May 2015

TOWARDS A BETTER UNDERSTANDING OF PRIMARY NEGATIVE SYMPTOMS: A LONGITUDINAL STUDY IN FIRST-EPISODE PSYCHOSIS

A thesis submitted to McGill University
in partial fulfillment of the requirements for the degree of
Ph.D. in Clinical Psychology

By

Michael Bodnar

Department of Psychology
Faculty of Science
McGill University, Montreal

Supervisor: Dr. Martin Lepage

Table of Contents

Abstract

Background: Schizophrenia is characterized by positive (hallucinations, delusions) and negative (blunted affect, avolition) symptoms. Negative symptoms can be classified as either primary (central to the illness) or secondary (induced by positive symptoms, depression, or extrapyramidal symptoms, for example). Primary negative symptoms have been more consistently and robustly related to a worse functional outcome and still represent an unmet therapeutic need. This project set out to increase our understanding of these core symptoms by: 1) exploring the proportion of primary and secondary negative symptoms among those who did not remit; 2) examining medication adherence and clinical insight (awareness of mental illness, belief in response to medication, and belief in need for treatment) in relation to primary negative symptoms; and 3) confirming previous neuroimaging markers of remission and exploring for markers of primary negative symptoms.

Participants and setting: The final sample included 385 first-episode of psychosis (275 diagnosed with schizophrenia or a related spectrum disorder) clients treated from January 2003 through April 2015 at the Prevention and Early Intervention Program for Psychoses at the Douglas Mental Health University Institute in Montreal, Canada. For the neuroimaging data, there were 101 first-episode of schizophrenia clients who completed a baseline MRI scan, of which, 75 completed a 1-year follow-up scan.

Main outcome measures: Remission was defined as achieving a global rating of mild or less on eight core symptoms (four positive and four negative) and maintained for six months (Andreasen et al. (2005) *Am J Psychiatry*, 162, 441-449). Primary negative symptoms (PNS) was defined as a global rating of moderate or worse severity on one negative symptom sustained for six months in the absence of clinically relevant positive, depressive, and extrapyramidal symptoms (Hovington et al. (2012) *BMC Psychiatry*, 12, 1-11). Ratings were based on the Scale for the Assessment of Negative Symptoms (Andreasen, N.C. (1984) Iowa City, University of Iowa) and the Scale for the Assessment of Positive Symptoms (Andreasen, N.C. (1984) Iowa City, University of Iowa).

Results: After one year of treatment, 20% of clients were considered to be full remission. Among the Non-Remitted clients, 40% presented with PNS and 28% with secondary negative symptoms (2nd-NS). Similar proportions were found after two years of treatment and 1-year outcome significantly predicted 2-year outcome. Clients with PNS and 2nd-NS displayed poorer insight on all three insight variables across the first year of treatment compared to all other clients. Intriguingly, insight did not alter as a function of medication adherence among the PNS clients, but did among the other clients with a significant effect observed for 'belief in the need for treatment'. Finally, smaller hippocampal tail and parahippocampal cortex (PHC) volumes were verified as markers of not achieving remission. Now, compared to the other Non-Remitted clients, those with PNS had a significantly smaller PHC volume but did not differ in hippocampal tail volume. Moreover, there was a significant decrease in right PHC volume in the PNS clients over the one year follow-up period with a trend-level decrease in the left PHC.

Conclusions: A large proportion of unremitted clients presented with PNS. In contrast to much of the current literature, clients with PNS do appear amenable to treatment; however, current treatments for PNS are rather inadequate and newer, more efficacious treatments are needed. A smaller PHC volume may represent a distinct neurobiological marker for PNS which could help guide future research in developing target-specific treatments. Moreover, this finding suggests that clients with PNS may represent a distinct subtype. The concept of remission may need to be reformulated to account for those presenting with PNS.

Résumé

Contexte théorique : La schizophrénie est caractérisée par la présence de symptômes positifs (hallucinations, idées délirantes) et négatifs (baisse de la motivation, anhédonie). Les symptômes négatifs peuvent être classifiés comme étant soit primaires (central à la maladie) ou secondaires (p.ex. induits par des symptômes positifs, dépressifs, ou extrapyramidaux). Les symptômes négatifs primaires sont généralement associés à une issue fonctionnelle défavorable. Malgré cela, aucune avenue thérapeutique ciblant spécifiquement ceux-ci n'est disponible à ce jour. Ainsi, ce projet vise à accroître notre compréhension de ces symptômes en: 1) explorant la proportion de symptômes négatifs primaires et secondaires présents auprès des individus atteignant la rémission; 2) examinant l'observance au traitement et le niveau de conscientisation ou *insight* (c.à.d. : prise de conscience du trouble de santé mentale, confiance quant à la réponse à la médication et croyance en la nécessité d'un traitement) en relation avec les symptômes négatifs primaires; et 3) confirmant les marqueurs d'imagerie cérébrale de la rémission mis en lumière par des études antérieures et en explorant la présence possible de marqueurs neuronaux des symptômes négatifs primaires.

Participants : L'échantillon final comprenait 385 clients diagnostiqués avec un premier épisode de psychose (275 diagnostics de schizophrénie ou d'un trouble du spectre de la schizophrénie) traités de Janvier 2003 à Avril 2015 au sein du programme d'évaluation, d'intervention et de prévention des psychoses de l'Institut Universitaire en Santé Mentale Douglas (Montréal, Canada). Quant aux données de neuroimagerie, 101 clients diagnostiqués avec un premier épisode de psychose ont pris part à un examen d'imagerie par résonance magnétique (IRM) initial. Parmi ceux-ci, 75 clients ont complété un IRM de suivi 1 an après le début du traitement.

Mesures de la rémission : La rémission a été définie comme un score d'intensité légère ou moindre sur huit symptômes cardinaux (quatre positifs et quatre négatifs) maintenu pendant six mois (Andreasen et al. (2005) Am J Psychiatry, 162, 441-449). Les symptômes négatifs primaires (SNP) ont été définis comme un score d'intensité modérée ou sévère sur un symptôme négatif et persistant pour une durée de six mois en l'absence de symptômes positifs, dépressifs et extrapyramidaux cliniquement significatifs (Hovington et al. (2012) BMC Psychiatry, 12, 1-11). Les échelles de symptômes utilisées ont été basées sur les mesures suivantes : 1) Scale for the Assessment of Negative Symptoms (Andreasen, N.C. (1984) Iowa City, University of Iowa); et 2)

Scale for the Assessment of Positive Symptoms (Andreasen, N.C. (1984) Iowa City, University of Iowa).

Résultats : Après un an de traitement, 20% des clients étaient considérés en rémission complète. Parmi les clients n'atteignant pas l'état de rémission, 40% présentaient des SNP et 28% présentaient des symptômes négatifs secondaires (2e-SN). Des proportions similaires ont été observées après deux ans de traitement et les résultats aux mesures à 1 an post-admission ont prédit significativement les résultats à 2 ans post-admission. Les clients avec des SNP et 2e-SN ont présenté un *insight* plus faible sur les trois variables de mesures de l'*insight* tout au long de la première année en comparaison avec le reste de l'échantillon. De façon surprenante, l'*insight* n'a pas été modifié par l'observance au traitement parmi les clients avec des SNP, mais un impact significatif de la « croyance en la nécessité d'un traitement » a été révélé pour les autres clients. Par ailleurs, des volumes plus faibles au niveau de la région postérieure de l'hippocampe et du cortex parahippocampique (CPH) ont été identifiés comme des marqueurs d'une rémission non atteinte. En comparaison avec les autres clients n'atteignant pas la rémission, les clients avec des SNP présentaient des volumes significativement plus petits au niveau du CPH. Aucune différence n'était observée pour la région postérieure de l'hippocampe. Enfin, une diminution significative du volume du CPH droit et une tendance vers une diminution significative du volume du CPH gauche ont été obtenues chez les clients avec des SNP durant la période de suivi d'un an.

Conclusions : Une proportion importante des clients n'atteignant pas la rémission présentait des SNP. Contrairement à ce qu'une vaste littérature actuelle suggère, les clients avec des SNP peuvent observer un traitement tout comme les autres patients ; toutefois, les traitements actuels ciblant les SNP sont inadéquats. Le développement de nouveaux traitements plus efficaces et adaptés à cette symptomatologie sont nécessaires. Finalement, un plus petit volume du cortex parahippocampique pourrait représenter un marqueur neurobiologique spécifique aux SNP et la découverte de ce marqueur permettra d'orienter la recherche future dans le développement de traitements spécifiques à des régions cérébrales. De plus, ce résultat suggère que les clients ayant des SNP peuvent représenter un sous-type distinct. Ainsi, une reformulation du concept de rémission pourrait être envisagée afin d'inclure les clients présentant des SNP.

Acknowledgements

There are many individuals to whom I owe my sincerest and greatest appreciation because without them my second thesis would not have been possible.

I would first like to thank Dr. Martin Lepage (James McGill Professor of Psychiatry). I said it before and I say it again, for everything, I thank you very much as both my research supervisor (again) and as my clinical supervisor. After I completed my first PhD with you in 2011, I thought to myself "That was so much fun, let's do it again!!" I think I may have exaggerated this story a smidgeon but when asked to do this all over again, without hesitation, you agreed. I know of no other person who would have done that, especially in a time when funding was (and still is) hard to come by. It has been a short 3 years and the time literally flew by. It was an honour and privilege to have worked with you and I hope we can continue to do so for many more years.

I would like to recognize all of the staff at PEPP. Without their efforts and the amazing treatment provided, I would not have had one of the best characterized samples to work with (again). My appreciation and admiration for the PEPP team has been exponentially increased as I had the privilege of working there as an intern psychologist. I can honestly say we truly have one of the most caring and dedicated treatment teams. And with that, I owe my greatest appreciation to all of the clients. I truly admire your efforts in providing us with the data we base all of this research on. I cannot begin to imagine what it must be like to experience such a life changing event and then go through all of those interviews and assessments. I extend a special thank you to those who further accepted to be part of our neuroimaging study.

To all past and present people from the Lepage lab. I am privileged knowing I will leave here (for how long this time, I don't know) knowing each of you have become and will remain my very good friends. To Karyne and Maike: the lab would literally be in shambles without the two of you; plus I would not have survived without Karyne's "candy drawer". To Carolina: your drive and passion for research are truly amazing; without hesitation I would be honoured to continue working with you (and it was great sharing an office with you). To Synthia: you have been a wonderful friend and always will be; soon you will be in Boston and then life will truly begin.

To the most wonderful and caring of all people who pushed me through the application process and got me on my way towards a second PhD. Giovanna and Chantale: I will always have the greatest appreciation for what you both did for me (and continue to do for all of students alike). I literally would not be where I am today without the two of you; thank you for everything.

To my clinical psychology cohort. I can honestly say to you all - Claire, Sue, Miriam, Molly, and Valérie - it was my honour to have met and gotten to know you so well over the past three years. I know we will continue to be friends no matter where our lives take us. And remember, we started the change to make the clinical program better at McGill that will continue for many years to come.

I also have the greatest appreciation for the support staff at the Montreal Neurological Institute Brain Imaging Centre. Ron, Dave, and Louise made collecting the imaging data a breeze for everyone involved. I would also like to thank the organizations that provided the requisite financial support. This project was funded by the Fonds de la recherche en santé du Québec and the Canadian Institute of Health Research awarded to Drs. Malla and Lepage.

To my beautiful Sara. You have carried me through the past three years with a never-ending love. You are truly an amazing person and the best thing that could have ever happened to me in my life. I truly do not have the words to express all that you mean to me.

Finally, to Mom and Тато. I wrote in my last thesis that “I am almost finished with school (well my PhD anyways)”. Who would have thought I would do this again? Nobody. Yet, no matter what I chose to do in life, you both have always told me you were proud of me and supported me in any decision. I know this has been the reason why I have been able to go on for so long, but I can finally say I am done. Мій другий теза закінчена, і гарна погода. Дякую тобі за все.

Preface & Contributions of Authors

All elements of this thesis are considered original scholarship and distinct contributions to the advancement of knowledge in the field of schizophrenia and the related psychoses.

Bodnar, M., Malla, A.K., Joober, R., & Lepage, M. (in preparation). Should remission include primary negative symptoms? A longitudinal behavioural and neuroimaging study involving first-episode schizophrenia.

Dr. Martin Lepage, Dr. Ashok K. Malla, and I designed this analysis. I undertook all statistical analyses and wrote the first draft of the manuscript. Drs. Malla and Joober managed all patient recruitment and clinical assessments.

Bodnar, M., Malla, A.K., Baer, L.H., Joober, R., & Lepage, M. (in preparation). A longitudinal study into clinical insight, primary negative symptoms, and medication adherence in first-episode of psychosis.

Dr. Lepage, Dr. Malla, and I designed this analysis. I undertook all statistical analyses. Dr. Lepage, Dr. Lawrence Baer, and I wrote the first draft of the manuscript. Drs. Malla and Joober managed all patient recruitment and clinical assessments.

Bodnar, M., Hovington, C.L., Buchy, L., Malla, A.K., Joober, R., & Lepage, M. (2014). Cortical thinning in temporo-parietal junction (TPJ) in non-affective first-episode of psychosis patients with persistent negative symptoms. *PLoS One, 9*(6), e101372.

Dr. Lepage, Dr. Malla, Dr. Hovington, and I designed this analysis. Dr. Lisa Buchy and I undertook all statistical analyses. I wrote the first draft of the manuscript. Drs. Malla and Joober managed all patient recruitment and clinical assessments. All authors contributed to revisions of the manuscript.

Bodnar, M., Malla, A.K., Makowski. C., Joober, R., & Lepage, M. (submitted). The effect of second-generation antipsychotics on hippocampal volume and verbal memory performance in first episode psychosis: a longitudinal neuroimaging study

Dr. Lepage, Dr. Malla, and I designed this analysis. Ms. Carolina Makowski and I undertook all statistical analyses. I wrote the first draft of the manuscript. Drs. Malla and Joober managed all patient recruitment and clinical assessments. All authors contributed to revisions of the manuscript.

Chapter 1

Introduction

1.1 Schizophrenia and the related psychoses

Schizophrenia and the related psychoses can affect anyone. Psychotic disorders are characterized by positive (e.g., hallucinations, delusions), negative (e.g., blunted affect, avolition), and cognitive symptoms (e.g., disorganized thinking, impaired memory and executive functioning). About 3% of all people aged 15 to 25 will experience a first-episode of psychosis. More specifically, there is a lifetime prevalence rate of 0.5 to 1.9% for schizophrenia (van Os & Kapur, 2009).

Schizophrenia is arguably the most serious of all the mental health disorders, interrupting young peoples' life trajectories through relapses and deterioration in social and cognitive functioning. Across the entire life-span, schizophrenia now ranks third in the world in causes of disability-adjusted life years (Cohen, Meesters, & Zhao, 2015). Moreover, a review examining healthcare cost found total expenditures for schizophrenia exceeded those for depression, dementia, or medical disorders across all age cohorts, except between the ages of 45 to 64 where dementia showed the highest expenditure (Bartels, Clark, Peacock, Dums, & Pratt, 2003).

As such, there is a growing need to better understand outcome – from remission to recovery – to help discover newer, improved treatments to offer a better outcome for more people and, at the same time, reduce the associated healthcare costs.

1.2 Outcome from schizophrenia

In general, there are two basic aspects to outcome: functional and clinical. Functional outcome can be defined as (or measured by), for example: the quality of life, stable employment, and the ability to live independently (Foussias, Agid, Fervaha, & Remington, 2014; Heering, Janssens, Boyette, van Haren, & investigators, 2015). Alternatively, clinical outcome is focused more on psychopathology and can be understood as: a therapeutic response to antipsychotics (Joober et al., 2002), number of hospitalizations (Verdoux, Liraud, Assens, Abalan, & van Os, 2002), persisting positive or negative symptoms, and achieving remission (Leucht, 2014).

A definition of remission exists for most non-psychiatric and many psychiatric illnesses, but no consensus regarding an internationally accepted definition existed for schizophrenia prior to 2005. The working group chosen to create the definition decided on a mild level rating or less on eight core signs and symptoms to be maintained for six months. (Andreasen et al., 2005). Since then,

the definition has been validated and has been described as a viable method to consistently measure clinical outcome; however, remission rates have been shown to vary from 17% to 78% (AlAqeel & Margolese, 2012; Lambert, Karow, Leucht, Schimmelmann, & Naber, 2010). There are a number of factors related to this heterogeneity of outcome, but one of the strongest predictors of not achieving remission has been more severe negative symptom levels at baseline and throughout the treatment process (AlAqeel & Margolese, 2012). Moreover, with respect to recovery, several large scale and long-term outcome studies identified negative symptoms, social functioning, medication adherence, and type of antipsychotic as predictors of recovery (Albert et al., 2011; Austin et al., 2013; Novick, Haro, Suarez, Vieta, & Naber, 2009; Shrivastava, Shah, Johnston, Stitt, & Thakar, 2010). In fact, negative symptom severity has been found as the most robust and consistent marker of a poorer outcome in general (Foussias et al., 2014; Leucht, 2014; Torrey, 2013).

1.3 What are negative symptoms?

According to consensus, there are five core negative symptoms: flat affect, alogia, avolition, asociality, and anhedonia (Kirkpatrick, Fenton, Carpenter, & Marder, 2006). As defined and measured by the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1984a), affective flattening is best described as a failure to express feelings, either verbally or non-verbally, when one would normally be expected to present an emotional response. Alogia is basically poverty of speech; i.e., a lack of additional content when engaged in conversation. Avolition is defined as lack of drive to perform activities or pursue meaningful goals, whereas apathy is a lack of interest or concern. Asociality is as it sounds; a disengagement in social activities or, alternatively, as a preference for solitary activities. Finally, anhedonia is seen as the general inability to experience pleasure from activities that should normally be found as enjoyable.

However, since first being fully described by Nancy Andreasen in 1982 (Andreasen, 1982), a number of changes in the understanding of these symptoms have occurred. One notable change involved anhedonia. Anhedonia has been refined as the original definition failed to account for differences between anticipatory (‘wanting’) and consummatory (‘liking’) pleasure. This change was driven by findings that revealed people with schizophrenia showed a lack of anticipatory pleasure seeking behaviour, whereas consummatory pleasure was found to be intact (Foussias et

al., 2014; Foussias, Siddiqui, Fervaha, Agid, & Remington, 2015). Although recent findings have revealed more ambiguous findings concerning these two parts of anhedonia, it is just one example of how definitions need to be dynamic to help improve of understanding of a complex illness like schizophrenia.

A second major change for the negative symptoms has come from factor analyses that have suggested there are two separate, yet related, subdomains of symptoms: *diminished expression* (affective flattening and poverty of speech) and *amotivation* (avolition/apathy, asociality, and anhedonia). This factor structure has been consistently found in both first-episode and enduring populations (Foussias et al., 2014; Messinger et al., 2011). These refinements in our understanding of the negative symptoms has led to the development of newer scales that can capture the abovementioned aspects (Kirkpatrick, 2014). As highlighted by Foussias et al (Foussias et al., 2015), the Brief Negative Symptom Scale (BNSS) (Kirkpatrick et al., 2011) and the Clinical Assessment Interview for Negative Symptoms (CAINS) (Kring, Gur, Blanchard, Horan, & Reise, 2013) both incorporate assessments of consummatory and anticipatory pleasure, and include refined evaluations of the two subdomains of symptoms. The BNSS also offers assessment for motivational deficits with separate ratings for internal experience (self-report) and the external behavioural indicators.

