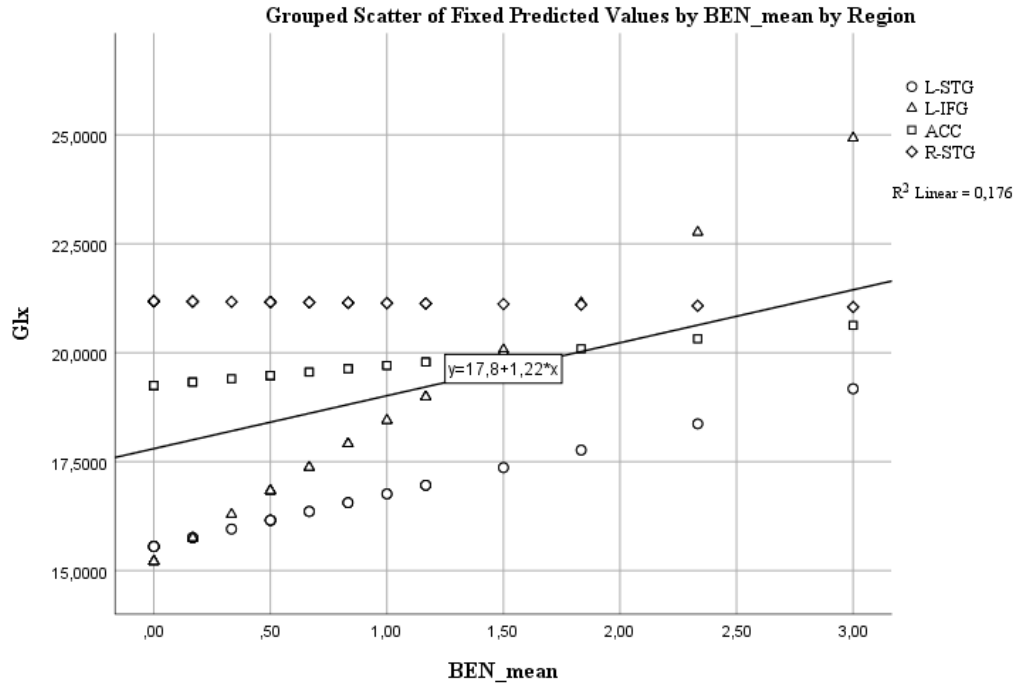

Figure 4. Scatter plot demonstrating differences in Glx levels (Institutional units) across regions for mean values of BAVQ-R sub-variable BEN. The correlation was positive, such that increased benevolence of AVHs is associated with increased levels of Glx across regions. Note that fixed predicted, not raw, values are used (*y*-axis).

In addition, a main effect of Region was found for these two analyses; (F(3,31.37) = 3,93, p=.02), (F(3,30.44) = 5,80, p=.01). Further inspection using pairwise comparisons showed that the difference in Glx levels is between L-STG and L-IFG compared to ACC and R-STG. In the first analysis, this was reflected by a difference between ACC to L-STG (p=.00) and L-IFG (n.s.) and between R-STG to L-STG (p=.00) and L-IFG (p=.03), whereas for the second analysis this was between ACC to L-STG (p=.00) and L-IFG (p=.04) and between R-STG to L-STG (p=.00) and L-IFG (p=.01). That is, for both analyses there was significantly more measured Glx in the ACC and R-STG than the other two regions. Coincidentally, although not significant, a similar trend of increased Glx in ACC and R-STG vs. L-STG and L-IFG was observed for all first analyses.

For the sub-variable ENG, although the correlation was positive such that increased engagement is associated with increased levels of Glx across regions, there was not found a significant main effect (F(1,38.18) = 1,22, n.s.). For this reason, an illustrative figure is not included for this sub-variable. Finally, there was a significant main effect for the OMN sub-variable (F(1,60.47) = 4,45, p=.04), the results of which are shown in Figure 5.