Of note, when this research study commenced in 2003, the gold standard for measuring negative symptoms was the SANS. As such, the work from this thesis was limited to the use of this scale and with definitions as originally defined by Andreasen in 1982 (Andreasen, 1982). The information provided above was to only highlight the changing understanding of what constitutes the negative symptoms.

1.4 A brief history of negative symptoms in schizophrenia

For many, the first descriptions of negative symptoms extends back to the earliest descriptions of schizophrenia by Kraepelin and Blueler (Bleuler, 1952; Kraepelin, 1919). Kraepelin began his book by declaring a "weakening…of volition" to describe dementia praecox (how schizophrenia was referred to then). Blueler went as far as to separate primary symptoms (e.g., abnormality in volition, complete lack of emotional and affective expressions) from accessory symptoms (e.g.,

delusions, hallucinations) (Bleuler, 1952). In what will become key later on, he described four types of schizophrenia that, of importance, included two types with predominant negative symptoms: 'hebephrenia', where accessory symptoms appear but do not dominate; and 'simple schizophrenia', where accessory symptoms are absent (Bleuler, 1952).

Blueler was not the first to describe hebephrenia. In fact, it was first described in 1863 by Karl Kahlbaum (Kahlbaum, Berrios, & Kraam, 2002), the mentor of Ewald Hecker who, in turn, produced a seminal article in 1871 describing multiple cases of people with hebephrenia. Hecker ultimately believed that hebephrenia stood "as a unitary mental illness in its own right" but needed evidence which could only be "provided by pathologic-anatomic facts" (Hecker & Kraam, 2009). He finally stated that "in hebephrenia one can only talk about recovery with defect" (Hecker & Kraam, 2009).

But prior to these systematic descriptions, the earliest mention of negative symptoms, as related to psychosis, was by Nikolai Gogol (Gogol, 1835). The novel, *Diary of a Madman*, provides a first-person account of a middle-aged man as he enters into psychosis. A number of passages highlight a volitional state. The book opens with "a strange occurrence has taken place to-day. I got up fairly late …When I heard it had long struck ten, I dressed as quickly as possible" (p.2). Near the end, before the man is taken to the asylum, he stated "to-day the office-messenger came and summoned me, as I had not been there for three weeks" (p.24). This story provided the first written account of someone not only entering psychosis but also of a low volitional lifestyle.

In the brief history provided above, as there are many more people who could be acknowledged from Wilhelm Griesinger (Kirkpatrick, 2014) to John Russell Reynolds and John Hughlings Jackson (Berrios, 1985), there is a general theme that negative symptoms have been central to schizophrenia and the related psychoses since first being described (Tandon et al., 2013).

1.5 Primary vs. secondary negative symptoms

From the abovementioned, it becomes clear that negative symptoms can be primary, or central to the illness, or secondary, manifest as a result of other symptoms. In 1988, William Carpenter and colleagues, set out to define and distinguish the two (Carpenter, Heinrichs, & Wagman, 1988) which was beautifully summarized by Brian Kirkpatrick in 2014:

> It is important to distinguish negative symptoms that are secondary to factors such as depression, a suspicious withdrawal, overwhelming psychotic symptoms, and extrapyramidal side effects from those symptoms that cannot be attributed to such factors. [Carpenter et al] termed symptoms that could not be attributed to these other factors—and therefore are due to the disease itself—primary symptoms, in contrast to symptoms secondary to these factors. People with schizophrenia who have enduring, primary symptoms were more likely to comprise a meaningful subtype than a negative symptom subtype that included patients with both primary and secondary symptoms (p.S102)

Thus, negative symptoms can be classified as primary (idiopathic) or secondary (manifest in relation to iatrogenic factors, environmental factors, or clinically relevant positive, depressive, or extrapyramidal symptoms).

To distinguish this subtype with primary negative symptoms within schizophrenia, Carpenter and colleagues (1988) coined the termed "deficit syndrome" and then created the Schedule for the Deficit Syndrome (SDS) (Kirkpatrick, Buchanan, McKenney, Alphs, & Carpenter, 1989) to help identify those with enduring, primary negative symptoms. However, the SDS requires specialized training and not all clinicians or researchers have this available to them. In response, Carpenter's group created the Proxy for the Deficit Syndrome (PDS) allowing the identification of deficit syndrome using the Brief Psychiatric Rating Scale (BPRS) (Kirkpatrick, Buchanan, Breier, & Carpenter, 1993; Overall & Gorham, 1962), which has been extended for use with the Positive and Negative Syndrome Scale for Schizophrenia (Goetz et al., 2007; Kay, 1987).

For both the SDS and PDS, symptom ratings must be maintained for at least 12 consecutive months to identify someone with deficit syndrome. Robert Buchanan highlighted that people with a first-episode of psychosis, who may be presenting with primary negative symptoms, may not have been in treatment long enough to use the SDS or PDS to confirm these symptoms are indeed primary (Buchanan, 2007). As such, he suggested an alternative concept - persistent negative symptoms. Although very similar to deficit syndrome, there were three key differences: 1) the time criterion was lowered to 6 consecutive months; 2) persistent negative symptoms included both primary and secondary negative symptoms; and 3) identification could be accomplished using any mainstream

rating scale (SANS, PANSS, BPRS) (Buchanan, 2007). In any case, primary negative symptoms could be identified in people with chronic (or enduring) schizophrenia or with a first-episode of psychosis.

Using the above mentioned scales, negative symptoms have been found to be present in 30–76% of people schizophrenia (Foussias et al., 2015). Intriguingly, primary negative symptoms make up a significant proportion having a prevalence rate of 15%-27% in people with a first-episode of psychosis (Buchanan, 2007; Chang et al., 2011; Hovington, Bodnar, Joober, Malla, & Lepage, 2012) and 15–30% in people with enduring schizophrenia (Foussias et al., 2015; Kirkpatrick et al., 2006). Such a high prevalence has further fuelled the debate as to whether or not those with primary negative symptoms truly make up a subtype within schizophrenia (very similar to what Bleuler had originally described as 'simple schizophrenia') (Bleuler, 1952).

1.6 A separate syndrome - primary negative symptoms

The idea there may be a stable, underlying illness within schizophrenia had researchers scrambling to find markers now that there were validated methods for distinguishing primary from secondary negative symptoms. Over the years following, those with primary negative symptoms, compared to those without, have been found to show a poorer awareness of mental illness (poorer insight) (Chang et al., 2011; Kirkpatrick, Castle, Murray, & Carpenter, 2000; Kosger, Sahin, Essizoglu, & Aksaray, 2014; Trotman, Kirkpatrick, & Compton, 2011), greater impairment of neurocognitive abilities (Galderisi et al., 2013; Galderisi et al., 2002; Kirkpatrick, Buchanan, Ross, & Carpenter, 2001), poorer social functioning, and worse functional outcome (Fervaha, Foussias, Agid, & Remington, 2014; Foussias et al., 2014; Kirkpatrick, 2014). More fundamentally, structural and functional neuroimaging studies attempted to find differences between the two groups that could provide a better understanding of the neurobiology of negative symptoms, but only disparate findings have been produced (Galderisi, Merlotti, & Mucci, 2015). Nonetheless, reviews have highlighted structural abnormalities in the frontal (namely, the prefrontal area) and medial temporal (namely, the parahippocampal cortex) areas as the strongest potential neurobiological markers (Galderisi et al., 2015; Hovington & Lepage, 2012). Although there are a multitude of other factors to strongly suggest that those with primary negative symptoms constitute a separate

illness within schizophrenia (Kirkpatrick, 2014), a clear neuroanatomical marker is needed to provide more definitive support of a separate syndrome as first underscored in 1871 by Hecker.

1.7 Costs of treating negative symptoms

In 2014, Sicras-Mainar and colleagues undertook an investigation to calculate the costs that negative symptoms in schizophrenia bear on the healthcare system over a 12-month period (Sicras-Mainar, Maurino, Ruiz-Beato, & Navarro-Artieda, 2014). To determine this, they separated their sample into those with one or more negative symptoms (n=588) versus those without any negative symptoms (n=532). They examined both direct healthcare costs (medical visits, lab tests, prescriptions, and so on) and indirect costs (work days lost), and found the overall cost associated for all patients was 2.1 million euros with a significantly higher average healthcare cost for those with negative symptoms versus those without negative symptoms (individual cost of 2,085 euros vs. 1,659 euros, respectively). Overall, the presence of negative symptoms significantly increased the burden on the healthcare system, mainly derived from an extraneous use of primary care. This study was completed in Spain so healthcare costs may vary from country to country, but the overall finding highlights more healthcare resources are being used by these individuals. Of note, a follow-up article by the same group found a sedentary lifestyle and lack of physical exercise, which is often observed in those with prominent negative symptoms, may contribute to metabolic syndrome development (dyslipidemia, hypertension, and diabetes mellitus were the most frequent comorbidities); part of the reason for increased primary care usage (Sicras-Mainar, Maurino, Ruiz-Beato, & Navarro-Artieda, 2015).

1.8 Rationale for the study

Negative symptoms are a core feature of schizophrenia and the related psychoses that are strongly linked to a poorer outcome. A number of factors have been associated with negative symptoms that do not remit including poorer medication adherence and poorer insight. For the most part, these factors been identified in studies using a cross-sectional design; very few studies have explored their dynamic nature as treatment progresses. Moreover, and key to better understanding the fundamental nature of negative symptoms, no studies have actually compared those with

primary negative symptoms to those with secondary negative symptoms. This would offer new insights towards a better understanding of primary negative symptoms.

As highlighted, schizophrenia itself is a costly illness to treat with negative symptom severity associated with an even higher cost. Unfortunately, there are currently no efficacious pharmacotherapies available to treat negative symptoms (Davis, Horan, & Marder, 2014; Moller & Czobor, 2015). There is a growing need to better understand the neurobiological underpinnings of these symptoms to help explain their aetiology and to potentially help develop newer treatments (Arango, Garibaldi, & Marder, 2013; Davis et al., 2014). Although neuroimaging results have been equivocal, no study to date has directly compared those with primary negative symptoms to those with secondary negative symptoms and there have been no longitudinal neuroimaging studies to date (Galderisi et al., 2015).

1.9 Specific aims

Aim 1 – Quantifying remission in terms of primary negative symptoms.

Clients were first separated into Remitted and Non-Remitted groups. The Non-Remitted group was then subdivided into subgroups comprised of those with primary negative symptoms, secondary negative symptoms, and not remitted due to transient symptoms (i.e. those not meeting the six-month time criterion). The aims were: 1) to reveal what proportion of people did not remit due to primary negative symptoms; 2) to determine how well 1-year outcome relates to (or predicts) 2-year outcome; and 3) to explore how the positive and negative symptom profiles differed among the groups and changed over the 24-month follow-up period.

Aim 2 – Longitudinal structural neuroimaging analysis.

Among the subgroups described above, grey matter volumes in the medial temporal lobe (entorhinal cortex, perirhinal cortex, parahippocampal cortex, hippocampus tail, anterior hippocampus, and amygdala) at baseline and at a 1-year follow-up were estimated using FreeSurfer, a fully-automated processing program. The aims were: 1) to verify smaller hippocampal tail and parahippocampal cortex volumes at baseline as markers of not achieving remission; 2) to compare baseline grey matter volumes of the medial temporal lobe structures

among the subgroups; and 3) to explore for grey matter changes over a 1-year period in the medial temporal lobe structures among the subgroups.

Aim 3 – Explore the dynamic nature of medication adherence and clinical insight.

Clinical insight comprised of three variables: awareness of mental illness, belief in response to medication, and belief in need for treatment. Clients were separated into three groups comprising primary negative symptoms, secondary negative symptoms, or non-primary negative symptoms. Each group was then subdivided into those who were Fully-Adherent or Partially-Adherent to antipsychotic medications. The aims were: 1) to verify those with primary negative symptoms did indeed have poorer insight and medication adherence; and 2) to explore how the clinical insight profiles differed among the six subgroups and changed over a 24-month follow-up period.

Chapter 2

General Methods

2.1 Treatment setting and participants

All participants were part of a longitudinal naturalistic outcome study of first-episode psychosis (FEP) treated in a specialised early intervention service, The Prevention and Early Intervention Program for Psychoses (PEPP), at the Douglas Mental Health University Institute in Montreal, Canada.

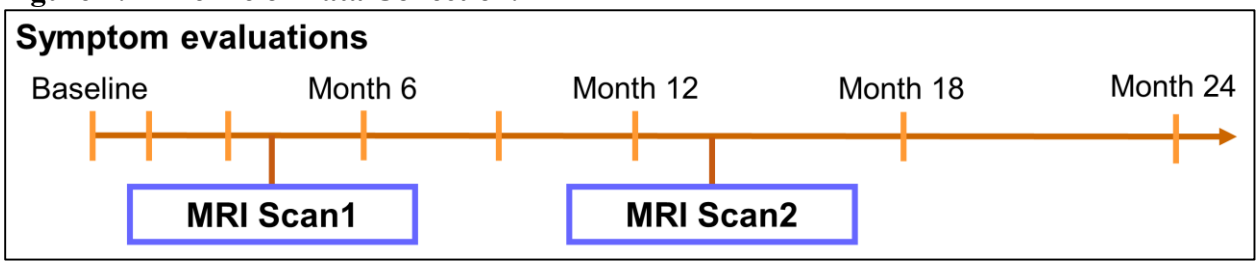
People aged 14–35 years from a predefined local catchment area experiencing either an affective or non-affective psychosis who had not taken antipsychotic medication for more than one month and had an IQ of 70 or above were consecutively admitted to the program as either in- or out-patients. There is no competing service and treatment is publicly funded. All PEPP clients were diagnosed according to DSM-IV criteria using the Structured Clinical Interview for DSM-IV (First, Spitzer, Gibbon, & Williams, 1998). Diagnoses were confirmed via consensus between the treating team and one of the on-staff research psychiatrists.

PEPP is a specialised early intervention service with integrated clinical, research, and teaching modules. The program involves a comprehensive approach with intensive medical and psychosocial management provided primarily through modified case management. Pharmacotherapy begins with a second generation antipsychotic medication (olanzapine, risperidone, quetiapine, ziprasidone, or aripiprazole) within the recommended doses for a period of 4 to 6 weeks followed by an assessment of therapeutic response. In case therapeutic response is not optimal or side effects develop, an alternate, second generation antipsychotic medication is prescribed. While specific treatment for psychosis begins with the initiation of antipsychotic medication, clients who initially refuse drug therapy are still provided with psychosocial support and education as well as their families. This may extend for weeks and, occasionally, for several months. In addition, all clients are provided modified assertive case management and interventions to assist in their recovery. For further details see (Iyer, Jordan, MacDonald, Joober, & Malla, 2015) or visit http://www.douglas.qc.ca/section/pepp-montreal-165?locale=en.

2.2 Longitudinal data collection

Initial clinical evaluations were conducted, on average, within one month after entry (Mean: 7.6 days, SD: 8.5, Range: -31 to 36) with interviews repeated at month 2, 3, 6, 9, 12, 18, and 24 past the first assessment; see Figure 1 for timeline of data collection.

Figure 1: Timeline of Data Collection.



2.2.1 Positive and negative symptoms

Positive and negative symptoms were assessed with the Hybrid Interview Schedule designed at PEPP that allows for the individual reconstruction of symptoms on the Positive and Negative Syndrome Scale (PANSS) (Kay, 1987), the Scale for the Assessment of Positive Symptoms (SAPS) (Andreasen, 1984b), the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1984a), and the Brief Psychiatric Rating Scale (BPRS) (Overall & Gorham, 1962).

Of particular note for this thesis, the SAPS and SANS each rate individual items and a global rating using a 6-point Likert scale [0 = absent, 1 = questionable, 2 = mild, 3 = moderate, 4 = marked, 5 = severe]. For the SAPS, there are four global symptoms: hallucinations (6 items); delusions (12 items); bizarre behaviour (4 items); and positive formal thought disorder (8 items). For the SANS, there are four global items: affective flattening (7 items); alogia (4 items); avolition-apathy (3 items); and anhedonia-asociality (4 items). The SANS also includes attention but this is no longer considered a part of the negative symptoms as confirmed through factor analyses (Foussias & Remington, 2010). Our raters at PEPP have established intra-class correlations (ICCs) of 0.89 and 0.71 on the SAPS and SANS, respectively.

2.2.2 Depressive symptoms

Depressive symptoms were assessed using the Calgary Depression Scale for Schizophrenia (CDSS) (D. Addington, Addington, & Schissel, 1990) which rates 9 items on 4-point Likert scale [0 = absent, 1 = mild, 2 = moderate. 3 = severe]. This scale has been validated to assess depressive symptoms in schizophrenia separate from negative symptoms (D. Addington, Addington, & Maticka-Tyndale, 1994; Muller, Muller, & Fellgiebel, 2006).

2.2.3 Medication adherence and antipsychotic dosage

Medication adherence was measured using a 5-point scale [0 = never (0%), 1 = very infrequently (1% to 25%), 2 = sometimes (26% to 50%), 3 = quite often (51% to 75%), 4 = fully (76% to 100%)] based on information obtained from patients and case managers. Patients were asked how often they missed a dose over the past month and adherence was calculated as a percent of prescribed doses taken; pill counting information was available for a subset of the sample. Pill count correlated highly to patient, family, and case manager reports of adherence; we have established an intra-class correlation of 0.84 using this technique (Cassidy, Rabinovitch, Schmitz, Joober, & Malla, 2010).

At each time-point, the type and dosage of antipsychotic prescribed were recorded with the dosage subsequently converted into chlorpromazine equivalents where necessary (Bollini et al., 2008; Jensen, 2012; Woods, 2003, 2011).

2.2.4 Clinical insight

Clinical insight was measured at each time point using a brief version of the Scale to Assess Unawareness of Mental Disorder (SUMD); items were rated using a 5-point Likert scale [1 = aware, 3 = somewhat, 5 = unaware] (Amador et al., 1993). For the purposes of this thesis, and as we have done so in previous work from the lab (Buchy, Bodnar, Malla, Joober, & Lepage, 2010; Lepage, Bodnar, Buchy, Joober, & Malla, 2010), exploration of insight was limited to the first three items: 'awareness of a mental illness' (Q1); 'awareness of response to medication' (Q2a); and 'belief in the need for treatment or would benefit from it' (Q2b). A sum of these three variables was also explored to provide a measure of overall clinical insight. Although ICCs for the SUMD

were not available, raters at PEPP have achieved an ICC of 0.79 for the insight item (G12) on the PANSS which shares many similarities with the SUMD-Q1.

2.2.5 Neuropsychological assessment

A first assessment, standardized cognitive battery was completed by all participants; tested and scored by a trained professional who was not involved with the treatment of the patient. Patients were assessed after the initiation of treatment and only when in a stable but not necessarily asymptomatic condition; assessments were conducted, on average, around three months after PEPP entry (Mean = 12.6 weeks, SD =9.4, Range = 0.1 - 54.0).

Of particular interest for this thesis, Full-scale IQ was estimated using either the Wechsler Adult Intelligence Scale – Third Edition (WAIS-III) (Wechsler, 1997a) or Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999). Also, verbal memory was assessed using either the Logical Memory subtest from the Wechsler Memory Scale - Third Edition (WMS-III) (Wechsler, 1997b) or the Shopping List subtest from CogState (Collie, Darby, & Maruff, 2001; Maruff et al., 2009). Two different scales or tests are listed for each variable of interest due to a change in our neuropsychological battery in December 2010. The protocol was changed to reduce the total assessment time from an average of 3 hours to an average of 2 hours.

2.2.6 Structural neuroimaging data

Among the clients followed at PEPP, as per ethics approval, only those over the age of 18 with no previous history of neurological disease or head trauma causing loss of consciousness were eligible for the neuroimaging study. Scanning took place only when clients were stable enough to tolerate the scanning session with suitability to participate reassessed on a weekly basis until our clinical and research team agreed that acute symptoms would not interfere with the protocol.

Scanning was carried out at the Montreal Neurological Institute (MNI) on a dedicated clinical research 1.5 Tesla Siemens Sonata whole-body Magnetic Resonance Imaging (MRI) scanner. The structural MRI sequence for each participant consisted of a high-resolution T1-image covering the whole brain and was acquired using a three-dimensional (3D) gradient echo pulse sequence with sagittal volume excitation (repetition time=22ms, echo time=9.2ms, flip angle=30º, 180 1mm

contiguous sagittal slices). The rectangular field-of-view for the images was 256mm (SI) x 204mm (AP).

Healthy controls were recruited through advertisements in local newspapers and were chosen only if they had no current or past history of 1) any Axis I disorders, 2) any neurological diseases, 3) head trauma causing loss of consciousness, and 4) a first-degree family member suffering from schizophrenia or related schizophrenia spectrum disorders. Healthy controls were included to provide normative data for brain imaging and neurocognitive measures. They were also chosen based on age, sex, handedness, and parental SES matched to the clients taking part in the neuroimaging study. Neurocognitive and neuroimaging data were collected for all healthy controls recruited.

2.2.7 Socio-demographic and other clinically relevant variables

Other pertinent data were obtained through a semi-structured interview conducted at baseline by trained research personnel from PEPP with the patient and the family member with the most contact with the patient both present. Additional necessary information was obtained from case managers, health records, and, whenever possible, school records. The interview included the Circumstances of Onset and Relapse Schedule (CORS) which includes material adapted from the Interview for Retrospective Assessment of Onset of Schizophrenia (IRAOS) (Hafner, 1998). From this interview, such variables as duration of untreated psychosis (DUP), duration of untreated illness (DUI), premorbid functioning levels, and socioeconomic status (SES) were obtained. Duration of untreated psychosis was calculated as the period between the time of onset of psychotic symptoms, at syndromal threshold based on the SCID, to adequate treatment with antipsychotics (30 days of continuous treatment or less if positive symptoms remitted) (Malla et al., 2002). Any previous periods of psychosis which had resolved spontaneously were added to the total calculation of DUP thus reflecting cumulative exposure to psychosis prior to treatment. Duration of untreated illness was defined as the time period from onset of any psychiatric symptoms (anxiety, depression, suicidal ideation, or social withdrawal) to adequate treatment with antipsychotics (Malla et al., 2006). Parental SES during upbringing was measured with the Hollingshead two-factor index of social position (Hollingshead, 1965) and handedness with the Edinburgh Handedness Inventory (Oldfield, 1971).

2.3 Defining remission

Following the proposed criteria by the Remission in Schizophrenia Working Group, remission was defined as a rating of mild (2) or less on all four global items of the SAPS (hallucinations, delusions, bizarre behaviour, and though disorder) and SANS (flat affect, alogia, avolition-apathy, or anhedonia-asociality), and maintained for at least six consecutive months (Andreasen et al., 2005).

2.4 Identifying primary and secondary negative symptoms

Primary negative symptoms can be identified using a variety of methods (Buchanan, 2007; Hovington et al., 2012; Kirkpatrick et al., 1993; Kirkpatrick et al., 1989). In the current study, primary negative symptoms were identified using the persistent negative symptom approach (Hovington et al., 2012).

Clients were identified with persistent (primary) negative symptoms (PNS) if they had a global rating of moderate (3) or more on at least one negative symptom (flat affect, alogia, avolition-apathy, or anhedonia-asociality) as measured with the SANS. To ensure negative symptoms were primary in nature, clients identified with PNS had to have a global rating of mild (2) or less on all global ratings of positive symptoms, as measured with the SAPS, a total score of 4 or less on the CDSS, and not present with extrapyramidal symptoms requiring anticholinergics. All criteria had to be maintained for at least 6 consecutive months.

From this definition, we are able to identify those with PNS and those with secondary negative symptoms (2nd-NS). People with 2nd-NS displayed severe negative symptoms for 6 consecutive months but in the presence of clinically relevant positive, depressive, and/or extrapyramidal symptoms.

2.5 Ethics statement

All research was conducted according to the guidelines laid out by the Declaration of Helsinki, and was approved by the Research Ethics Board of the Douglas Mental Health University Institute and the McGill University Faculty of Medicine Research Ethics Board. All participants provided

written informed consent prior to engaging in any research-related activity, and were free to withdraw from the study at any time; verbal consent was not considered adequate.

Particular to the clients, for the collection and disposition of clinically-based data, if a client was under 18 years of age or deemed incapable to properly represent themselves, written informed consent was obtained from the next of kin, caretaker, or legal guardian. The capacity for individual clients to provide consent was determined by the individual treating team (psychiatrist, case manager, and clinical evaluator) and confirmed by one of our in-staff research psychiatrists. For the collection and disposition of the neuroimaging data, only those aged 18 years and over were recruited from the PEPP clinic, and only after obtaining written informed consent for the collection and disposition of clinically-based data.

Chapter 3

Preamble to Article 1

For the first manuscript in this thesis, PubMed and Google Scholar were searched for articles and reviews published between January 2005 and May 2015 that included the search terms "remission" and "schizophrenia" and identified two main reviews that highlighted the heterogeneity of remission rates, with negative symptom severity as a strong determiner of not achieving remission. A second search was conducted with no time constraints for articles and reviews exploring primary negative symptoms alone and those that examined them in relation to neuroimaging; the search terms included "schizophrenia", "psychosis", "primary negative symptoms", "persistent negative symptoms", "deficit syndrome", "MRI", "neuroimaging", "hippocampus", and "parahippocampus". This search identified five main reviews, two pertaining to neuroimaging. The behavioural reviews summed that primary negative symptoms represent an unmet therapeutic need and people presenting with these symptoms may represent a distinct subtype within schizophrenia. The neuroimaging reviews highlighted the main areas of interest have been the frontal region and medial temporal lobe; however, findings have not been consistent and all studies have been cross-sectional - no study to date had looked for changes in grey matter volume over time.

The results for Article 1 have not been published but the manuscript is ready for submission. The prepared manuscript contained a 'Supplementary Material' file that has been included in this thesis as a separate section directly following the manuscript. This extra material provides a complete description of methods and results, as well as supplementary Figures and Tables.

Article 1: Should remission include primary negative symptoms? A longitudinal behavioural and neuroimaging study involving first-episode schizophrenia.

Abstract

Background. Remission in schizophrenia equates positive and negative symptoms in determining outcome. With treatments for primary negative symptoms rather inadequate and these symptoms more robustly related to functional outcome, should positive and negative symptom remission be separate.

Methods: Our sample included 275 first-episode of schizophrenia clients treated through an early intervention service; 101 had a baseline MRI scan and 75 had a 1-year follow-up scan. Following the remission definition (i.e., mild or less severity on positive and negative symptoms for six months), clients formed Remitted and Non-Remitted groups; the latter was further subdivided. Those not meeting the time criterion formed the 'Non-Remitted-transient' subgroup. The remainder were separated based on the presence or not of primary negative symptoms (PNS; defined as a moderate or worse severity on one negative symptom sustained for six months in the absence of positive, depressive, and extrapyramidal symptoms). Those presenting with the latter formed the secondary negative symptom (2nd-NS) subgroup. Subgroup proportions were compared at 1-year and 2-year outcome. Differences in parahippocampus, hippocampus, and amygdala volumes, estimated using FreeSurfer, were explored among the subgroups.

Findings: At 1-year and 2-year outcome, 40% of unremitted clients presented with PNS. Smaller hippocampal tail and parahippocampal cortex (PHC) volumes were verified as markers of not achieving remission. Compared to the other Non-Remitted clients, those with PNS had a significantly smaller PHC volume ($p=0.021$) but did not differ in hippocampal tail volume ($p=0.424$). Finally, there was a significant decrease in right PHC volume in the PNS clients over the one year follow-up period ($p=0.002$) with a trend-level decrease on the left ($p=0.085$).

Interpretation: A large proportion of unremitted clients had PNS who presented with a distinct neurobiological marker (smaller PHC volume), suggesting these clients may represent a distinct subtype. With no efficacious treatments currently available for PNS, the concept of remission may need to be reformulated to account for PNS. The PHC may help guide future research for in developing target-specific treatments.

1. Introduction

Schizophrenia is characterized by positive (hallucinations, delusions) and negative (diminished expression, amotivation) symptoms. The remission definition in schizophrenia has equated the importance of these symptoms in determining clinical outcome (1). However, negative symptoms have been related to a poorer functional outcome, more robustly for primary negative symptoms (PNS) as opposed to broadly-defined negative symptoms and secondary negative symptoms (2nd-NS; severe negative symptoms in presence of clinically relevant positive, depressive, or extrapyramidal symptoms) (2, 3). Thus, identifying unremitting symptoms at multiple levels (e.g., PNS vs. 2nd-NS vs. Remitted) would highlight where current treatments may not be as effective and aid in identifying the underpinnings (e.g., neurobiological markers) of not achieving remission and of schizophrenia itself.

In first-episode of schizophrenia (FES), smaller grey matter (GM) volume in the hippocampus tail (4) and parahippocampal cortex (PHC) (5, 6) have been identified as markers of not achieving remission. In these studies, parahippocampal volume, but not hippocampal volume, was related to negative symptom severity (5, 6). Moreover, smaller GM volume (7) and a thinner cortex (8) were found in FES patients identified with PNS (versus non-PNS). This suggested reduced PHC volume may be a neurobiological marker of not achieving remission but, in particular, of unremitting primary negative symptoms.

Recent reviews of the neurobiology of negative symptoms highlighted multiple areas across the brain with a preponderance in the frontal and temporal areas (9, 10). Interestingly, no study has explicitly examined the multidimensional nature of negative symptoms (e.g., PNS vs. 2nd-NS) or explored the dynamic nature of these markers over time; all studies have been cross-sectional (9). There is a growing need to better understand the neurobiological underpinnings of negative symptoms to better understand their aetiology and to aid in developing newer treatments to help more people achieve a better outcome (11, 12).

In this naturalistic outcome and longitudinal neuroimaging study, we first explored the dynamic nature of remission by comparing FES clients separated into subgroups (PNS, 2nd-NS, Non-Remitted broadly defined, and Remitted) based on symptom severity at two different time points (1-year vs. 2-year outcome). We hypothesized (1) 1-year outcome would strongly predict 2-year

outcome and (2) those with PNS would make up at large proportion of those not achieving remission. Next, GM volumes in the medial temporal lobe (parahippocampus, hippocampus, and amygdala) at baseline and changes over a 1-year period were compared among the subgroups. We hypothesized (3) Non-Remitted patients would have a smaller PHC and hippocampus tail volume at baseline and (4) compared to the other groups, the PNS subgroup would have the smallest PHC volume at baseline and would show GM loss in the PHC over the 1-year follow-up period.

2. Materials and Methods

2.1 Treatment setting.

All clients were recruited and treated through the Prevention and Early Intervention Program for Psychoses (PEPP-Montreal), a specialized early intervention service at the Douglas Mental Health University Institute in Montreal, Canada. People aged 14 to 35 years from a defined local catchment area experiencing either a non-affective or affective first-episode of psychosis (FEP) who had not taken antipsychotic medication for more than one month, with an IQ greater than 70, were consecutively admitted to the program as either in- or out-patients. Since 2003, we have provided treatment to over 600 clients. Our initial sample included 388 clients who took advantage of the full 2-year program and had nearly-complete clinical research data sets. Our final sample was limited to 275 first-episode of schizophrenia (FES) clients since we were exploring remission as formulated for schizophrenia (1). All diagnoses were determined using the SCID-IV and validated through consensus with a research-staff psychiatrist. See supplementary material for more information regarding PEPP and all subsequent methods and materials.

2.2 Longitudinal data description.

Clinical data were collected near entry and at months 2, 3, 6, 9, 12, 18, and 24 thereafter; baseline assessment occurred, on average, 6.9 days after entry (SD=8.0, Range:-18-34). Key data were collected using the Scale for the Assessment of Negative Symptoms (SANS), Scale for the Assessment of Positive Symptoms (SAPS), and Calgary Depression Scale for Schizophrenia (CDSS). For the neuroimaging study, we collected 224 baseline [90 controls; 134 FEP (101 FES)] and 136 one-year follow-up [46 control; 90 FEP (76 FES] scans on a 1.5T MRI system. FreeSurfer v5.3 was used to automatically obtain grey matter volumes for the amygdala, hippocampus (tail and anterior portions), and parahippocampus (perirhinal, entorhinal, and parahippocampal cortices).

2.3 Ethics statement.

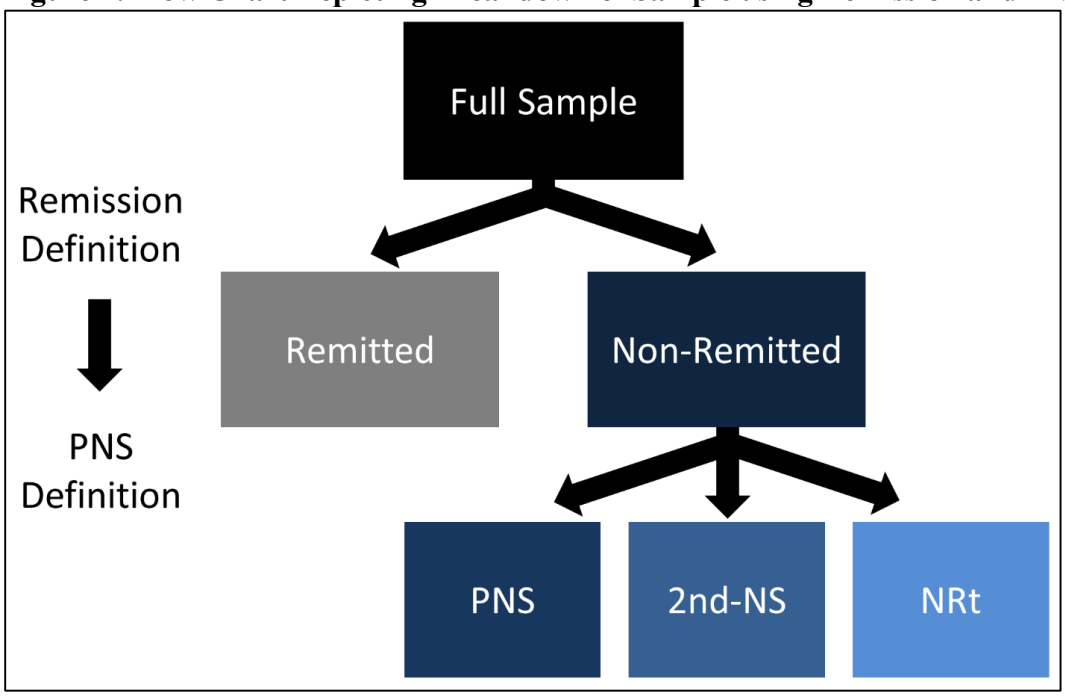
After a comprehensive description of the study was provided, written informed consent was obtained from all participants; verbal consent was not considered adequate. All clients were free to withdraw from research-based activities at any point without compromising treatment. Research protocols were approved by by the Research Ethics Boards of the Douglas Mental Health University Institute and the McGill University Faculty of Medicine.

2.4 Subdividing the sample based on remission & primary negative symptoms.

Remission was defined as a rating of mild or less on all four global items of the SAPS (hallucinations, delusions, bizarre behaviour, and though disorder) and SANS (flat affect, alogia, avolition-apathy, or anhedonia-asociality), and maintained for at least six consecutive months (1). Primary negative symptoms (PNS) were identified using the persistent negative symptom approach (13, 14) with PNS defined as having a global rating of moderate or more on at least one negative symptom as measured by the SANS. To ensure symptoms were primary in nature, clients also had to have a global rating of mild or less on all symptoms as measured by the SAPS, a total score of 4 or less on the CDSS, and not present with extrapyramidal symptoms requiring anticholinergic treatment. All criteria had to be maintained for at least six consecutive months.

In the end, there were four subgroups. First, the sample was separated into Remitted and Non-Remitted groups. Then, using the PNS definition, the Non-Remitted group was subdivided into three subgroups: PNS, 2nd-NS (secondary negative symptoms), and NRt (Non-Remitted transient; those who did not meet the six-month time criterion. See Figure 1 for a flow-chart summary.

Figure 1: Flow Chart Depicting Breakdown of Sample using Remission and PNS Definitions.



Abbreviations: PNS, primary negative symptoms; 2nd-NS, secondary negative symptoms; NRt, non-remitted transient symptoms.

2.6 Statistical analyses.

The sample was subdivided at two separate time periods, from month 6 to 12 and from month 18 to 24, to compare the underlying symptomatic profiles (subgroups) at 1-year outcome to 2-year outcome. Comparisons were mainly descriptive with cross-tabulation used to determine the strength of the relationship among the subgroup patterns. Positive and negative symptom totals were explored using Generalised Estimating Equations. Finally, the neuroimaging volumetric data were explored using a repeated-measures ANOVA, followed by one-way ANOVAs for between-group comparisons and paired t-tests for within-group comparisons.