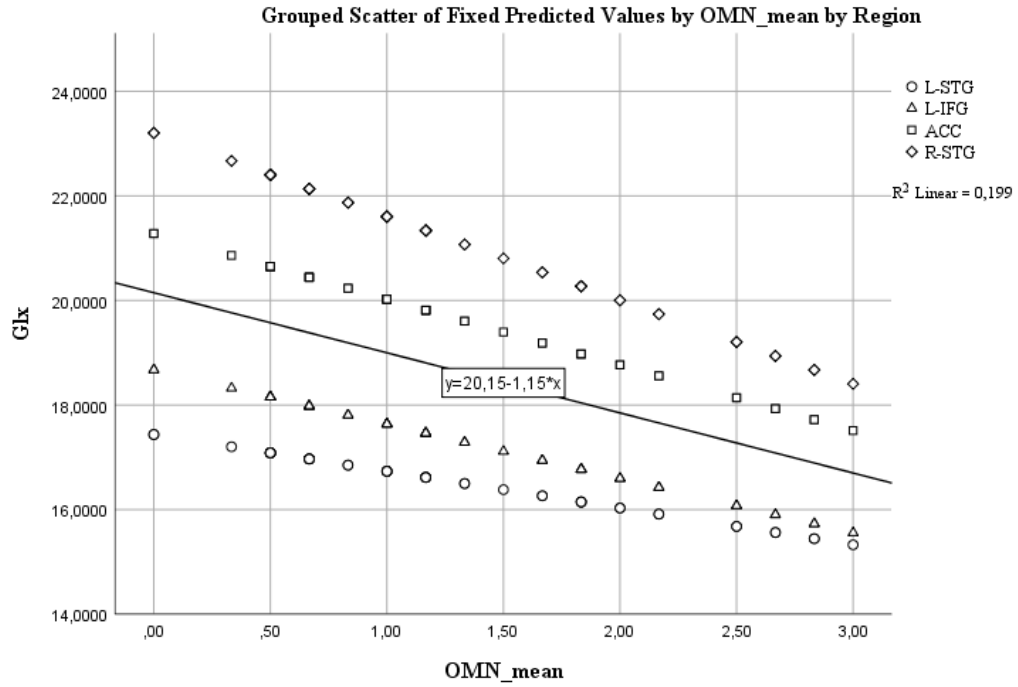

Figure 5. Scatter plot demonstrating differences in Glx levels (Institutional units) across regions for mean values of BAVQ-R sub-variable OMN. The correlation was negative, such that increased omnipotence of AVHs is associated with decreased levels of Glx across regions. Note that fixed predicted, not raw, values are used (*y*-axis).

Second Analysis

The results revealed correlation coefficients between mean medication response across all time-points for all BAVQ-R sub-variables. However, none were statistically significant at the .05 level. Refer to Table 1 below for results from the second analysis.

Table 1. The results from the second analysis showing Spearman's rank correlation coefficients for mean medication response for all BAVQ-R sub-variables across all time-points, the associated *p*-value and number of participants.

MAL mean	Correlation coefficient	-.05
	Sig. (2.tailed)	.75
	N	39
BEN mean	Correlation coefficient	.07
	Sig. (2.tailed)	.70
	N	39
OMN mean	Correlation coefficient	.05
	Sig. (2.tailed)	.75
	N	39
ENG mean	Correlation coefficient	.02
	Sig. (2.tailed)	.90
	N	39
RES mean	Correlation coefficient	.11
	Sig. (2.tailed)	.51
	N	39

Discussion

To sum, the core findings from the first analyses showed a statistically significant negative correlation between the BAVQ-R sub-variable RES and measured Glx values across all regions. This was also found for sub-variables OMN and MAL. In other words, given that glutamate is used to refer to Glx, as noted in a previous section of this thesis, all measured regions showed lower levels of glutamate with higher levels of malevolence, omnipotence and resistance to AVHs – which may, considering the given operationalization, translate to higher degree perceived or appraised negative emotional valence of AVHs. Thus, the first hypothesis (H1) is confirmed by the significant correlation between negatively appraised emotional valence of AVHs and lower levels of glutamate in regions previously associated with auditory hallucinations (L-STG, R-STG, L-IFG, ACC). Finally, the hypothesis is confirmed by a significant finding in the opposite direction, showing a positive correlation for sub-variable BEN and levels of Glx across all regions. For the second set of

analyses pertaining to the second (H2) hypothesis, no statistically significant correlations were found. Hence, there was no significant difference in response to antipsychotic (dopaminergic) medication among patients who experience differential degrees of positive/negative emotional valence in AVHs. That is, the clinical response did not vary as a function of the emotional valence of AVHs in schizophrenia, thereby disconfirming the second hypothesis. Taken together, both findings highlight the complex relationship existing between glutamate and phenomenological characteristics of the AVH experience in schizophrenia such as perceived emotional valence. Another layer of complexity is added when attempting to interpret and extract the potential existence of meaningful sub-types from this relationship. In the final sections of this thesis, resolvement of this complexity will be sought by discussing the findings from all analyses and their respective hypotheses. Insights and findings from other relevant studies will be incorporated to facilitate this discussion. Subsequently, limitations will be presented and discussed before ending this thesis in a final conclusion and recommendations for future research.

Glutamate and Emotional Valence of “Voices”

H1: “The emotional valence of AVHs in schizophrenia is associated with levels of glutamate in brain regions previously associated with auditory hallucinations (L-STG, R-STG, L-IFG, ACC)”.

The results appear to confirm the first (H1) hypothesis. Given the assumed positive association between BAVQ-R sub-variables MAL and RES, with the operationalization of the positive/negative perceived emotional valence constructs, it was expected that measured levels of Glx across all regions would be associated with scores on the RES and MAL sub-variables. The finding of a statistically significant negative correlation between both RES and MAL and measured Glx across regions suggest that perceived (negative) emotional valence of AVHs is associated with lower levels of glutamate across regions. Overall, this finding is in line with other studies showing an association between glutamate and AVH in schizophrenia using MRS (e.g. Ćurčić-Blake et al., 2017b; Hjelmervik et al., 2019; Hugdahl et al., 2015). However, the findings from this thesis adds to existing literature a novel aspect of the AVH experience, as it is based on phenomenological or the affective appraisals of AVHs, rather than the severity of AVHs. Considering the positive correlations between the construed malevolence of AVHs and resistance on one hand (e.g. Chadwick & Birchwood, 1994; Sayer et al., 2000; van der Gaag et al., 2003) and between resistance and depression and anxiety on the other (Chadwick et al., 2000), one may infer that the negative emotional appraisal of AVHs is related to its severity. Intuitively, this makes sense, as depression and anxiety both could be argued to be “severe”, in that they are related to an increased risk for detrimental outcomes such as suicide (Chesney, Goodwin & Fazel, 2014). If the severity of AVHs coincides with its negatively valenced appraisal, the results from this thesis generally appear to