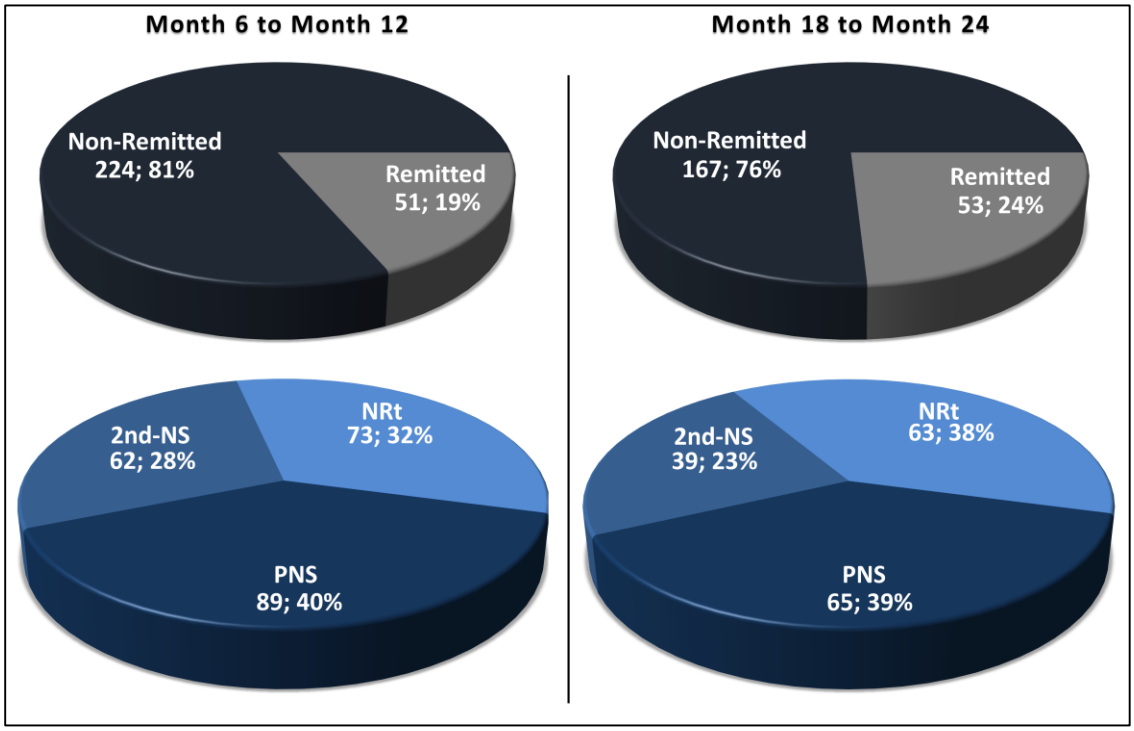
3. Results

3.1 The symptomatic profiles at two different 6-month time periods.

At 1-year outcome, 51 clients (19%) achieved full remission. Of the 224 Non-Remitted clients, 89 (40%) were not remitted due to PNS; noteworthy, 62 (70%) presented with avolition and/or anhedonia. Intriguingly, only one client displayed primary flat affect and two had primary alogia. Moreover, the 24 clients displaying a mix of PNS presented with avolition (n=5), anhedonia (n=4), or both (n=12). Remarkably, when the negative symptoms were removed from the remission

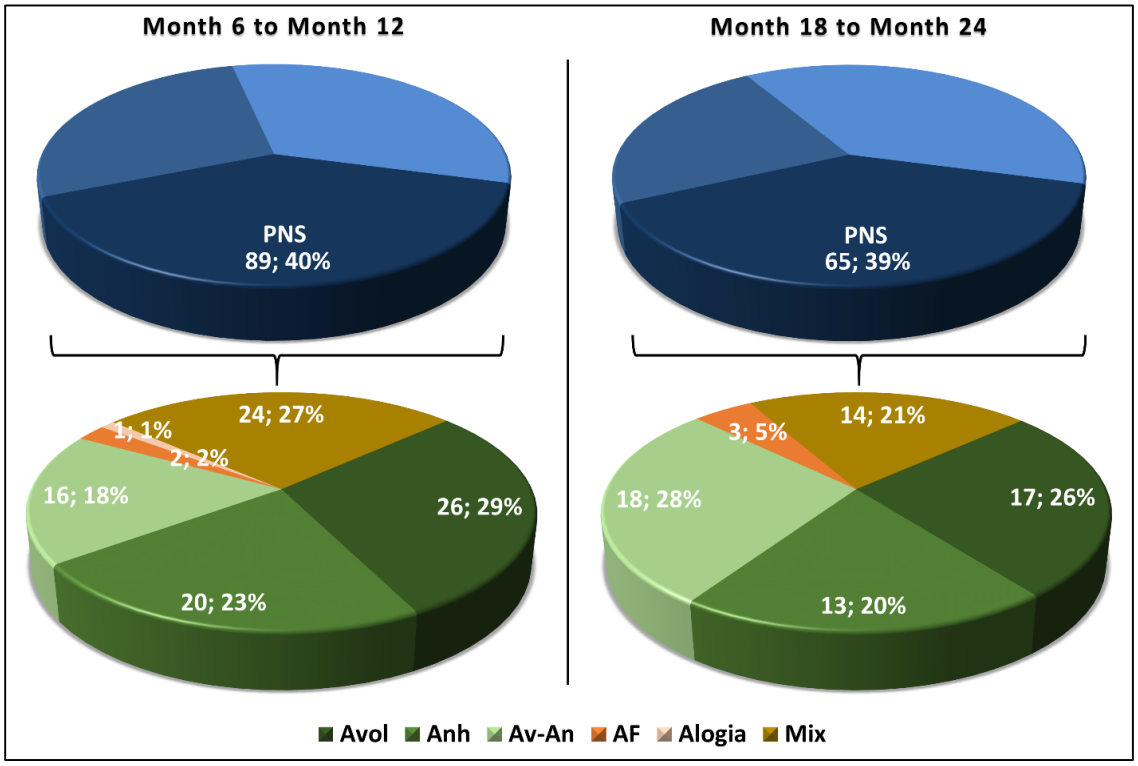
definition, 135 (49.1%) were in sustained positive symptom remission; removing the time criterion increased the number to 180 (65.5%) in positive symptom remission at Month 12. At 2-year outcome, 53 clients (24.1%) were in full remission; 113 (51.4%) were in sustained positive symptom remission; and 142 (64.5%) were in remission at Month 24. Among the 167 NR clients, 65 40% were not remitted due to PNS; 48 of them had avolition, anhedonia, or both. Of note, only three clients presented with primary flat affect and none with primary alogia. Overall, subgroup percentages were similar at both 1-year and 2-year outcome. See Figure 2 and Figure 3 for pie-chart summaries.

Figure 3: Remission Rate and Non-Remitted Quantification at Two separate 6-month Outcome Periods.



Abbreviations: PNS, primary negative symptoms; 2nd-NS, secondary negative symptoms; NRt, non-remitted transient symptoms. At 1-year outcome, among the 62 clients with 2nd-NS, negative symptoms were secondary alone due to depression (n=11), extra-pyramidal symptoms (n=3), and positive symptoms (n=41). At 2-year outcome, among the 39 clients with 2nd-NS, negative symptoms were secondary alone due to depression (n=3), extra-pyramidal symptoms (n=3), and positive symptoms (n=28).

Figure 3: Pie-chart Depicting Primary Negative Symptom Breakdown at Two separate Outcome Periods.



Abbreviations: PNS, primary negative symptoms; Avol, avolition-apathy; Anh, anhedonia-asociality; Av-An, avolition-apathy & anhedonia-asociality; AF, affective flattening. Percentages are calculated in relation to number of PNS (n=89). Mix was a combination of the four core negative symptoms.

3.2 How well does 1-year outcome relate to 2-year outcome?

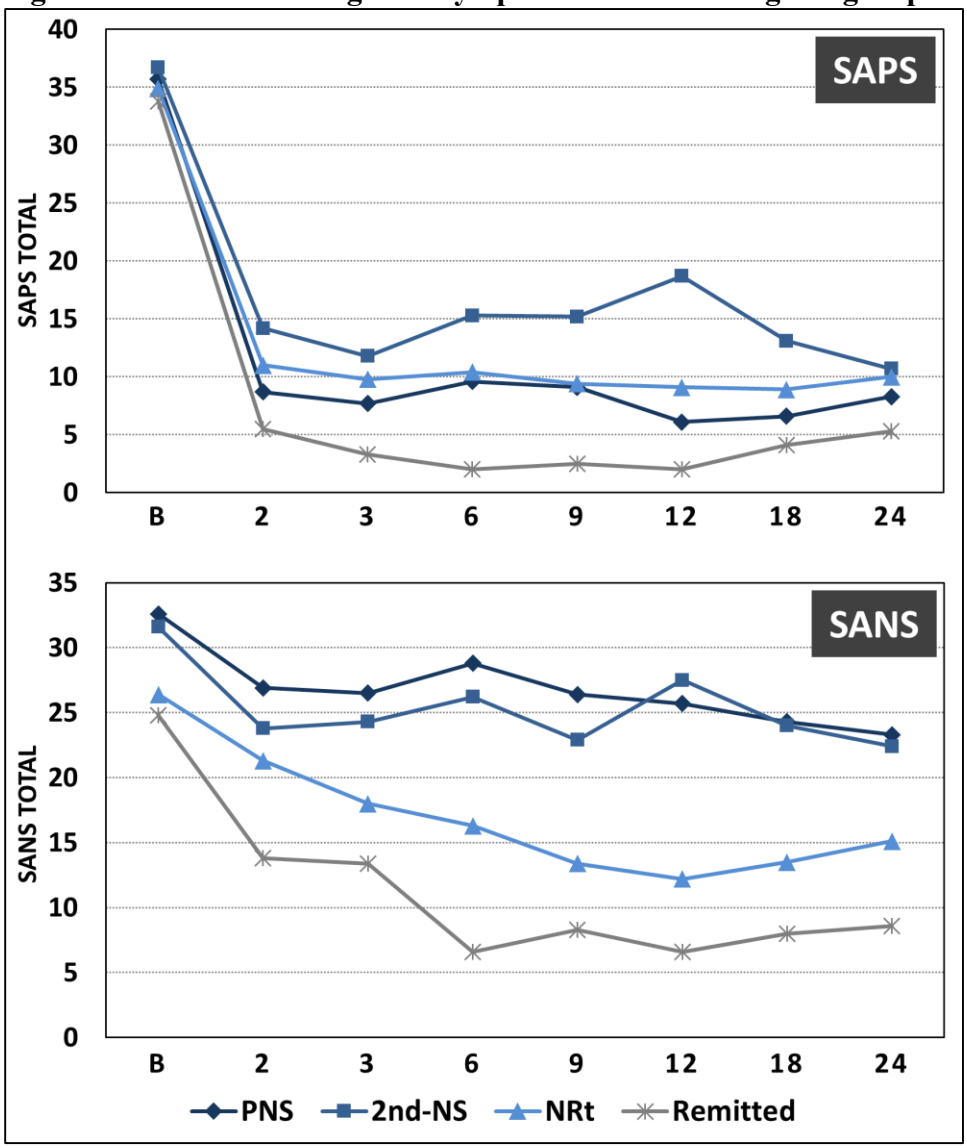
A highly significant relationship was found between the 1-year and 2-year outcome (χ^2=100.19,df=9,p<0.001). After two years of treatment, 56% continued to have PNS, 39% remained with 2nd-NS, 40% continued to show transient symptoms, and 62% remained remitted. See supplementary Table S1 for cross-tabulation results.

3.3 Symptomatic profiles.

See Figure 4 for SAPS and SANS profiles and supplementary Figure S1 for the CDSS profile over the 24-month treatment period. See supplementary material for full description of results; a brief summary for the SAPS and SANS is provided. For the SAPS, all subgroups had equal totals at Baseline and showed a significant decrease from Baseline to Month 2. All subgroups remained stable thereafter except the 2nd-NS subgroup which had a large increase from Month 9 to Month 12 followed by a significant decrease from Month 12 to Month 18; an effect driven by 12 clients

who relapsed and then recovered. Overall, the PNS and NRt subgroups did not significantly differ at any timepoint and the Remitted subgroup had the lowest totals. For the SANS, distinct profiles were already present starting at Baseline. The PNS and 2nd-NS subgroups had the highest totals that remained steady over time and did not differ from one another. The NRt subgroup displayed a gradual reduction over time whereas the Remitted subgroup had the lowest totals overall and showed significant decreases from Baseline to Month 2 and again Month 3 to Month 6. In fact, all subgroups, displayed a significant decrease from Baseline to Month 2.

Figure 4: Positive and Negative Symptom Profiles among Subgroups.



Abbreviations: SANS, Scale for the Assessment of Negative Symptoms; SAPS, Scale for the Assessment of Positive Symptoms; PNS, primary negative symptoms; 2nd-NS, secondary negative symptoms; NRt, non-remitted transient symptoms.

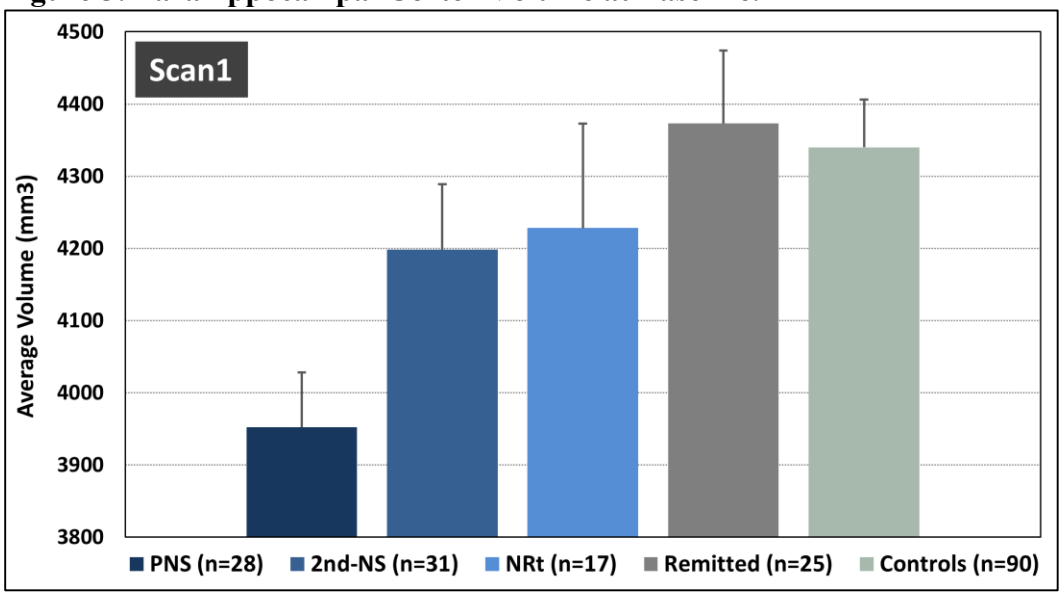
3.4 Basic socio-demographic descriptions.

Overall, there was a significant effect of education where the Controls and Remitted subgroup completed the most years. Now, particular to the neuroimaging subsample, the Controls had a significantly higher Full-Scale IQ compared to all FES subgroups, which did not differ from one another. Also, the PNS subgroup completed Scan1 closer to PEPP entry than the other subgroups. See supplementary material for complete description of results and Table S2, S3, S4 for the data.

3.5.1 Medial temporal lobe volumes at Scan1 (Baseline).

Significant between-group differences were limited to the parahippocampal cortex (PHC); see Figure 5. The PNS subgroup had the smallest volume that significantly differed from the Controls and the Remitted and 2nd-NS subgroups; there was a trend-level difference with the NRt subgroup.

Figure 5: Parahippocampal Cortex Volume at Baseline.

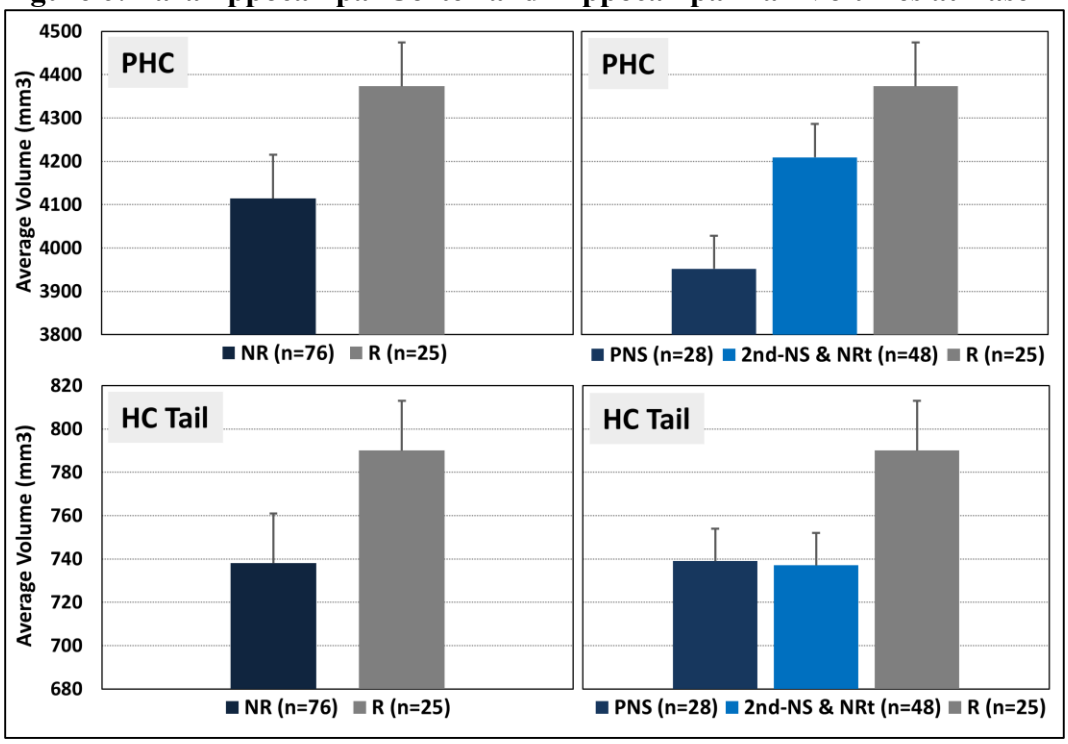


Abbreviations: PNS, primary negative symptoms; 2nd-NS, secondary negative symptoms; NRt, non-remitted transient symptoms. Error bars represent standard error. The PNS had significantly smaller volumes compared to all groups except for the NRt.

Of note, supplemental analyses verified the Non-Remitted group had a significantly smaller PHC volume compared to the Remitted group. Further, when removed from the Non-Remitted group, the PNS subgroup was found to significantly differ from the 'Non-Remitted without PNS' subgroup and the Remitted and 'Non-Remitted without PNS' no longer significantly differed. A second supplementary analysis confirmed the Non-Remitted group had a significantly smaller hippocampal tail volume compared to the Remitted group; see supplementary Figure S2. Further,

the PNS subgroup and 'Non-Remitted without PNS' subgroup had significantly smaller volumes compared to the Remitted group. However, the PNS and 'Non-Remitted without PNS' subgroups did not significantly differ; see Figure 6 for comparisons.

Figure 6: Parahippocampal Cortex and Hippocampal Tail Volumes at Baseline.



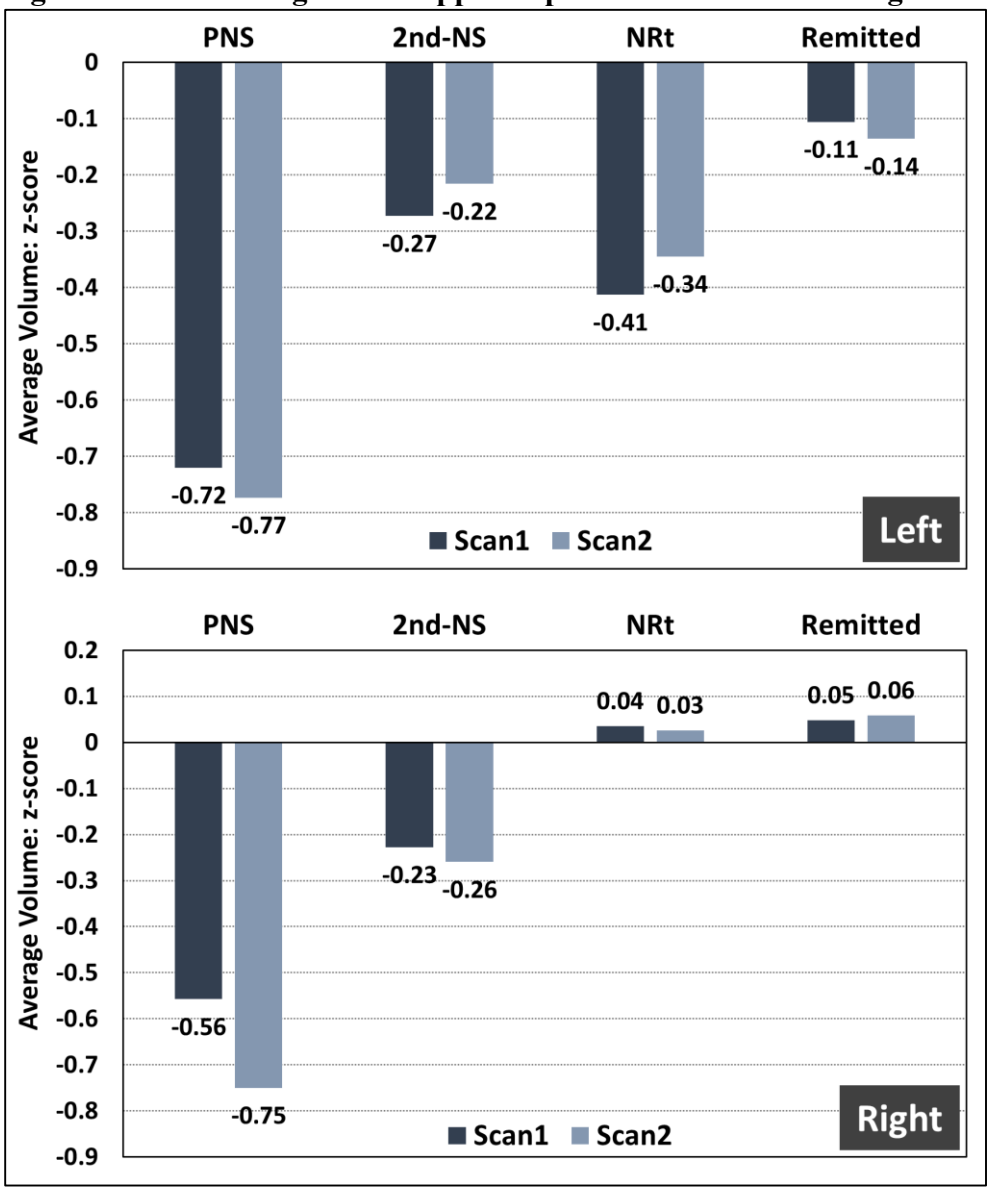
Abbreviations: PHC, parahippocampal cortex; HC Tail, hippocampus tail; PNS, primary negative symptoms; 2nd-NS, secondary negative symptoms; NRt, non-remitted transient symptoms; NR, non-remitted; R, remitted. On the left side, the Non-Remitted significantly differed from Remitted for both structures. On the right side, the PNS clients were removed from the Non-Remitted subgroup. For the PHC, PNS significantly differed from 2nd-NS & NRt and Remitted; the 2nd-NS & NRt and Remitted did not significantly differ. For the HC Tail, PNS and 2nd-NS_NRt subgroups significantly differed from the Remitted but not from each other.

3.5.2 Volume changes from Scan1 and Scan2 (1-year follow-up).

Analyses revealed a significant 'Time x Side x Region x Group' interaction with significant between-group differences again limited to the PHC at both Scan1 and Scan2; see Figure 7. Focusing on Scan2, the PNS subgroup had the smallest left volume that significantly differed from the Controls and the Remitted subgroup. There was a trend-level difference with the 2nd-NS subgroup and no significant difference with the NRt subgroup. On the right side, the PNS subgroup again had the smallest volume that significantly differed from the Controls, the Remitted subgroup, and the NRt subgroup; there was a trend-level difference with the 2nd-NS subgroup. Paired t-tests

revealed the PNS subgroup showed a significant decrease in right PHC volume and a trend-level decrease in left PHC volume. See supplementary Figure S3a for a scatter-plot of right PHC volume change and Figure S3b for a scatter-plot of left PHC volume change. See supplementary material for complete description, statistical values, and volumetric data.

Figure 5: Left and Right Parahippocampal Cortex Volume Change.



Abbreviations: PNS, primary negative symptoms; 2nd-NS, secondary negative symptoms; NRt, non-remitted due to transient symptoms. Values are standardised to the control group at each scan as they showed negligible change over the 1-year follow-up. Of note, at Scan2, the PNS subgroup had significantly smaller right volume compared to all subgroups, except for a nearly significant difference with the 2nd-NS subgroup.

4. Discussion

In this naturalistic outcome study, we examined the proportion of unremitting negative symptoms in first-episode of schizophrenia (FES) clients receiving treatment from an early intervention service. Our sample was subdivided into subgroups including: Remitted, PNS (primary negative symptoms), 2nd-NS (secondary negative symptoms), and NRt (non-remitted transient symptoms). Among the subgroups, we examined symptomatic profiles over the 2-year follow-up period and explored grey matter (GM) volumetric differences in the medial temporal lobe at baseline and changes therein over a 1-year follow-up period.

To start, we found nearly 40% of FES patients did not achieve remission due to PNS after one year of treatment; a percentage that remained constant after the second year. Of those with PNS, around 70% displayed amotivation (i.e., avolition and/or anhedonia) and less than 5% displayed emotional expressivity (i.e., alogia and/or flat affect). Regarding the symptomatic profiles, all patients, regardless of subgroup, displayed noteworthy improvements in both positive and negative symptoms over the first two months of treatment. From here, two findings stood out: 1) the PNS subgroup showed a strong antipsychotic response as evidenced by the low positive symptom totals up to Month 24, and 2) the PNS and 2nd-NS subgroups displayed higher negative symptom totals starting at Baseline that unwavered over the 24-month follow-up period.

For the neuroimaging analyses, using a fully-automated technique, smaller volumes in the hippocampal tail and parahippocampal cortex (PHC) at Scan1 were verified in Non-Remitted clients compared to Remitted clients. Compared to the other Non-Remitted clients, those with PNS had a significantly smaller PHC volume but did not differ in hippocampal tail volume, highlighting the PHC not only as a marker of not achieving remission but as a marker unremitting PNS. Finally, the PNS subgroup alone displayed a significant loss in PHC volume from Scan1 to Scan2 (1-year follow-up).

4.1 The parahippocampal cortex, schizophrenia, and primary negative symptoms.

A recent meta-review confirmed there is indeed reduced GM volume in the parahippocampal gyrus (PHG) in people with schizophrenia compared to healthy controls; a finding less pronounced in studies involving FES (15, 16). However, two separate studies examining the entorhinal cortex (one of three structures comprising the PHG), found smaller volumes in FES patients compared to

controls (17, 18). Interestingly, a smaller volume was actually found in patients who were non-delusional compared to those who were delusional (18); however, the opposite was found when exploring the PHG as a whole (19). These findings suggest volumetric differences in the PHG may be cortex specific and perhaps related to specific symptomatic profiles. In support of this, patients with prominent negative symptoms were found to have a smaller PHG volume compared to controls (20). Moreover, using two different fully-automated MRI data analysing techniques (voxel-based morphometry and cortical thickness), we previously found reduced GM in the PHG in FEP clients identified with PNS compared to non-PNS clients and healthy controls (7, 8).

The current study extended the above findings by employing a much larger sample size, longitudinal MRI data, and a different fully-automated technique (FreeSurfer) to estimate GM volumes. Our results not only verified the hippocampus tail and PHC as neurobiological markers of not achieving remission (4-6), but revealed the PHC as a specific marker of unremitting PNS. Further investigations are warranted to confirm this, but these findings strongly suggest the PHC as a region-of-interest in future studies exploring for and designing target-specific interventions aimed at treating PNS.

4.2 Negative symptoms and not achieving remission.

When the remission definition was first proposed in 2005, its primary intent was to allow clinicians to consistently quantify outcome and researchers to easily compare results (1); since then, the definition has been validated and found to be clinically meaningful (21, 22). Remission rates, however, have been shown to vary from 17% to 78%, with the majority of this variation due to studies not using the 6-month time component (21). For example, Emsley et al (23) showed that 70% of patients met the cut-off criteria for symptom ratings, but only 23.6% met both the symptom cut-off and time criteria. In fact, much debate has been raised about the time criterion with one study showing a 3-month criteria (vs. the 6-month criteria in determining remission) was as powerful in predicting functional outcome (24). Moreover, this study found that remission rates reached 84% for positive symptoms alone but dropped to 54% when the negative symptoms were included (24). Our study supported this idea by showing 55% did not achieve remission due to negative symptoms alone. In fact, negative symptom severity has been revealed as a strong predictor of not achieving remission (21) and of a poorer outcome in general (3, 25).

These findings together beg-the-question of whether or not remission, at the present time, is better served by including the negative symptoms or not. Arguments for exclusion come from multiple avenues. First, negative symptoms appear to be a stronger indicator of a poorer functional outcome compared to positive symptoms (2, 3), suggesting these symptoms may not be entirely equal in determining overall outcome. Second, from our current findings, those with primary negative symptoms may represent a distinct subtype within schizophrenia (26). With no efficacious pharmacotherapies currently available to treat primary negative symptoms (11, 12, 27), unlike the plethora of medications available for effectively treating the positive symptoms (27, 28), so should the same definition apply to those with PNS? An argument against excluding them comes from a recent large-scale review highlighting negative symptoms, regardless if primary or secondary, do reduce over time and are not as stable as previously assumed (29).

Excluding the negative symptoms may be somewhat extreme, but change is needed to better account for those presenting with primary negative symptoms. In these cases, perhaps an entirely separate definition of negative symptom remission may be necessary, such as a percent reduction, or that remission rates should be presented separately for the positive and negative symptoms. Regardless, more studies are needed to support that unremitting PNS make up a large proportion of those not achieving full remission to fully consider the above arguments.

4.3 Limitations

Our results are strengthened in that our patients are largely previously untreated with antipsychotic medications, from a defined catchment area treated in an early intervention service, not exclusively as in-patients, and therefore truly represent FES patients with varying severity. However, there are a number of limitations to consider. First, avolition and anhedonia/asociality were overly represented in our sample; very few patients presented with alogia or flat affect. Thus, our results may be more representative of those with a primary amotivation factor as opposed to all negative symptoms in general. However, as argued by Foussias et al (30), all negative symptoms can be argued to fit under the general concept of avolition. Nevertheless, more studies are needed to verify our findings and to verify if they are indeed specific to avolition. Second, a recent review revealed that indeed GM volumes are affected by the use of specific antipsychotic medications (31). Although we co-varied for dosage, this did not account for the effects that individual antipsychotics may have on specific regions, namely the PHC, if any.

4.4 Summary

We found that primary negative symptoms (PNS) are over-represented in FES patients not achieving remission. Further, those identified with PNS may even represent a distinct subtype with a distinct neurobiological marker (namely, the parahippocampal cortex). For decades following the discovery of the first antipsychotic, positive symptom reduction was considered the cornerstone of measuring outcome. Over the past 40 years, however, negative symptoms have reclaimed part of the spotlight, culminating with the remission definition in schizophrenia equating the importance of both the positive and negative symptoms in determining outcome (1). However, considering the vast number of effective treatments available to treat the positive symptoms compared none currently available for primary negative symptoms (27, 28), this may have been premature at our present time. In the full honesty, remission should still include the negative symptoms until viable and efficacious treatments become available, studies should considering reporting positive and negative symptom remission separately.

5. References

1. Andreasen NC, Carpenter WT, Jr., Kane JM, Lasser RA, Marder SR, Weinberger DR. Remission in schizophrenia: proposed criteria and rationale for consensus. Am J Psychiatry. 2005;162:441-449.
2. Fervaha G, Foussias G, Agid O, Remington G. Impact of primary negative symptoms on functional outcomes in schizophrenia. Eur Psychiatry. 2014;29:449-455.
3. Foussias G, Agid O, Fervaha G, Remington G. Negative symptoms of schizophrenia: clinical features, relevance to real world functioning and specificity versus other CNS disorders. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology. 2014;24:693-709.
4. Bodnar M, Malla AK, Czechowska Y, Benoit A, Fathalli F, Joober R, Pruessner M, Pruessner J, Lepage M. Neural markers of remission in first-episode schizophrenia: a volumetric neuroimaging study of the hippocampus and amygdala. Schizophrenia research. 2010;122:72-80.
5. Bodnar M, Harvey P-O, Malla AK, Joober R, Lepage M. The parahippocampal gyrus as a neural marker of early remission in first-episode psychosis: a voxel-based morphometry study. Clinical Schizophrenia & Related Psychoses. 2011;4:217-228.
6. Bodnar M, Malla AK, Joober R, Lord C, Smith E, Pruessner J, Lepage M. Neural markers of early remission in first-episode schizophrenia: A volumetric neuroimaging study of the parahippocampus. Psychiatry Res. 2012;201:40-47.
7. Benoit A, Bodnar M, Malla AK, Joober R, Lepage M. The structural neural substrates of persistent negative symptoms in first-episode of non-affective psychosis: a voxel-based morphometry study. Front Psychiatry. 2012;3:42.
8. Bodnar M, Hovington CL, Buchy L, Malla AK, Joober R, Lepage M. Cortical thinning in temporo-parietal junction (TPJ) in non-affective first-episode of psychosis patients with persistent negative symptoms. PLoS One. 2014;9:e101372.

9. Galderisi S, Merlotti E, Mucci A. Neurobiological background of negative symptoms. Eur Arch Psychiatry Clin Neurosci. 2015.
10. Hovington CL, Lepage M. Neurocognition and neuroimaging of persistent negative symptoms of schizophrenia. Expert Rev Neurother. 2012;12:53-69.
11. Arango C, Garibaldi G, Marder SR. Pharmacological approaches to treating negative symptoms: a review of clinical trials. Schizophrenia research. 2013;150:346-352.
12. Davis MC, Horan WP, Marder SR. Psychopharmacology of the negative symptoms: current status and prospects for progress. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology. 2014;24:788-799.
13. Buchanan RW. Persistent negative symptoms in schizophrenia: an overview. Schizophrenia Bulletin. 2007;33:1013-1022.
14. Hovington CL, Bodnar M, Joober R, Malla AK, Lepage M. Identifying persistent negative symptoms in first episode psychosis. BMC psychiatry. 2012;12:224.
15. Shepherd AM, Laurens KR, Matheson SL, Carr VJ, Green MJ. Systematic meta-review and quality assessment of the structural brain alterations in schizophrenia. Neurosci Biobehav Rev. 2012;36:1342-1356.
16. Williams LM. Voxel-based morphometry in schizophrenia: implications for neurodevelopmental connectivity models, cognition and affect. Expert Rev Neurother. 2008;8:1049-1065.
17. Joyal CC, Laakso MP, Tiihonen J, Syvalahti E, Vilkman H, Laakso A, Alakare B, Rakkolainen V, Salokangas RK, Hietala J. A volumetric MRI study of the entorhinal cortex in first episode neuroleptic-naive schizophrenia. Biol Psychiatry. 2002;51:1005-1007.
18. Prasad KM, Patel AR, Muddasani S, Sweeney J, Keshavan MS. The entorhinal cortex in first-episode psychotic disorders: a structural magnetic resonance imaging study. Am J Psychiatry. 2004;161:1612-1619.
19. Prasad KM, Rohm BR, Keshavan MS. Parahippocampal gyrus in first episode psychotic disorders: a structural magnetic resonance imaging study. Prog Neuropsychopharmacol Biol Psychiatry. 2004;28:651-658.
20. Sigmundsson T, Suckling J, Maier M, Williams S, Bullmore E, Greenwood K, Fukuda R, Ron M, Toone B. Structural abnormalities in frontal, temporal, and limbic regions and interconnecting white matter tracts in schizophrenic patients with prominent negative symptoms. Am J Psychiatry. 2001;158:234-243.
21. AlAqeel B, Margolese HC. Remission in schizophrenia: critical and systematic review. Harv Rev Psychiatry. 2012;20:281-297.
22. Lambert M, Karow A, Leucht S, Schimmelmann BG, Naber D. Remission in schizophrenia: validity, frequency, predictors, and patients' perspective 5 years later. Dialogues in clinical neuroscience. 2010;12:393-407.
23. Emsley R, Rabinowitz J, Medori R, Early Psychosis Global Working G. Remission in early psychosis: Rates, predictors, and clinical and functional outcome correlates. Schizophrenia research. 2007;89:129-139.
24. Cassidy CM, Norman R, Manchanda R, Schmitz N, Malla A. Testing definitions of symptom remission in first-episode psychosis for prediction of functional outcome at 2 years. Schizophrenia Bulletin. 2010;36:1001-1008.
25. Kirkpatrick B. Progress in the study of negative symptoms. Schizophrenia Bulletin. 2014;40 Suppl 2:S101-106.

26. Kirkpatrick B, Buchanan RW, Ross DE, Carpenter WT, Jr. A separate disease within the syndrome of schizophrenia. Arch Gen Psychiatry. 2001;58:165-171.
27. Moller HJ, Czobor P. Pharmacological treatment of negative symptoms in schizophrenia. Eur Arch Psychiatry Clin Neurosci. 2015.
28. Leucht S, Cipriani A, Spineli L, Mavridis D, Orey D, Richter F, Samara M, Barbui C, Engel RR, Geddes JR, Kissling W, Stapf MP, Lassig B, Salanti G, Davis JM. Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: a multiple-treatments meta-analysis. Lancet. 2013;382:951-962.
29. Savill M, Banks C, Khanom H, Priebe S. Do negative symptoms of schizophrenia change over time? A meta-analysis of longitudinal data. Psychological medicine. 2015;45:1613-1627.
30. Foussias G, Remington G. Negative symptoms in schizophrenia: avolition and Occam's razor. Schizophrenia Bulletin. 2010;36:359-369.
31. Vita A, De Peri L, Deste G, Barlati S, Sacchetti E. The effect of antipsychotic treatment on cortical gray matter changes in schizophrenia: does the class matter? A meta-analysis and metaregression of longitudinal magnetic resonance imaging studies. Biol Psychiatry. in press.