point towards the opposite direction than the aforementioned MRS studies (i.e. Hjelmervik et al., 2019; Hugdahl et al., 2015). In the latter study, increased glutamate was found in patients with high symptom load (i.e. severity) versus low symptom load (Hugdahl et al., 2015). In the former study, increased glutamate was found in L-STG together with decreased glutamate in ACC in patients with more severe hallucinations than patients with less severe hallucinations (Hjelmervik et al., 2019). In this thesis, with the results from the first analyses, no difference was observed between regions for e.g. BAVQ-R sub-variable RES. That is, patients with high resistance/negative appraisals/severity of AVHs had low glutamate across all regions (L-STG, R-STG, L-IFG, ACC). As such, the results are in contrast to the results of the Hugdahl et al., (2015) study, and also seem to contrast the Hjelmervik et al., (2019) study in some regions (i.e. L-STG) but not others (i.e. ACC). However, it is important to note that for aforementioned studies (i.e. Hugdahl et al., 2015), decreased levels of glutamate was found for all patients, both high- and low severity subgroups, compared to healthy controls. Hence, in that regard (low glutamate considered as a marker for illness), the results from this thesis appear to be in agreement with the direction of other studies.

Similarly to the sub-variables RES and MAL, in the first analyses, OMN was found to be significantly and negatively correlated with levels of glutamate across all regions. Together with the factor-analysis of the BAVQ-R scale showing a strong positive correlation between the factors OMN and MAL (Strauss et al., 2018) and given that higher omnipotence (and malevolence) of AVHs have been associated with more depression and anxiety (Chadwick et al., 2000), this finding would be expected based on the results from the RES and MAL sub-variables. In other words, because OMN, RES and MAL all can be argued to reflect negatively valenced appraisals/severity of AVHs, it would be expected that one follows in the same direction as the others. Thus, with regard to the Hugdahl et al., (2015) and Hjelmervik et al., (2019) studies, similar lines of reasoning as for sub-variables RES and MAL could be argued to be drawn for sub-variable OMN.

Finally, for the sub-variable BEN, there also was a significant correlation with levels of glutamate. However, in contrast to RES, MAL and OMN, a significant positive correlation was found, such that positive appraisals/less severe AVHs is associated with more glutamate across regions. To some extent, this lends support to the factor-analysis of Strauss et al., (2018) of the BAVQ-R, suggesting RES, MAL and OMN to belong to a separate factor from BEN and ENG. Thus, taken together it appears that negative and positive emotional valence of AVHs in schizophrenia is associated with decreased and increased glutamate across all measured regions, respectively. Furthermore, this also seems to support the assumption made prior to presenting the hypotheses of this thesis, as an inverse relationship was found for negative and positive emotional valence to levels of Glx.

Adopting the view that emotional valence is related to severity, the findings above could

lend support to models such as the two-fold excitation/inhibition model of Hugdahl (2009) by way of dysfunction or hypofunction at NMDAR units. In essence, this may be argued to occur in two principal ways. First, hypofunction at NMDAR units across regions may consequently contribute to an inability of frontal regions (i.e. ACC, L-IFG) to suppress activity generated by temporal regions (i.e. L-STG, R-STG) – in turn decreasing input to inhibitory GABA interneurons – which contributes to an increase in overall glutamate levels across regions. Thus, it may be that increased glutamate levels reflect a compensatory effect in response to antagonized or otherwise hypo-functional NMDAR units (Ćurčić-Blake et al., 2017b). Alternatively, because it is not known whether such a compensatory effect occurs, it may be that increased hypofunction at NMDAR units across regions reflects decreased levels of glutamate. As such, low levels of glutamate in frontal regions may contribute to a lack of cognitive resources necessary to inhibit activity of temporal regions (Hjelmervik et al., 2019). Based on this and the fact that it is not known whether glutamate levels are up-regulated in a compensatory response, patients with more severe/negative AVHs may have more dysfunction at NMDAR units, which contributes to a reduction in levels of glutamate across regions.

Nevertheless, in the case of a compensatory effect – that is, elevated glutamate as a response to NMDA dysfunction – this could exert excito-toxic effects over time (Plitman et al., 2014). In that sense, this may have contributed to the often observed neuroanatomical abnormalities in schizophrenia such as cortical thinning and GM reductions (Plitman et al., 2014) – and as such, given that there is more glutamate located in GM than in WM (Bustillo et al., 2011) – a reduction in glutamate would be expected as a function of time and AVH severity/negative emotional valence. This latter point is also supported by the meta-analysis of Modinos et al., (2013), finding severity of AVH to be related to GM reductions in bilateral STG, although with a dominance for L-STG reduction (see also Neckelmann et al., 2006). This could then imply a reduction in glutamate levels across all regions over time, with the cognitive, behavioural and affective consequences that might follow. Hence, if patients with more positively/less negatively valenced AVHs have a larger amount of GM than patients with less positive/more negative AVHs, the results from the first analyses makes sense. However, as the Glx measurements were corrected for the amount of GM/WM within voxels for each patient from T1-weighted structural image, a potential reduction in GM would statistically balance out by averages. Based on this, the findings do not support the idea of decreased GM volume/thickness (i.e. decreased glutamate) with increased severity/negative AVHs.

However, reductions in GM volume and thickness does not necessarily equate to reduced levels of glutamate. For example, although prospective studies have shown that early-onset psychosis generally and AVH specifically is associated with progressive reductions in cortical volume (Allen et al., 2008; Arango et al., 2008), abnormally increased neuronal density have also

been reported from tissue of older patients with schizophrenia (Selemon, Rajkowska & Goldman-Rakic, 1995). Thus, it may be the neuronal integrity/density or viability that is important for the observed levels of glutamate – of which NAA compounds act as a marker (Bustillo et al., 2017) – rather than GM thickness and volume. Hence, it is possible that patients in the Hjelmervik et al., (2019) study had “normal” GM volume and thickness together with abnormal GM integrity/viability/density. In GM, higher levels of NAA compounds suggests higher neuronal density or compactation (Rae, 2014). Thus, in order to control for the potential effects of neuronal density confounding the results from the first analyses, an a-posteriori multivariate regressional analysis was performed using linear mixed modelling. This was run using similar parameters as those described for Glx in the first analyses, although replacing Glx values with values for NAA. From this, no significant results were found for levels of NAA across regions as a function of scores on BAVQ-R sub-variables, suggesting normal GM density and indicating that neuronal viability do not vary across measured regions as a function of perceived emotional valence of AVHs in schizophrenia.

In sum, from the above discussion, the most likely interpretation of the results is low glutamate as a function of NMDA hypofunction. This means that patients experiencing AVHs with negative emotional valence have more severe dysfunctions at NMDAR units and therefore lower levels of glutamate. However, this explanation infers negative and positive emotional valence to be a question of severity in the same manner as the P3 PANSS sub-scale. One would in this case expect the P3 control analysis to be significant. However, this was not the case, suggesting that it is unlikely that severity per se (as defined by P3) can explain the associations between BAVQ-R sub-variables and Glx. Therefore, considering the above in light of this, the first compensatory response explanation (increased glutamate) may be specific to positive emotional valence and not decreased severity, whereas the second approach (decreased glutamate) may be specific to negative emotional valence and not increased severity. In this sense, although speculative, negative and positive emotional valence of “voices” are not more and less severe per se, respectively, but rather reflect two different sub-types of patients in which one group has a glutamatergic compensatory response (more glutamate) to NMDA hypofunction whereas the other group does not.

Besides the shortcomings of the “severity” construct and how it is measured – to which will be addressed under “limitations” – the above findings from the first analyses may to varying extent represent expected and unexpected results. Given the results from the Bestelmeyer et al., (2017) study suggesting the non-relevance of positive vs. negative emotional valence, as an increase from neutral to extreme value would reflect in increased bilateral (e.g. STG) activity in both cases, it might have been expected that the current results show an increase or decrease in levels of glutamate across all regions as a function of emotional valence wherein the relative positive or

negative value does not matter. Of course, all of this assumes that increased activity as measured with fMRI translate to increased levels of glutamate as measured with MRS, an assumption that is generally supported given the glutamate/GABA-glutamine cycle (Bak et al., 2006; Rae, 2014). However, aside from the limited inferences that can be made from the BOLD signal in fMRI investigations, as will become clear through the text below, this relationship is not always straight-forward and thus is often subject to speculative and indirect interpretations based on theoretical assumptions. Returning to the results from the current thesis, what is found in the analyses is a difference in levels of glutamate across all regions as a function of positive vs. negative emotional valence. That is, contrary to what may have been expected based on the findings of Bestelmeyer et al., (2017), the results from this thesis seems to suggest an inverse relationship between the two measures of emotional valence (i.e. positive vs. negative) with levels of Glx. Although, as mentioned, this may reflect fundamental methodological differences and limitations pertaining to what and how results from fMRI studies may translate to results from MRS studies and furthermore how a measure of an increase in activity from neutral to extreme values would relate to this, the results from the current analyses may nevertheless provide a novel contribution, suggesting that the value and direction (i.e. positive vs. negative) of emotional valence in AVHs matter – at least in schizophrenia patients.

Finally, it may be that the relationship between symptom severity and levels of glutamate is indirect (Merritt et al., 2013). For example, the results from the Bustillo et al., (2011) study suggest that negative symptoms may be secondary to cognitive deficits associated with decreased levels of glutamate. Given that negative symptoms represent a constellation of symptoms such as amotivation, anhedonia and generally blunted affect (Correll & Schooler, 2020), it is further possible that this subsequently influence how and to what extent AVHs are perceived to be of negative and/or positive emotional valence. As such, although speculative, the decreased levels of glutamate found across regions in the first analyses of this thesis for BAVQ-R sub-variables could contribute to cognitive deficits which in turn contribute to anhedonia and blunted affect, subsequently reducing the extent and likelihood of appraising AVHs with negative and/or positive emotional valence.