Supplementary Material for Article 1

1. Materials and Methods

1.1 Treatment setting.

The Prevention and Early Intervention Program for Psychoses (PEPP-Montreal) is a specialized early intervention service with integrated clinical, research, and teaching modules at the Douglas Mental Health University Institute in Montreal, Canada. Treatment involves a comprehensive approach towards recovery with intensive medical and psychosocial management provided primarily through modified assertive case management. Pharmacotherapy for all patients, regardless of initial diagnosis, begins with a second-generation antipsychotic (olanzapine, risperidone, paliperidone, quetiapine, or aripiprazole) within the recommended doses. If therapeutic response is not optimal within 4-6 weeks or significant side effects emerge, a different second-generation antipsychotic is prescribed. While treatment for psychosis begins with an antipsychotic, patients who refuse drug therapy are still provided with all available psychosocial interventions, especially case management and family intervention. For program details see http://www.douglas.qc.ca/page/programme-pepp?locale=en.

1.2 Longitudinal clinical data collection.

A full battery of scales are administered as part of the PEPP protocol but the key scales employed in this study included the Scale for the Assessment of Negative Symptoms (SANS) (1), the Scale for the Assessment of Positive Symptoms (SAPS) (2), and the Calgary Depression Scale for Schizophrenia (CDSS) (3). These data were collected at entry and at months 2, 3, 6, 9, 12, 18, and 24 thereafter. Over the 2-year period, our evaluators have established an inter-class correlation of 0.89 and 0.71 on the SAPS and SANS, respectively. Evaluations were performed by research assistants who received extensive training and supervision; inter-reliability was measured at least once a year; symptom raters were not involved in the treatment process.

At each assessment, the type and dosage of antipsychotic prescribed were noted and converted into chlorpromazine equivalents (4, 5). Medication adherence was also measured at each time point using a 5-point scale [0 = never adherent (0%), 1 = very infrequently adherent (1% to 25%), 2 = sometimes adherent (26% to 50%), 3 = quite often adherent (51% to 75%), 4 = fully adherent (76% to 100%)] based on composite information obtained from the patient, family members, and treating team. Patients were asked how often they missed a dose over the past month and adherence was calculated as a percentage of prescribed doses taken; pill counting information was available for a subset of the sample. Pill count correlated highly to patient, family, and case manager reports of adherence; we have established an intra-class correlation of 0.84 using this technique (6).

Data on sex (male/female), education level (years completed), Full-scale IQ (7, 8) were obtained at baseline. Additionally, parental socio-economic status during upbringing (9) and handedness (10) were obtained for the neuroimaging subsample.

1.4 Longitudinal structural MRI data acquisition & processing.

For the neuroimaging study, only PEPP clients over 18 years of age were considered to partake. The only non-mechanical exclusion criteria included head trauma causing loss of consciousness. A non-clinical healthy control group was also recruited from the local catchment area through advertisements placed in newspapers or on bulletin boards. Non-mechanical exclusion criteria for the healthy controls included a current or past history of any Axis I disorder, any neurological disease, head trauma causing loss of consciousness, or a first-degree relative diagnosed with a schizophrenia-spectrum disorder. Mechanical exclusion criteria for all participants were MRI scanning based that included, for example, having un-removable metal in body, claustrophobia, having a pace-maker, being pregnant, and so on.

Scanning was completed at the Montreal Neurological Institute on a 1.5T Siemens whole-body MRI system. For each participant, T1 MR images were acquired using a 3D gradient-echo pulse sequence (TR=22ms; TE=9.2ms; flip angle=30º; FOV=256mm SI x 204mm AP; 180 sagittal slices; voxel size=1mm^3). The same scanner and identical parameters were used at both Scan1 (baseline) and Scan2 (1-year follow-up.

To obtain grey matter volumetric data, T1 images were automatically processed in FreeSurfer v5.3 (http://surfer.nmr.mgh.harvard.edu) using the longitudinal stream (11, 12). For each participant, Scan1 and Scan2 were: 1) cross-sectionally processed ("recon-all…-all"); 2) used to create a within-subject template ("recon-all…-base"); and 3) longitudinally processed ("recon-all…-long"). This provided volumetric data for the amygdala and parahippocampus (perirhinal, entorhinal, and parahippocampal cortexes). Volumetric data for the hippocampus were obtained using the 'hippo-subfields' stream (13). All final volumes were presented in cubic millimeters. A post-processing visual inspection of each scan was conducted for quality control.

We obtained 224 baseline MRI scans (Scan1) that included 134 FEP clients and 90 controls. For the clients, several participants were removed due to: affective diagnosis (n=23); organic lesion (n=2); missing key clinical data (n=5); and MRI data processing errors (n=3); no controls were removed. The subgroups of the remaining 101 FES clients included: PNS, n=28; 2nd-NS, n=31; NR-Neg, n=7; NR-Pos-Mix, n=10; Remitted, n=25.

We also obtained 136 1-year follow-up scans (Scan2) that included 90 FEP clients and 46 controls. For the clients, several participants were removed due to: affective diagnosis (n=14); missing key clinical data (n=1); and MRI data processing errors (n=5); two controls were also removed due to a data processing errors. Of the 71 clients, the subgroups included: PNS, n=21; 2nd-NS, n=23; NRt, n=13; Remitted, n=13.

1.5 Statistical analyses

Socio-demographic variables were analysed with one-way ANOVAs for continuous variables or Kruskall-Wallis H tests for nominal variables. All analyses were conducted using SPSS 22 (IBM Corp., Armonk, NY, USA) and were two-tailed with a critical *p*-value of 0.05, except where noted.

1.4.1 Entire FES sample

Generalized Estimating Equations (GEE) were used to longitudinally analyse SAPS, SANS, and CDSS Totals across all time points. The GEE analysis is considered a multivariate extension of the generalized linear model to analyze repeated measurements or other correlated observations. There are several advantages inherent to GEE for examining a large, longitudinal data set including its robust nature to accommodate violations of normality (homogeneity of variance) and incomplete data (exclude missing observations within a subject and not exclude the entire subject).

1.4.2 Neuroimaging FES subsample

Baseline medial temporal grey matter volumes were analysed using a repeated-measures ANCOVA with 'Group' (PNS, 2nd-NS, NRt, Remitted, Controls) as the between-group factor with 'Side' (Left, Right) and 'Region' (Amygdala, Hippocampus Tail, Anterior Hippocampus, Perirhinal Cortex, Entorhinal Cortex, Parahippocampal Cortex) as the within-group factors; analysis was one-tailed. Follow-up data were analysed using a repeated-measures ANCOVA with 'Group' as the between-group factor and 'Time' (Scan1, Scan2), 'Side', and 'Region' as the within-group factors; analysis was one-tailed. All analyses included a matching covariate, estimated using Propensity Score Matching (14), that matched clients and controls on age at scan, sex, handedness, parental SES during upbringing, and education. Antipsychotic dosage (CPZ/month) was added as a separate covariate as it could not be matched to the controls. Note, for the baseline analysis, age included was Age at Scan1 and 'CPZ/month' was the average antipsychotic dosage in chlorpromazine equivalents (mg/day) per month from entry until Scan1. For the follow-up analysis, age included was Age at Scan1 and Age at Scan2; the 'CPZ/month' was the average dosage calculated from entry until Scan2.

2. Results

2.1 Positive and negative symptom totals (GEE analysis of entire sample).

For SAPS Totals, there were significant main effects of 'Time' (Wald χ^2=961.90, df=7, p<0.001) and 'Group' (Wald χ^2=99.44, df=3, p<0.001) and a significant 'Time x Group' interaction (Wald χ^2=171.90, df=21, p<0.001); see Figure 2 for symptom profile. Exploring the interaction, there were no significant differences among the subgroups at Baseline (all p>0.304) and all subgroup totals significantly decreased from Baseline to Month 2 (all p<0.001). From this point forward only the 2nd-NS displayed notable alterations; there was a trend-level increase (relapse) from Month 9 to Month 12 (p=0.091) followed by a significant decrease (improvement) from Month 12 to Month 18 (p=0.005). The PNS and NRt subgroups had totals that did not significantly differ from one another at any timepoint (all p>0.063).

The Remitted subgroup had the lowest totals compared to all subgroups that significantly differed from the other subgroups until Month 12 (all $p<0.001$); at Month 18 Remitted < 2nd-NS ($p<0.001$) & NRt ($p=0.030$). Importantly, by Month 24, there was only one significant between-group difference (2nd-NS > Remitted, $p=0.014$).

For SANS Totals, there were significant main effects of 'Time' (Wald $\chi^2=251.76$, df=7, $p<0.001$) and 'Group' (Wald $\chi^2=197.88$, df=3, $p<0.001$) and a significant 'Time x Group' interaction (Wald $\chi^2=164.00$, df=21, $p<0.001$); see Figure 2 for symptom profile. Exploring the interaction, distinct profiles were present. At Baseline, the PNS and 2nd-NS subgroups, which did not differ from one another ($p=0.658$), had significantly higher totals compared to the NRt and Remitted subgroups (all $p>0.005$), which also did not differ from one another ($p=0.523$). From Month 2 to Month 24, the Remitted subgroup had significantly lower totals at each time point compared to the other three subgroups (all $p<0.020$). Moreover, the PNS and 2nd-NS subgroups did not significantly differ over the 2-year period (all $p>0.099$). All of the subgroups significantly decreased from Baseline to Month 2 (all $p<0.001$). The NRt subgroup showed an additional decrease from Month 2 to Month 3 ($p=0.001$); the Remitted subgroup also showed an additional decrease from Month 3 to Month 6 ($p<0.001$). The PNS was largely unwavering except for an increase from Month 3 to Month 6 ($p=0.035$) followed by a decrease from Month 6 to Month 9 ($p=0.023$). The 2nd-NS subgroup showed a similar pattern expect they showed an increase from Month 9 to Month 12 ($p=0.009$) followed by a decrease from Month 12 to Month 18 ($p=0.009$).

For CDSS Totals, there were significant main effects of 'Time' (Wald $\chi^2=165.32$, df=7, $p<0.001$) and 'Group' (Wald $\chi^2=22.86$, df=3, $p<0.001$) and a significant 'Time x Group' interaction (Wald $\chi^2=56.16$, df=21, $p<0.001$). As seen in Figure S2 the average totals were not clinically meaningful beyond Month 2, thus results were minimally interpreted. Exploring the interaction, all subgroups had a significant decrease from Baseline to Month 2 (all $p<0.001$). Past Month 2, there were a few significant changes between successive time points (NRt: Month 2 to Month 3, $p=0.032$; PNS: Month 9 to Month 12, $p=0.013$; 2nd-NS: Month 2 to Month 3, $p=0.003$; Month 18 to Month 24, $p=0.014$; Remitted: Month 9 to Month 12, $p=0.022$; Month 12 to Month 18, $p=0.035$); the remaining were non-significant (all $p>0.070$). Of particular interest, at Month 24, there were no significant between-group differences (all $p>0.387$).

2.2 Socio-demographic characteristics.

Three sets of analyses were conducted; 1) entire FES sample; 2) participants with only Scan1; and 3) participants with both Scan1 and Scan2.

For the entire sample, there was a significant effect of education where Remitted subgroup completed significantly more years of education then the PNS subgroup. For both neuroimaging analyses, there were no significant differences among the subgroups on any variable of interest except on Entry until Scan1, education, and Full-Scale IQ. The PNS subgroup had Scan1 completed closer to entry than the other subgroups; a difference that significantly differed from only the Remitted subgroup. The Controls completed more years of education and had a higher Full Scale IQ that significantly differed from all FES subgroups.

2.3 Neuroimaging.

2.3.1 Medial temporal lobe grey matter volumes at Scan1 (Baseline).

The repeated-measures ANCOVA revealed a significant 'Region x Group' interaction ($F_{5,183}=3.97$, $p=0.001$). Further analyses revealed a significant between-group difference in the parahippocampal cortex (PHC; $F_{4,184}=2.86$, $p=0.013$). The PNS subgroup had the smallest PHC volume that significantly differed from the Controls ($p=0.001$; Cohen's $d=0.67$), the Remitted subgroup ($p=0.003$; Cohen's $d=0.95$), and the 2nd-NS subgroup ($p=0.040$; Cohen's $d=0.54$); there was a trend-level difference with the NRt subgroup ($p=0.091$; Cohen's $d=0.58$); see Figure 3A for PHC volumes among the subgroups.

Based on these results, we examined the most basic level of separation and a one-way ANCOVA (using the same covariates) confirmed the Non-Remitted group had a significantly smaller PHC volume compared to the Remitted group ($F_{1,97}=5.83$, $p=0.009$; Cohen's $d=0.52$); see Figure 3B. Next, the clients with PNS were separated from the Remitted group. A comparison among the PNS, Non-Remitted (without PNS), and Remitted subgroups revealed a significant between-group effect ($F_{2,96}=5.13$, $p=0.004$). Further analyses revealed the PNS subgroup had a significantly smaller volume compared to both the Remitted ($p=0.001$, Cohen's $d=0.95$) and Non-Remitted (without

PNS) (p=0.021, Cohen's d=0.53) subgroups. Interestingly, the Remitted and Non-Remitted (without PNS) subgroups now no longer significantly differed (p=0.060, Cohen's d=0.32); see Figure 3C.

As another supplement to the baseline analysis and based on previously published findings, we examined hippocampal tail volumes at the most basic level of separation. The one-way ANCOVA (using the same covariates) confirmed the Non-Remitted group had a significantly smaller hippocampal tail volume compared to the Remitted group ($F_{1,97}$=4.62, p=0.017; Cohen's d=0.51); see Figure S3B. As above, the clients with PNS were separated from the Remitted group. A comparison among the PNS, Non-Remitted (without PNS), and Remitted subgroups revealed a tend-level between-group effect approaching significance ($F_{2,96}$=2.31, p=0.053). Nonetheless, further analyses were carried out and revealed the PNS subgroup (p=0.048, Cohen's d=0.53) and Non-Remitted (without PNS) subgroup (p=0.021, Cohen's d=0.50) had a significantly smaller volumes compared to Remitted subgroup. Interestingly, the PNS and Non-Remitted (without PNS) subgroups did not significantly differ from one another (p=0.424, Cohen's d=0.02); see Figure S3C. See Figure S3A for hippocampal tail volumes among the subgroups.

2.3.2 Medial temporal lobe grey matter volumes at Scan1 and Scan2 (1-year follow-up).
The repeated-measures ANCOVA revealed a significant 'Time x Side x Region x Group' interaction ($F_{5,106}$=2.13, p=0.034). Further analyses revealed significant between-group differences in PHC at Scan1 on the left ($F_{4,107}$=2.15, p=0.040) and right ($F_{4,107}$=2.08, p=0.045) sides. There were also significant differences at Scan2 on the left ($F_{4,107}$=2.14, p=0.041) and right ($F_{4,107}$=3.37, p=0.006) sides. See Figure 4 for left and right PHC volume change at both Scan1 and Scan2.

Exploring Scan1 comparisons, the PNS subgroup had the smallest left PHC volume that significantly differed from the Controls (p=0.004; Cohen's d=0.74) and the Remitted subgroup (p=0.037; Cohen's d=0.63); there was no significant difference with the 2nd-NS (p=0.119; Cohen's d=0.49) or NRt (p=0.289; Cohen's d=0.28) subgroups. The PNS subgroup also had the smallest right PHC volume that significantly differed from the Controls (p=0.005; Cohen's d=0.61) and the Remitted subgroup (p=0.028; Cohen's d=0.76); there was a trend-level difference with the NRt subgroup (p=0.062; Cohen's d=0.69) and no significant difference with the 2nd-NS subgroup (p=0.255; Cohen's d=0.39).

Exploring Scan2 comparisons, the PNS subgroup had the smallest left PHC volume that significantly differed from the Controls (p=0.003; Cohen's d=0.80) and the Remitted subgroup (p=0.035; Cohen's d=0.65); there was a trend-level difference with the 2nd-NS subgroup (p=0.058; Cohen's d=0.61) and no significant difference with the NRt subgroup (p=0.171; Cohen's d=0.40). The PNS subgroup also had the smallest right PHC volume that significantly differed from the Controls (p<0.001; Cohen's d=0.84), the Remitted subgroup (p=0.007; Cohen's d=1.02), and the NRt subgroup (p=0.015; Cohen's d=0.94); there was a trend-level difference with the 2nd-NS subgroup (p=0.064; Cohen's d=0.57).

Paired t-tests revealed the PNS subgroup showed a significant decrease in right PHC volume (t_{20}=3.21, p=0.002; Cohen's d=0.28) and a trend-level decrease in left PHC volume (t_{20}=1.43, p=0.085; Cohen's d=0.09). The PNS (t_{20}=1.97, p=0.062; Cohen's d=0.12), NRt (t_{12}=2.12, p=0.056; Cohen's d=0.10), and Remitted (t_{12}=1.79, p=0.099; Cohen's d=0.13) subgroups showed trend-level decreases in the left anterior hippocampus.

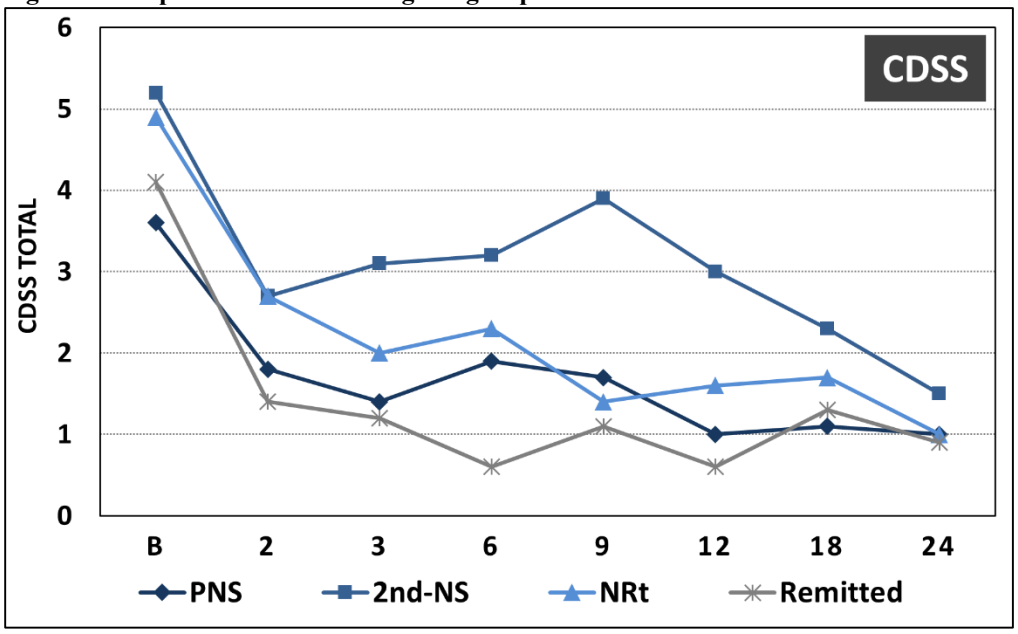
3. References

1. Andreasen NC: Modified Scale for the Assessment of Negative Symptoms (SANS). Iowa City, University of Iowa; 1984.
2. Andreasen NC: Scale for the Assessment of Positive Symptoms (SAPS). Iowa City, University of Iowa; 1984.
3. Addington D, Addington J, Maticka-Tyndale E. Assessing depression in schizophrenia: the Calgary Depression Scale. The British Journal of Psychiatry. 1993:39-44.
4. Woods SW. Chlorpromazine equivalent doses for the newer atypical antipsychotics. The Journal of clinical psychiatry. 2003;64:663-667.
5. Woods SW: Chlorpromazine Equivalent Doses for Atypical Antipsychotics: An Update 2003-2010. 2011. pp. 1-8.