In addition to the main effects of BAVQ-R sub-variables discussed above, in two of the analyses a main effect was also found for Region. Given the involvement and specific role of right hemisphere language regions (e.g. R-STG) in prosodic and emotional aspects of speech processing (Allen et al., 2012) and findings of increased activity in response to negative emotional valence of AVHs (Sommer et al., 2008), it may have been expected that levels of glutamate in this region (R-STG) differ from that of other implicated regions as a function of emotional valence. However, despite two analyses showing a significant effect of region (i.e. higher glutamate in ACC and R-

STG vs. L-STG and L-IFG), the absence of a significant interaction between BAVQ-R sub-variables with region suggests that glutamate in R-STG is not uniquely associated with the emotional valence of AVHs. Given that emotional valence and severity of AVHs may represent distinct phenomena, it would be interesting to further explore this within the context of a dichotic listening framework. As such, failing to show REA, which is common in schizophrenia (Hugdahl, 2009), would be indicative of functional deficits in left hemisphere regions (e.g. L-STG) given the phonological features of presented syllables, whereas failing to show a hypothesized left ear advantage (LEA) would be indicative of functional deficits in right hemisphere regions (e.g. R-STG) as this area is involved in emotional aspects of speech processing. However, how such prosody and affective aspects could be incorporated into- and represented by syllables within such a paradigm in a way that reliably measures an hypothesized LEA is difficult to imagine, as emotional comprehension involves analysing acoustic cues, deriving emotional significance from such cues and then applying those to higher order cognition (i.e. more than just comparing syllables spoken sadly vs. neutrally). Furthermore, each of these processes may have widely different brain correlates (Schirmer & Kotz, 2006). Nonetheless, considering the argument above, it is important to note that no significant interactions were found in the current analyses for any BAVQ-R sub-variable between regions. Therefore, none of the implicated regions can by themselves help provide any explanation as to how levels of glutamate relate to the emotional valence of AVHs in schizophrenia.

In sum, although the above discussion of the first analyses seem to lend support to previous studies in some respects but not others, it is revolving around a novel aspect of AVHs in schizophrenia not explicitly investigated by those studies and as such, many of the arguments may be speculative. This in mind, given the non-significant results of NAA and control analyses suggesting that AVH severity and emotional valence represent distinct phenomena, what might provide the best explanation for the findings of the first analyses is a combination of approaches to the excitation-inhibition model, suggesting that increased glutamate across regions is uniquely (i.e. not explained by severity) associated with positive emotional valence and that decreased glutamate across regions is uniquely associated with negative emotional valence.

Emotional Valence of "Voices" and Medication Response

H2: "The clinical response to antipsychotic (dopaminergic) medications will vary as a function of the emotional valence of AVHs in schizophrenia".

The results appear to disconfirm the second (H2) hypothesis. This is based on the non-significant results of the bivariate correlational analysis for all BAVQ-R sub-variables to clinical response (measured with P3 item of PANSS) to antipsychotic (dopaminergic) medications. Additionally, effect sizes (rho) were small for all sub-variables – the largest being for RES,

indicating that the clinical response to antipsychotic medications increase (i.e. improvement of symptoms) with increased resistance/severity/negative AVHs, although to a low (.11) degree. Because there was no significant difference between positive and negative emotional valence groups in clinical response, this does not support the idea of sub-types of schizophrenia based on differences between underlying neurochemical (glutamatergic vs. dopaminergic) pathologies and provide limited support for the suggestion that phenomenological qualities of AVHs, such as emotional valence, could be used to predict clinical response to antipsychotic medication (Sommer et al., 2018). However, this does not preclude the possibility that there still might exist sub-types based on differences within underlying neurochemical (glutamatergic) pathologies. For instance, no currently available antipsychotic medications directly target the glutamate system (Falkenberg et al., 2014). It may therefore be argued that what can be inferred from the results is limited to the effects of (dopaminergic) antipsychotic medications, which in themselves, there is limited information within research literature regarding their heterogeneous neuro-pharmacodynamic effects (Gardner et al., 2005). Moreover, taken together with the above, the findings from the Kegeles et al., (2000) study that suggests that glutamatergic projections, via NMDAR units, regulate activity of midbrain dopaminergic neurons, adds to the idea that glutamatergic systems, mechanisms or processes may mediate the response to (dopaminergic) antipsychotic medications. Furthermore, given that antipsychotic medications not only act at receptors for dopamine but also act on various other receptor types to a varying degree (Gardner et al., 2005), it is plausible that levels of glutamate and the glutamatergic system is influenced downstream from cascading dopaminergic (and other neurotransmitters) processes that have yet to be identified and validated by studies.

Taken together, based on the above, this may very well constitute a basis for the notion of (glutamatergic) sub-types. However, given that no current antipsychotic medications directly target the glutamate system, this argument and idea of (glutamatergic) sub-types is difficult to validate and thus remains highly speculative. It would be interesting to see if, in the future, the potential development of effective antipsychotic medications directly targeting glutamatergic systems would reflect in changes to the results of the second analysis based on the current (H2) hypothesis. Moreover, it is difficult to be certain whether the results of absence of significant correlations between specific phenomenological aspects of the AVH experience, such as emotional valence and clinical responsiveness to antipsychotic medications, because research into phenomenological aspects of AVHs in general is very limited (Johnsen et al., 2013). For example, as reported in this review of fMRI investigations into the neuro-psychopharmacology of auditory hallucinations, only changes related to limited subjective aspects such as perceived loudness of AVHs is associated with the use of antipsychotic medications over time (Johnsen et al., 2013). Other aspects - such as developments in the perceived emotional valence of AVHs in schizophrenia over time with

antipsychotic medications - has through thorough search and review of relevant literature not yielded any results. As such, to the best of this author's knowledge, there has not been any previous studies that have investigated this specific aspect. Although clinical drug trials have reported on changes in symptom scores over time, this is typically not reported for single symptom scores (e.g. P3 item of the PANSS) but rather is reported as group mean changes of sub-scores or overall psychopathology (Johnsen et al., 2013). Hence, as noted elsewhere, as the P3 item does not exclusively measure AVHs and does not accurately measure the perceived or appraised emotional valence of AVHs, no clear indication of the effects of antipsychotic medications over time can be extracted from this. It is therefore difficult to draw any lines to the results of the second analysis showing no significant correlation and responsiveness/non-responsiveness in (AVH) symptoms with negatively/positive AVHs. However, the results may have been confounded by limitations imposed by the chosen statistical and/or methodological approach and thus, the discussion of the current results will continue into and be elaborated upon throughout the next and final section of this thesis.

Limitations

In general, there are several issues both within and between AVH research in schizophrenia pertaining to aspects such as methodology and construct validity that may confound their results, thereby reducing the direction and strength of inferences that can be made to the overall phenomenon of interest and to the results of this thesis. For example, a potential overarching problem with most research into auditory hallucinations relates to the terms used by researchers to refer to the phenomenon, as auditory hallucinations and AVH appears to be used interchangeably within literature, with some authors referencing research on auditory hallucinations when in fact AVHs is what has been studied (e.g. Allen et al., 2012). While it is true that AVHs are auditory hallucinations, the opposite may not always be assumed and thus may constitute a problem insofar that they represent different phenomena with different neuropsychological correlates. Thus, although the dataset used in this thesis specifically investigates AVHs, problems with validity may nevertheless arise when comparing and discussing results across other studies. Specifically, in addition to the above, there are also other problems within this thesis and its analyses that may ultimately be reflected in its results. Before concluding this thesis, in this final section, as an extension of the above discussion, these specific and general aspects will be presented and discussed in order to elucidate just how certain changes to them may yield different results – thereby changing their subsequent implications – and may promote and facilitate cohesion across fields of research into AVHs in schizophrenia.

Both in general and specific to this thesis, there may be problems with how participants and constructs are defined, operationalized and measured. The diagnostic category of schizophrenia to

select participants into a study at worst may present a serious problem to validity of research on phenomenological aspects of the disorder, as there is no one symptom that is pathognomonic (Jablensky, 2006). The phenomenological similarities among patients selected for research purposes based only on this assessment may have no single symptom in common. Hence, the diagnostic category cannot accurately predict which pathophysiological attributes will be common to patients diagnosed with schizophrenia. Thus, how a patient group is defined (e.g. in a state-study vs. trait-study) may contribute to variations in the degree to which they reportedly hallucinate between different studies. As such, they may meet the criteria for AVHs in one study but not another. It is therefore important to properly define groups based on, for instance, degree of hallucination severity (Allen et al., 2012). However, as previously pointed out in the discussion of the first analysis of this thesis, separate problems arise regarding the “severity” of AVHs.

First, to measure the severity of AVHs, studies generally rely on scales and their items, such as the P3 (hallucination) item of the PANSS. To some extent, this could be argued to represent an effective and beneficial approach, as it promotes objectivity and standardization across studies. However, in addition to the previously mentioned potential drawback of measuring AVH severity with the P3 item (i.e. not modality-specific), there are problems with subjectivity relating to the collection of data, as this is obtained through a clinical interview. Although interviewers require proper and rigorous training in the use of the scale and interpretation of responses to ensure reliability (Opler, Yavorsky & Daniel, 2017), some bias may nevertheless be reflected in their rating of responses. For example, if a patient with AVH additionally presents with paranoid delusions, they may be suspicious of the interviewer and therefore may withhold information which could help inform about the severity of AVHs. Thus, factors such as the ability and/or willingness of the patient to provide accurate information about their symptoms, combined with the experience and skill of the interviewer to accurately interpret this information, signal the extent to which such measures are useful. Second, studies investigating the severity of AVHs do not exclusively rely on the same scale (e.g. PANSS). Although there are relatively high correlations among scales used to assess and score severity of symptoms (Lyne, Kinsella & O'Donoghue, 2012), some differences among them may nevertheless constitute potential bias and contribute to inconsistencies in the literature. Third, as the severity of symptoms in schizophrenia vary and may fluctuate over the temporal course of the illness (Keshavan et al., 2011), the time at which measurements are obtained is critical to the final outcome, as scales such as the PANSS only measure symptoms based on the previous week. Hence, what is regarded as “severe” one week may no longer be so the following week.