6. Cassidy CM, Rabinovitch M, Schmitz N, Joober R, Malla A. A comparison study of multiple measures of adherence to antipsychotic medication in first-episode psychosis. Journal of Clinical Psychopharmacology. 2010;30:64-67.
7. Wechsler D: Wecshler Adult Intelligence Scale - 3rd Edition. San Antonio, TX, The Psychological Corporation; 1997.
8. Wechsler D: Wechsler Abbreviated Scale of Intelligence. San Antonio, TX, The Psychological Corporation; 1999.
9. Hollingshead A: Two-Factor Index of Social Position. New Haven, CN, Yale University Press; 1965.
10. Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia. 1971;9:97-113.
11. Reuter M, Fischl B. Avoiding asymmetry-induced bias in longitudinal image processing. NeuroImage. 2011;57:19-21.
12. Reuter M, Schmansky NJ, Rosas HD, Fischl B. Within-subject template estimation for unbiased longitudinal image analysis. NeuroImage. 2012;61:1402-1418.
13. Van Leemput K, Bakkour A, Benner T, Wiggins G, Wald LL, Augustinack J, Dickerson BC, Golland P, Fischl B. Automated segmentation of hippocampal subfields from ultra-high resolution in vivo MRI. Hippocampus. 2009;19:549-557.
14. Rubin DB. Estimating causal effects from large data sets using propensity scores. Annals of internal medicine. 1997;127:757-763.

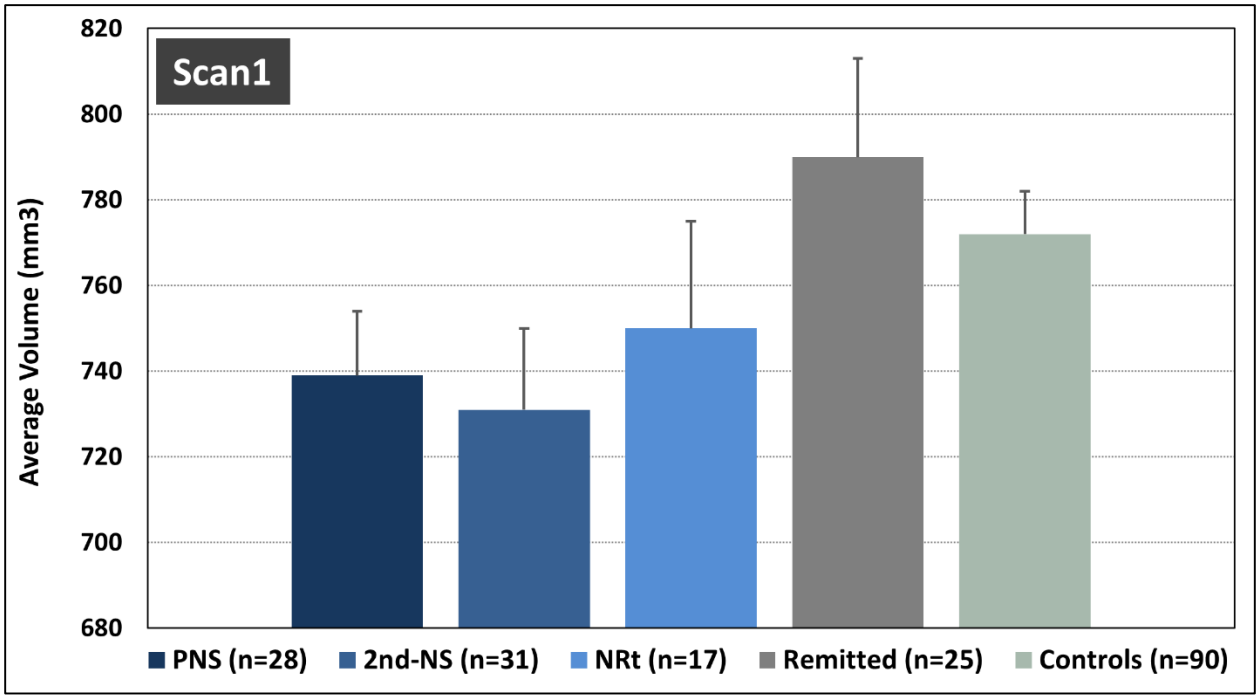
Supplementary Figures

Figure S1: Depression Totals among Subgroups.



Abbreviations: CDSS, Calgary Depression Scale for Schizophrenia; PNS, primary negative symptoms; 2nd-NS, secondary negative symptoms; NRt, non-remitted due to transient symptoms.

Figure S2: Hippocampal Tail Volume at Baseline.



Abbreviations: PNS, primary negative symptoms; 2nd-NS, secondary negative symptoms; NRt, non-remitted due to transient symptoms. Error bars represent standard error. Between-group differences were not explored as omnibus F-test was not significant.

Figure S3a: Scatterplot of Right Parahippocampal Cortex Grey Matter Volume Change.

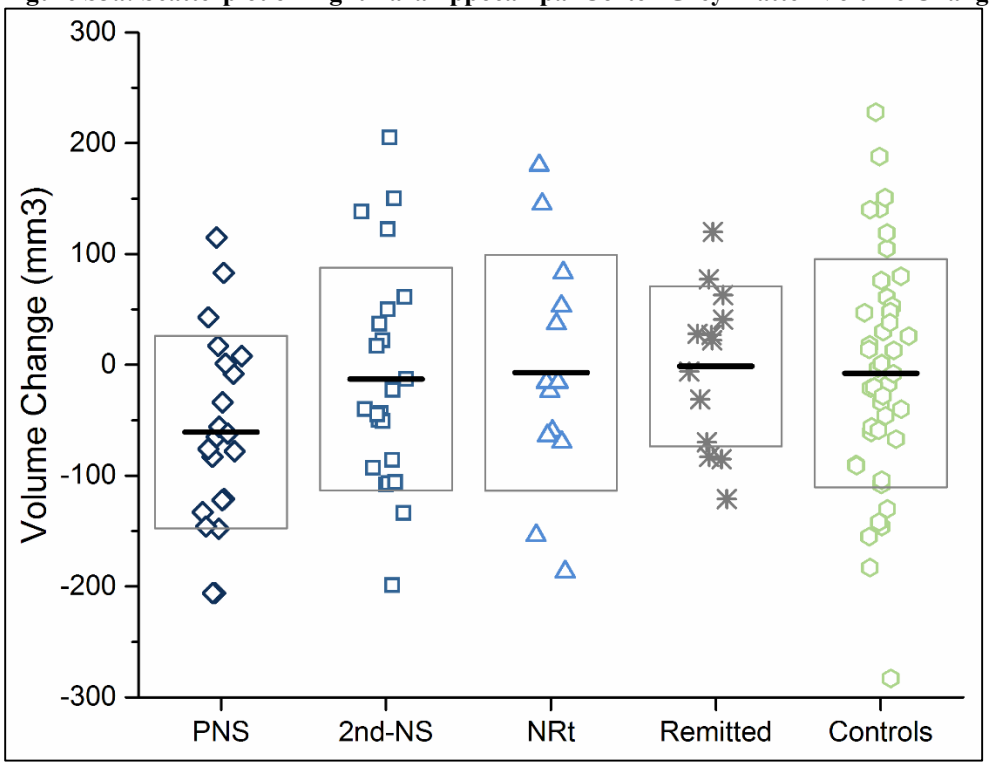
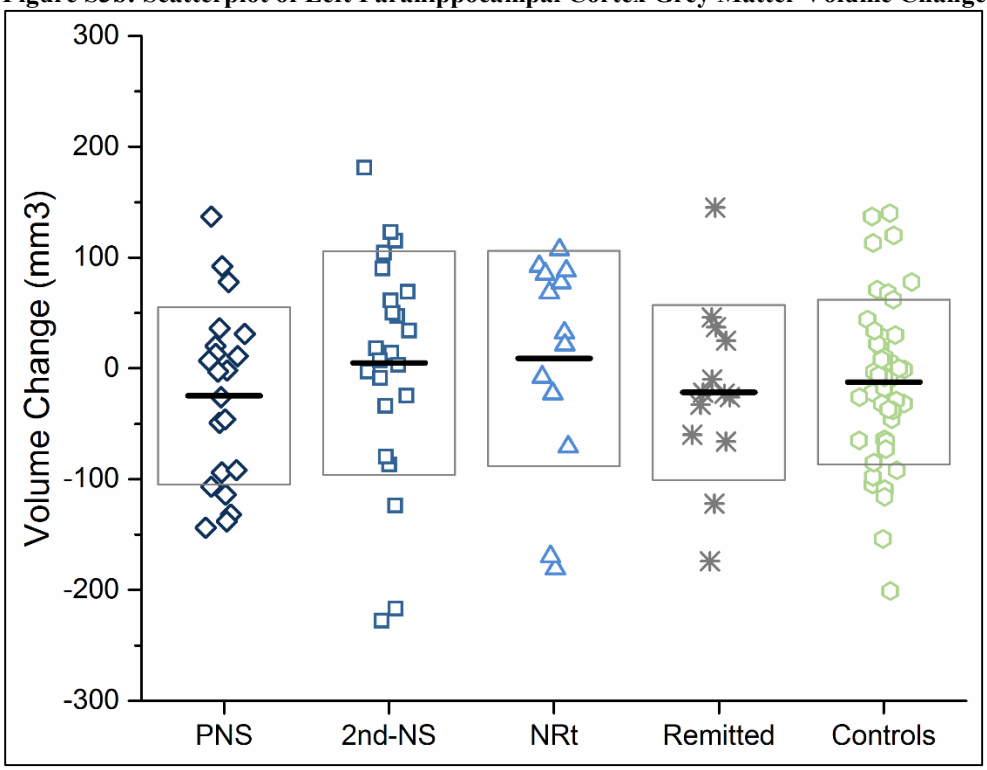


Figure S3b: Scatterplot of Left Parahippocampal Cortex Grey Matter Volume Change.



Abbreviations: PNS, primary negative symptoms; 2nd-NS, secondary negative symptoms; NRt, non-remitted due to transient symptoms. The thick black bar represents the mean for each group while the grey box represents the standard deviation.

Supplementary Tables

Table S1: Cross-tabulation of Subgroups at 1-year outcome and 2-year outcome for Entire FES Sample.

	1-Year Outcome					
	PNS	2nd-NS	NRt	Remitted		Totals (2-Year)
2-Year Outcome						
PNS	39 (55.7%)	17	9	0		65
2nd-NS	12	20 (39.2%)	5	1		38
NRt	13	12	23 (40.4%)	14		62
Remitted	6	2	20	24 (61.5%)		52
Totals (1-Year)	70	51	57	39		217

Abbreviations: PNS, primary negative symptoms; 2nd-NS, secondary negative symptoms; NRt, non-remitted due to transient symptoms. Table highlights (see percentages) clients who maintained the same group membership from 1-year outcome to 2-year outcome. The smaller overall sample size (n=217 vs. n=275) was a result analyses being limited to those who were able to be categorised at both 1-year and 2-year outcome.

Table S2: Characteristics of the Entire FES Sample.

	Non-Remitted			Remitted (n=51)	Analysis			
	PNS (n=89)	2nd-NS (n=62)	NRt (n=73)		F	χ^2	d.f.	p
Age at Entry, years: mean (s.d.)	22.8 (4.3)	23.2 (4.4)	23.2 (4.2)	24.8 (4.5)	2.55	--	3,271	0.056
Education, years: mean (s.d.) [n]	10.8 (2.6) [85]	11.6 (3.3) [60]	11.7 (3.0) [69]	12.6 (3.4) [48]	3.54	--	3,258	**0.015**[a]
Full-Scale IQ, mean (s.d.) [n]	93.0 (14.5) [76]	95.6 (17.9) [54]	94.9 (13.7) [58]	99.1 (14.1) [45]	1.57	--	3,229	0.198
Male, n (%)	68 (76.4)	51 (82.3)	53 (72.6%)	31 (60.8)	--	7.13	3	0.068

Abbreviations: PNS, primary negative symptoms; 2nd-NS, secondary negative symptoms; NRt, non-remitted due to transient symptoms.

[a] Education, number of years completed. Remitted > PNS (p=0.001).

Table S3: Characteristics of the Neuroimaging with only Scan1.

	Non-Remitted			Remitted (n=25)	Controls (n=90)	Analysis			
	PNS (n=28)	2nd-NS (n=31)	NRt (n=17)			F	χ^2	d.f.	p
Age at Scan1, years: mean (s.d.)	23.5 (4.0)	23.7 (3.7)	22.6 (3.5)	25.4 (4.5)	24.4 (3.2)	2.03	--	4,186	0.092
Entry to Scan1, months: mean (s.d.)	3.4 (1.6)	4.3 (2.1)	4.3 (1.7)	4.8 (2.1)	----	2.72	--	3,97	**0.049**[a]
Parental SES, mean (s.d.)	3.5 (1.1)	3.4 (1.2)	3.0 (1.2)	3.1 (1.2)	3.1 (1.1)	--	4.31	4	0.366
Education, years: mean (s.d.)	11.1 (2.3)	12.0 (2.1)	12.1 (2.9)	12.2 (2.6)	14.3 (2.5)	13.00	--	4,186	**<0.001**[b]
Full-Scale IQ, mean (s.d.) [n]	96.3 (18.8)	97.0 (16.7)	99.5 (13.4)	99.2 (17.0)	109.8 (14.6) [86]	6.82	--	4,182	**<0.001**[c]
Right handed, n (%)	24	28	14	21	81	--	1.53	4	0.822
Male, n (%)	22	22	13	18	58	--	2.67	4	0.614
Entry to Scan1 - CPZ/month, mean (s.d.)	192 (130)	152 (126)	228 (167)	151 (137)	----	1.55	--	3,97	0.206
Entry to Scan1 - Adherence, percent: mean (s.d.)	85.3 (21.3)	82.7 (22.4)	87.9 (17.3)	88.6 (19.8)	----	0.42	--	3,97	0.738

Abbreviations: PNS, primary negative symptoms; 2nd-NS, secondary negative symptoms; NRt, non-remitted due to transient symptoms; SES, socioeconomic status (ranked: 1=high to 5=low).

[a] Time from PEPP entry until Scan1. PNS < Remitted (p=0.006)

[b] Education, number of years completed. Controls > all FES subgroups (all p<0.001).

[c] Full-Scale IQ (WAIS-III & WASI-I). Controls > all FES subgroups (all p<0.016).

Table S4: Characteristics of the Neuroimaging with Scan1 & Scan2.

	Non-Remitted			Remitted (n=13)	Controls (n=44)	Analysis			
	PNS (n=21)	2nd-NS (n=23)	NRt (n=13)			F	χ^2	d.f.	p
Age at Scan1, years: mean (s.d.)	24.1 (4.3)	24.1 (3.7)	22.4 (3.3)	25.6 (3.6)	23.9 (3.3)	1.25	--	4,109	0.296
Age at Scan2, years: mean (s.d.) [n]	25.2 (4.3)	25.2 (3.7)	23.5 (3.3)	26.7 (3.6)	25.0 (3.4)	1.24	--	4,109	0.297
Entry to Scan1, months: mean (s.d.)	3.2 (1.4)	4.5 (2.1)	4.0 (1.8)	5.3 (1.5)	----	4.15	--	3,66	**0.009**[a]
Interscan Interval, months: mean (s.d.) [n]	13.4 (1.2)	13.2 (1.3)	13.3 (1.5)	13.1 (1.2)	12.7 (1.3)	1.31	--	4,109	0.270
Parental SES, mean (s.d.)	3.7 (1.0)	3.1 (1.2)	3.1 (1.2)	3.0 (1.0)	3.3 (0.9)	--	5.53	4	0.237
Education, years: mean (s.d.)	11.2 (2.4)	12.0 (2.1)	12.3 (3.3)	12.3 (2.8)	14.3 (2.5)	6.68	--	4,109	**<0.001**[b]
Full-Scale IQ, mean (s.d.) [n]	97.1 (19.7)	99.0 (17.6)	97.2 (14.6)	101.9 (19.2)	111.1 (14.6) [42]	3.80	--	4,107	**0.006**[c]
Right handed, n (%)	17 (81.0)	20 (87.0)	10 (76.9)	11 (84.6)	38 (86.4)	--	0.97	4	0.914
Male, n (%)	15 (71.4)	15 (65.2)	10 (76.9)	10 (76.9)	24 (54.5)	--	4.12	4	0.391
Entry to Scan1 - CPZ/month, mean (s.d.)	188 (131)	173 (135)	211 (175)	136 (146)	----	0.64	--	3,66	0.595
Entry to Scan1 - Adherence, percent: mean (s.d.)	83.4 (22.2)	85.7 (22.4)	86.7 (18.9)	83.8 (25.9)	----	0.08	--	3,66	0.971
Entry to Scan2 - CPZ/month, mean (s.d.)	147.4 (125.2)	199.7 (166.2)	219.3 (176.3)	114.1 (104.9)	----	1.59	--	3,66	0.201
Entry to Scan2 - Adherence, percent: mean (s.d.)	84.2 (16.9)	79.0 (21.2)	83.4 (15.4)	84.5 (17.8)	----	0.39	--	3,66	0.760

Abbreviations: PNS, primary negative symptoms; 2nd-NS, secondary negative symptoms; NRt, non-remitted due to transient symptoms; SES, socioeconomic status (ranked: 1=high to 5=low).

[a] Time from PEPP entry until Scan1. PNS < 2nd-NS (p=0.019) & Remitted (p=0.001).

[b] Education, number of years completed. Controls > all FES subgroups (all p<0.013).

[c] Full-Scale IQ (WAIS-III & WASI-I). Controls > all FES subgroups (all p<0.011), except vs. Remitted (p=0.087).

Table S5: Baseline Raw Volumetric Data for Medial Temporal Lobe Structures among Subgroups.