This is also the case for the BAVQ-R, such that, hypothetically, AVHs may be appraised with negative emotional valence one week and positive emotional valence the next. If this is true, then inconsistencies within both the analyses of this thesis and between studies could reflect

variability at which points in time measurements were obtained. In particular, this may have contributed to the non-significant results of the second analysis, as BAVQ-R measures were obtained at (seven) different time-points between patients. In order to overcome or control for this potential confounding effect, the approach of aggregating the means of the P3 scores of each patient across all time-points into a new variable representing mean medication responsiveness for each patient was chosen in the second analysis. However, as the BAVQ-R was administered at varying points in time – some administered before and some after measuring P3 item of the PANSS – it is possible that this may have skewed the results in a way that reflects how the emotional valence of AVHs are appraised after medication response. Because the hypothesis (H2) states that the medication response will vary as a function of the emotional valence, what is of interest is the emotional valence prior to the onset of the potential effects of antipsychotic medications over time. Therefore, using the means in such a way for the second analysis may not have been feasible, as this assumes homogeneity within the group across measures. An alternative approach could have been to only include patients wherein BAVQ-R was measured before the effects of medication (i.e. at the first time-point). However, as such an approach would require omitting the vast majority of patients, leaving only a total of 11 patients, this was deemed inappropriate. This is because using such a small sample would increase the likelihood of a false positive finding (Type I error) and reduce the external validity of the results, as such a small sample arguably would comparably be less able to ensure sufficient statistical power to be able to extrapolate and generalize the results of the analysis to the overall population. Coincidentally, this is a limitation that pertains to the findings of most studies of AVH in schizophrenia (Allen et al., 2012). Thus, a clear limitation to the present study relates to the fact that the BAVQ-R was measured at different and inconsistent points in time. If all patients had been scored on the BAVQ-R prior to P3 (i.e. at the first time-point), thereby increasing the size of the available sample, this second approach would have been favourable in the second analysis. A third approach, adding to the selected approach, could have been to control for the effects of time by including the time-points (1-7) as a covariate in the analysis. However, since none of the included variables were normally distributed, a non-parametric test (Spearman) had to be chosen, which do not allow the inclusion of covariates into its analysis.

Another limitation to both analyses, particularly the second analysis, is that a few patients in the dataset used first generation (i.e. “typical”) either alone or in addition to second generation (i.e. “atypical”) antipsychotic medications, while others used second generation medications only. This was not accounted for in the current study or other studies based on the same dataset (e.g. Hjelmervik et al., 2019), neither was it specified which particular antipsychotic drugs patients were taking. This is in part because of relatively high variability between the different kinds of antipsychotic drugs patients were taking. It is therefore not possible to ascertain whether potential

neuro-psychopharmacodynamic differences between drugs may have contributed to the results of the second analysis. In the future, studies investigating such aspects could benefit from only including patients that are on the same drug (e.g. Clozapine).

In a more general sense, because it is rare that studies are conducted on medication-naive patients due to both practical and ethical concerns, most information of auditory hallucinations in various investigations (e.g. fMRI) are obtained from subjects concurrently receiving antipsychotic medication (Johnsen et al., 2013). This is an issue insofar that they still experience hallucinations during the time of investigation, despite being on medication, which suggests this population being resistant to their effects. Thus, as pointed out by the authors, what can be inferred regarding the exact mechanisms involved in the therapeutic action of such medications is limited and as such, imposes limitations on how neural correlates of AVH respond to antipsychotic medications.
A related limitation concerning hypothesized sub-types in schizophrenia is that, as noted by Geisler et al., (2015), neuropsychological differences between identified sub-types could to some extent reflect variability in unrelated and external illness-related variables. For instance, use of various medications may cause new symptoms such as extrapyramidal symptoms, and also may contribute to the modification of existing symptoms such as, for example, anhedonia. Considering this latter example and extending upon it, a schizophrenia patient that experience and appraise their AVHs with negative emotional valence, that is also diagnosed with major depression and is prescribed antidepressant medications, may over time experience improvement in symptoms. Consequently, as the subjective experience of pleasure improve, they may be more inclined to appraise their AVHs with positive emotional valence. Given that nearly half of all patients in the first and second analyses had another diagnosis in addition to schizophrenia (N=19), it is quite possible that this may have confounded the results. Moreover, some patients (N=12) also used other medications (e.g. SSRI/SNRI, benzodiazepines, etc.) in addition to antipsychotics.

Aforementioned intra- and inter-individual neuro-psychopharmacodynamic differences may not be exclusive to antipsychotic medications but also could be argued to apply to unrelated medications (e.g. antidepressant medication). As such, there may still be unexplored interactions at multiple genetic, molecular, neurophysiological, cognitive and behavioural levels between, for example, a specific antipsychotic and specific antidepressant drug, which ultimately may reflect in the final results of the current analyses. With regard to the second analysis, clinical responsiveness to antipsychotic medications was based on hallucination (P3) symptoms, however, it would be interesting to see whether there also was an improvement in negative symptoms, which could to some extent help explain the results through its potential mediating effects on how the emotional valence of AVHs is appraised. As such, although perhaps indirectly, the results from the second analysis could help inform about the results of from the first analysis. Another related limitation

may exist in the relationship between AVHs or psychosis in schizophrenia and substance use disorder (Kavanagh et al., 2004). Considering the association between psychosis, dopamine and the use of stimulants such as amphetamines (Howes et al., 2015) – which coincidentally has been shown to be used more frequently in patients with psychotic disorders than the general population by 160% in a Norwegian sample (Ringen et al., 2008) – a clear disadvantage of the current dataset is that a potential history of substance use and dependency was not probed and biological drug-tests not administered before inclusion into the study. Besides the obvious shortcomings of various substances, such as amphetamines, potentially affecting the glutamatergic system indirectly through altered dopaminergic signalling (e.g. Underhill et al., 2014), severity of AVHs and by what emotional valence they are perceived in the patient group, the use of most such substances constitute a criminal offence and furthermore may be associated with social stigma in such a way that they go unreported.

Other limitations of the present thesis relate to technical aspects of MRS as a method (not previously mentioned in relevant sections of this thesis) and constraints set by the data on which the analyses are based. For example, pertaining to MRS as a method, when investigating the ACC using ^{1}H-MRS procedures, most studies place the voxels in one hemisphere only or across both hemispheres (Falkenberg et al., 2014). This makes it difficult to discern the role of possible inter-hemispheric interactions that may affect glutamatergic regulation. For example, based on their results, the authors suggest that a reduction of glutamate levels in the left ACC may disrupt cognitive control processing as a function of high glutamate levels in the right ACC. Hence, it may be that the link between cognitive control and glutamate in schizophrenia is mediated through contra-hemispheric processes within the same regions (i.e. ACC). Another example concerns its sensitivity at readily available field strengths. That is, at 3 Tesla there usually is sufficient sensitivity to estimate many metabolites in several regions of the brain (Rae, 2014). However, besides that the resonances of some metabolites such as glutamate cannot be resolved from others such as glutamine given factors such as coupling and peak overlap in the spectra, quantification of glutamate within small subcortical regions such as the amygdala is limited at this field strength (Nacewicz et al., 2012). From their findings regarding the reliability of MRS to estimate metabolites in the amygdala, the authors note that due to its proximate spatial localization the method is highly sensitive to partial inclusion of basolateral and centro-medial regions, further arguing the importance of specifying accurate details of voxel placement, particularly within subcortical regions.

Although this latter point does not specifically represent a problem of the current thesis per se, as no estimations from such subcortical regions were obtained, it acts to highlight a limitation to the present thesis concerning the dataset on which analyses were based – namely that estimations were obtained from- and thus limited to four voxels in the bilateral STG, ACC and L-IFG (see

Figure 1). Another area of interest that was not measured and included in the current data is the R-IFG, which is associated with cognitive evaluation of the emotional significance of speech (Schirmer & Kotz, 2006) and also show increased activity associated with AVHs (Sommer et al., 2008). Given such associations, it would be interesting to see whether estimates of glutamate in limbic structures (e.g. amygdala) and R-IFG vary as a function of the emotional valence of AVHs. Future MRS investigations into phenomenological aspects of AVHs, particularly emotional aspects, may benefit from including and placing voxels within R-IFG and subcortical regions, being aware of- and accounting for potential limitations in MRS sensitivity in recordings from subcortical regions etc.

As a concluding remark to this section, it is again important to note that although it appears to be of considerable length, some of the limitations presented above do not purely reflect problems with this thesis and its results. Rather, this section should be regarded as an extension of the previous section, adopting a reflective approach to potential problems with the results of this thesis and to related research in general. That is, some of the presented arguments relates to and reflects potential rather than concrete and explicit limitations specific to the analyses of this thesis.

Conclusion

The general and overarching goal of this thesis was to investigate the relationship between thoughts, emotions and behaviour related to AVHs in schizophrenia and their underlying pathophysiological factors, as increased understanding of the neurobiological underpinnings of effective pharmacological intervention and specific symptoms (i.e. AVH) may be crucial in order to disentangle the heterogeneity that characterizes the disorder. Some authors (e.g. Sommer et al., 2018) have taken such heterogeneity to suggest that phenomenological aspects of hallucinations may inform about which specific pathophysiological factors that contribute to the hallucinations and that there may be sub-types of schizophrenia based on such differences. Although the current project cannot by and in itself disentangle the pathological, etiological and clinical heterogeneity that characterizes schizophrenia and its associated symptoms (i.e. AVH), as it adopts an explorative approach and adds to existing research and literature a novel aspect of the AVH experience – namely, the appraised emotional valence – its findings may nevertheless contribute to an increased understanding of the complex relationship between neurophysiological- chemical and psychopathological factors on one hand and cognitive, affective and behavioural abnormalities on the other.

Using an available MRS dataset, this project has demonstrated a significant association between levels of glutamate in brain regions previously associated with AVHs in schizophrenia (L-STG, R-STG, L-IFG, ACC) and how these hallucinations are appraised in emotional valence.

Limitations notwithstanding, there was no significant association between emotional valence and clinical responsiveness to the use of antipsychotic (dopaminergic) medications, suggesting that inferring sub-types of schizophrenia based on similarities and differences in neurochemical and phenotypical factors may be unwarranted – at least for glutamatergic vs. dopaminergic sub-types.

However, given that there may be sub-types based in glutamatergic systems and mechanisms, this may nevertheless prove true. As such, there may be glutamatergic sub-types in which one group compensate for NMDAR dysfunction (increased glutamate), whereas the other group does not (decreased glutamate). If this is the case, the emotional valence of AVHs may act as a marker for this, being relatively more positive and negative for the former and latter group, respectively. Therefore, proper assessment of emotional valence may prove invaluable both to fields of research in general and specifically to successful treatment of patients experiencing AVHs in schizophrenia.

Although no currently available antipsychotic medications specifically target the glutamate system (Falkenberg et al., 2014), as noted by Poels et al., (2014), with the rapid and continual technological and methodological development of MRS techniques, future studies and drug development of novel and putative (e.g. glutamatergic) medications may benefit from investigating glutamatergic indices post-treatment. Through this, future studies may be able to further elucidate the role of glutamate, thereby increasing our understanding of AVHs as a symptom and schizophrenia as a disorder.

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