		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Left Parahippocampal Cortex	PNS	28	2034	272	51	1544	2720
	2nd-NS	31	2180	266	48	1512	2767
	NRt	17	2131	345	84	1723	3133
	Remitted	25	2274	294	59	1409	2940
	Control	90	2268	377	40	1083	3383
	Total	191	2208	342	25	1083	3383
Right Parahippocampal Cortex	PNS	28	1918	230	44	1515	2256
	2nd-NS	31	2019	285	51	1252	2549
	NRt	17	2096	291	71	1669	2822
	Remitted	25	2099	285	57	1562	2521
	Control	90	2073	320	34	1378	2907
	Total	191	2047	299	22	1252	2907
Left Entorhinal Cortex	PNS	28	1819	507	96	944	3282
	2nd-NS	31	1849	415	75	810	2870
	NRt	17	1958	587	142	1185	3564
	Remitted	25	1838	273	55	1277	2257
	Control	90	1852	367	39	1121	2893
	Total	191	1855	408	30	810	3564
Right Entorhinal Cortex	PNS	28	1662	415	78	930	2680
	2nd-NS	31	1582	361	65	763	2377
	NRt	17	1765	581	141	1056	3293
	Remitted	25	1656	325	65	1188	2476
	Control	90	1666	374	39	1049	2947
	Total	191	1659	393	28	763	3293
Left Perirhinal Cortex	PNS	28	2560	507	96	1860	3399
	2nd-NS	31	2625	505	91	1782	3847
	NRt	17	2685	587	142	1991	4044
	Remitted	25	2582	450	90	1902	3607
	Control	90	2685	496	52	1592	4040
	Total	191	2643	499	36	1592	4044
Right Perirhinal Cortex	PNS	28	1786	317	60	1271	2630
	2nd-NS	31	1731	259	46	1313	2245
	NRt	17	1870	428	104	1244	2881
	Remitted	25	1832	310	62	1221	2722
	Control	90	1785	270	28	990	2445
	Total	191	1790	296	21	990	2881
Left Hippocampus Tail	PNS	28	356	43	8	283	446
	2nd-NS	31	353	50	9	285	477
	NRt	17	360	52	13	266	444
	Remitted	25	381	55	11	309	572
	Control	90	376	50	5	277	495
	Total	191	369	50	4	266	572
Right Hippocampus Tail	PNS	28	383	48	9	296	501
	2nd-NS	31	377	63	11	235	505
	NRt	17	390	60	15	257	506
	Remitted	25	409	65	13	302	631
	Control	90	396	49	5	295	536
	Total	191	392	55	4	235	631
Left Anterior Hippocampus	PNS	28	2996	319	60	2370	3704
	2nd-NS	31	2976	334	60	2474	3917
	NRt	17	3031	372	90	2277	3623
	Remitted	25	3148	279	56	2732	3936
	Control	90	3104	359	38	2445	4258
	Total	191	3067	343	25	2277	4258
Right Anterior Hippocampus	PNS	28	3069	328	62	2453	3832
	2nd-NS	31	3056	367	66	2603	4136
	NRt	17	3070	416	101	2393	3742
	Remitted	25	3170	345	69	2688	4110
	Control	90	3169	336	35	2592	4196
	Total	191	3127	349	25	2393	4196
Left Amygdala	PNS	28	1544	194	37	994	1862
	2nd-NS	31	1506	189	34	1190	2224
	NRt	17	1595	205	50	1191	1937

	Remitted	25	1528	211	42	1125	2013
	Control	90	1552	203	21	1041	2213
	Total	191	1544	200	14	994	2224
Right Amygdala	PNS	28	1620	158	30	1306	1893
	2nd-NS	31	1580	208	37	1124	2043
	NRt	17	1623	223	54	1289	2002
	Remitted	25	1578	255	51	1026	2189
	Control	90	1618	216	23	1145	2151
	Total	191	1607	212	15	1026	2189

Table S6: Baseline & Follow-up Raw Volumetric Data for Medial Temporal Lobe Structures among Subgroups.

		N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Left Parahippocampal Cortex Scan1	PNS	21	2055	286	62	1544	2720
	2nd-NS	23	2186	268	56	1512	2767
	NRt	13	2145	396	110	1723	3133
	Remitted	13	2235	314	87	1409	2615
	Control	44	2266	293	44	1649	2807
	Total	114	2194	307	29	1409	3133
Left Parahippocampal Cortex Scan2	PNS	21	2031	269	59	1551	2718
	2nd-NS	23	2191	268	56	1693	2857
	NRt	13	2154	388	108	1700	3221
	Remitted	13	2214	326	90	1287	2582
	Control	44	2253	287	43	1707	2789
	Total	114	2184	303	28	1287	3221
Right Parahippocampal Cortex Scan1	PNS	21	1904	238	52	1515	2249
	2nd-NS	23	2007	302	63	1252	2549
	NRt	13	2089	328	91	1669	2822
	Remitted	13	2093	282	78	1562	2400
	Control	44	2078	312	47	1533	2907
	Total	114	2035	299	28	1252	2907
Right Parahippocampal Cortex Scan2	PNS	21	1843	214	47	1428	2141
	2nd-NS	23	1994	311	65	1390	2723
	NRt	13	2082	328	91	1645	2905
	Remitted	13	2092	304	84	1441	2430
	Control	44	2074	308	46	1602	2848
	Total	114	2018	304	28	1390	2905
Left Entorhinal Cortex Scan1	PNS	21	1787	509	111	944	3282
	2nd-NS	23	1885	400	83	1301	2870
	NRt	13	2043	652	181	1185	3564
	Remitted	13	1922	224	62	1474	2257
	Control	44	1876	405	61	1244	2893
	Total	114	1885	441	41	944	3564
Left Entorhinal Cortex Scan2	PNS	21	1846	475	104	1145	3265
	2nd-NS	23	1936	438	91	1277	3059
	NRt	13	2066	601	167	1199	3434
	Remitted	13	1882	277	77	1467	2367
	Control	44	1893	411	62	991	3168
	Total	114	1911	438	41	991	3434
Right Entorhinal Cortex Scan1	PNS	21	1695	455	99	930	2680
	2nd-NS	23	1595	310	65	1172	2179
	NRt	13	1807	658	183	1056	3293
	Remitted	13	1759	322	89	1310	2476
	Control	44	1685	356	54	1056	2727
	Total	114	1691	406	38	930	3293
Right Entorhinal Cortex Scan2	PNS	21	1677	401	88	970	2414
	2nd-NS	23	1635	357	74	1010	2368
	NRt	13	1829	694	192	980	3390
	Remitted	13	1742	351	97	1269	2494
	Control	44	1691	369	56	998	2768
	Total	114	1699	415	39	970	3390
Left Perirhinal Cortex Scan1	PNS	21	2561	482	105	1860	3399
	2nd-NS	23	2608	524	109	1866	3847
	NRt	13	2803	626	174	1991	4044
	Remitted	13	2596	378	105	1902	3181
	Control	44	2736	486	73	1860	3542
	Total	114	2670	499	47	1860	4044
Left Perirhinal Cortex Scan2	PNS	21	2598	417	91	1965	3360
	2nd-NS	23	2656	517	108	1852	3847
	NRt	13	2797	578	160	2009	3904
	Remitted	13	2554	451	125	1604	3267
	Control	44	2748	512	77	1541	3488
	Total	114	2685	496	46	1541	3904
Right Perirhinal Cortex	PNS	21	1805	348	76	1271	2630
	2nd-NS	23	1730	227	47	1356	2237

Scan1	NRt	13	1912	469	130	1244	2881
	Remitted	13	1873	320	89	1506	2722
	Control	44	1794	241	36	1213	2323
	Total	114	1806	301	28	1213	2881
Right Perirhinal Cortex Scan2	PNS	21	1751	310	68	1350	2497
	2nd-NS	23	1770	330	69	1294	2687
	NRt	13	1914	478	132	1193	2892
	Remitted	13	1881	337	93	1409	2801
	Control	44	1792	252	38	1187	2238
	Total	114	1804	318	30	1187	2892
Left Hippocampus Tail Scan1	PNS	21	361	43	9	283	446
	2nd-NS	23	363	51	11	296	477
	NRt	13	352	53	15	266	417
	Remitted	13	394	65	18	314	572
	Control	44	373	54	8	279	495
	Total	114	369	53	5	266	572
Left Hippocampus Tail Scan2	PNS	21	358	40	9	282	448
	2nd-NS	23	366	49	10	291	472
	NRt	13	359	58	16	239	424
	Remitted	13	390	49	14	315	502
	Control	44	370	53	8	281	486
	Total	114	368	50	5	239	502
Right Hippocampus Tail Scan1	PNS	21	383	40	9	319	465
	2nd-NS	23	388	60	12	306	505
	NRt	13	386	68	19	257	506
	Remitted	13	431	75	21	322	631
	Control	44	386	50	7	295	509
	Total	114	391	57	5	257	631
Right Hippocampus Tail Scan2	PNS	21	377	45	10	307	475
	2nd-NS	23	391	61	13	300	517
	NRt	13	387	65	18	263	513
	Remitted	13	435	67	19	363	636
	Control	44	384	49	7	295	513
	Total	114	390	57	5	263	636
Left Anterior Hippocampus Scan1	PNS	21	2994	293	64	2522	3704
	2nd-NS	23	2991	373	78	2474	3917
	NRt	13	2995	387	107	2277	3623
	Remitted	13	3224	291	81	2732	3936
	Control	44	2994	269	41	2445	3624
	Total	114	3020	317	30	2277	3936
Left Anterior Hippocampus Scan2	PNS	21	2962	265	58	2509	3526
	2nd-NS	23	3014	376	78	2466	4040
	NRt	13	3033	394	109	2365	3737
	Remitted	13	3262	316	88	2694	3980
	Control	44	2991	272	41	2429	3547
	Total	114	3026	321	30	2365	4040
Right Anterior Hippocampus Scan1	PNS	21	3044	276	60	2650	3621
	2nd-NS	23	3061	380	79	2603	4136
	NRt	13	3026	430	119	2393	3742
	Remitted	13	3278	351	97	2688	4110
	Control	44	3090	300	45	2592	4067
	Total	114	3090	337	32	2393	4136
Right Anterior Hippocampus Scan2	PNS	21	3044	274	60	2599	3775
	2nd-NS	23	3038	379	79	2539	4086
	NRt	13	3067	413	114	2419	3738
	Remitted	13	3298	370	103	2666	4112
	Control	44	3093	309	47	2587	4169
	Total	114	3093	341	32	2419	4169
Left Amygdala Scan1	PNS	21	1518	206	45	994	1862
	2nd-NS	23	1478	152	32	1190	1738
	NRt	13	1573	210	58	1191	1937
	Remitted	13	1515	228	63	1125	1901
	Control	44	1495	185	28	1162	1899
	Total	114	1507	189	18	994	1937
Left Amygdala	PNS	21	1509	214	47	1097	1867
	2nd-NS	23	1475	162	34	1193	1841

Scan2	NRt	13	1547	232	64	1275	1980
	Remitted	13	1526	219	61	1229	2025
	Control	44	1499	168	25	1160	1863
	Total	114	1505	188	18	1097	2025
Right Amygdala Scan1	PNS	21	1595	154	34	1306	1880
	2nd-NS	23	1582	196	41	1124	2022
	NRt	13	1630	231	64	1289	2002
	Remitted	13	1552	277	77	1026	2189
	Control	44	1586	196	30	1145	2117
	Total	114	1588	201	19	1026	2189
Right Amygdala Scan2	PNS	21	1574	140	31	1321	1811
	2nd-NS	23	1577	167	35	1211	1886
	NRt	13	1611	187	52	1353	1916
	Remitted	13	1594	214	59	1130	1960
	Control	44	1595	178	27	1191	2053
	Total	114	1589	172	16	1130	2053

Preamble to Article 2

As the second manuscript in this thesis, PubMed and Google Scholar were again searched for articles and reviews published with no time constraints that included the search terms “insight”, “clinical insight”, “awareness”, “adherence”, “compliance”, “schizophrenia”, “psychosis”, “primary negative symptoms”, “persistent negative symptoms”, and “deficit syndrome”. This search identified two main reviews and several key articles. The findings could be summed that nonadherence in psychosis has been linked to multiple factors including poorer insight and more severe positive and negative symptoms. Moreover, those with primary negative symptoms were identified to have poorer insight, but the interaction of these two factors in relation to adherence had not been explored. The findings were mainly consistent among the articles; however, only one study that explored primary negative symptoms and insight had been longitudinal –all other studies to date had been cross-sectional. Importantly, no study prior to this analysis had compared those with primary negative symptoms and secondary negative symptoms.

The results for Article 2 have not been published but the manuscript is ready for submission. The prepared manuscript contained a ‘Supplementary Material’ file that has been included in this thesis as a separate section directly following the manuscript. This extra material provides a complete description of methods and results, as well as supplementary Figures and Tables.

Article 2: A longitudinal study into clinical insight, primary negative symptoms, and medication adherence in first-episode of psychosis

Abstract

Importance: Negative symptoms are consistently related to a worse functional outcome and represent an unmet therapeutic need in people with psychosis. Future pharmacological interventions for negative symptoms, when available, may face barriers similar to antipsychotics, such as, poor insight and medication adherence.

Objective: To examine changes in clinical insight in relation to primary negative symptoms and medication adherence early on in treatment.

Design: A 12-month longitudinal analysis, as part of an ongoing, naturalistic outcome study based in an early intervention integrated clinical-research service.

Setting: Prevention and Early Intervention Program for Psychoses (PEPP), Douglas Mental Health University Institute, McGill University.

Participants: Clinical sample of 385 first-episode of psychosis (FEP) patients treated from January 2003 through December 2014.

Main Outcome Measures: Clinical insight: awareness of illness, belief in response to medication, and belief in need for medication. Persistent (primary) negative symptoms were defined as the presence of at least one negative symptom rated at a moderate level for at least six consecutive months and not secondary to positive, depressive, and extra-pyramidal symptoms. Final groups included: PNS (primary negative symptoms), 2nd-NS (secondary negative symptoms), and non-PNS. Full adherence was defined as taking prescribed medication greater than 75% of the time over nine consecutive months.

Results: Final groupings: 119 PNS (72 Fully-Adherent), 74 2nd-NS (43 Fully-Adherent), and 192 non-PNS (110 Fully-Adherent). Patients with PNS and 2nd-NS displayed poorer insight on all three dimensions across the 12-month period compared to the non-PNS group. Insight did not alter as a function of adherence in PNS patients but did among the 2nd-NS and non-PNS groups (largest effect observed for 'belief in need for medication').

Conclusions and Relevance: Patients identified with PNS displayed poor overall clinical insight over the first 12-months of treatment, regardless if adherent or not to medication. However, those with PNS showed little change in insight into the need for treatment regardless of adherence levels. In sum, PNS patients appear amenable to treatment but current treatments appear to be inadequate, newer, more efficacious treatments are needed.

1. Introduction

Regardless of illness stage, primary negative symptoms (PNS) are prevalent in about 25% of people with psychosis (1-3). Currently, no efficacious pharmacotherapies are available (4-6); however, viable options may eventually become available following the work from any one of the numerous ongoing clinical trials (4, 5). As with antipsychotic medications, one overriding concern with providing pharmacotherapy treatment to people with psychosis may be nonadherence (7).

Nonadherence in psychosis has been linked to cognitive impairment, substance misuse, depression, poorer insight, and more severe positive and negative symptoms (7). Of particular interest, poorer insight and PNS have been shown to be strongly associated with one another (2, 8-12), but how these two factors interact with or relate to adherence has not been explored. This is of critical importance as the future pharmacological treatments for negative symptoms may very well face this same issue of poor adherence as related to PNS and poorer insight.

A striking limitation of the previous studies investigating PNS and insight has been the cross-sectional design implemented by most studies. In the lone longitudinal study, poorer insight at the start of treatment was found to significantly predict PNS in first-episode of psychosis (FEP) patients after a 3-year follow-up (2). This study, however, could not establish whether insight had improved or not over that time since insight was only measured at baseline. This may be an important aspect to consider, especially from a treatment perspective, as we previously found insight actually improved over the first three months in a large cohort of FEP patients (13); supporting a result from a previous longitudinal FEP study (14). Thus, a longitudinal design is necessary to capture the dynamic nature of insight early on, especially in relation to PNS and adherence.

A second limitation shared by these studies, except for one (11), was that all employed a single overall measure of insight. The multidimensional nature of insight (15-17) may have a direct impact on understanding treatment adherence as some FEP patients, despite having a lack of awareness of mental illness, have been shown to be aware of their need for medication (18). Additionally, negative symptom severity has been related to decreased motivation to obtain and take medications (7, 19) and to poorer awareness of illness (13, 18). It is therefore crucial to understand the relationship among PNS, adherence, and insight factors such as ‘awareness of response to medication’ and ‘belief in the need for medication’ (20) which could aid with the

utilization of pharmacological treatments likely to be available for treatment of negative symptoms.

This study set out to identify when, and if, specific insight dimensions (awareness of mental illness, awareness of response to medication, and belief in the need for treatment) changed over a 12-month period following admission into an early intervention FEP clinic in those with primary negative symptoms (PNS), secondary negative symptoms (2nd-NS), and those without (non-PNS), in relation to level of medication adherence (Fully-Adherent vs. Partially-Adherent). We hypothesized, over the 12-month period (1) all patients will show an improvement in insight within the first three months of starting treatment; (2) PNS and 2nd-NS patients will display poorer insight across all dimensions; and (3) PNS, together with poorer insight, would manifest in poorer adherence.

2. Materials and Methods

2.1 Participants & Treatment Setting.

All patients were treated at the Prevention and Early Intervention Program for Psychoses (PEPP-Montreal), a specialized early intervention service with integrated clinical, research, and teaching modules, at the Douglas Mental Health University Institute in Montreal, Canada. People aged 15 to 35 years from the local catchment area suffering from either affective or non-affective psychosis who had not taken antipsychotic medication for more than one month and an IQ higher than 70 were consecutively admitted to the program as either in- or out-patients. For program details see http://www.douglas.qc.ca/page/programme-pepp?locale=en.

Patients completed a baseline evaluations, on average, 7.0 days after entry (SD=8.0; Range:-26.0-36.0); follow-up evaluations were completed at months 2, 3, 6, 9, and 12 thereafter. Evaluations were performed by research personnel, not involved in actual treatment, who receive extensive training and supervision; reliability is measured at least once a year; intra-class correlations (ICC) are listed with each scale.

After a comprehensive description of the study was provided, written informed consent was obtained from all participants. All clients were free to withdraw from research-based activities at any point without compromising treatment. Research protocols were approved by by the Research Ethics Boards of the Douglas Mental Health University Institute and the McGill University Faculty of Medicine.

2.2 Identifying Persistent Negative Symptoms.

As already published, (1), patients were identified with persistent (primary) negative symptoms (PNS) if they had a global rating of moderate (3) or more on at least one negative symptom (flat affect, alogia, avolition-apathy, or anhedonia-asociality) as measured with the Scale for the Assessment of Negative Symptoms (SANS; ICC=0.71) (21). To ensure negative symptoms were primary in nature, PNS patients had to have a global rating of mild (2) or less on all global ratings of positive symptoms, as measured with the Scale for the Assessment of Positive Symptoms (SAPS; ICC=0.89) (22), a total score of 4 or less on the Calgary Depression Scale for Schizophrenia (CDSS) (23), and not present with extrapyramidal symptoms requiring anticholinergics. All criteria had to be maintained for at least 6 consecutive months (between month 6 and 12). Thus, from this definition, we are able to identify those with PNS and those with secondary negative symptoms (2nd-NS). People with 2nd-NS displayed severe negative symptoms but in the presence of clinically relevant positive, depressive, or extrapyramidal symptoms.

2.3 Medication Adherence

An overall level of medication adherence was calculated by averaging adherence ratings from Month 3 to Month 12; as we previously found clinical stability to begin around Month 3 (13). The PNS and non-PNS patient groups were separated into Fully-Adherent (overall level > 75% or > 3) and Partially-Adherent (overall level ≤ 75%); adherence greater than 75% is considered clinically efficacious (24). Medication adherence was measured based on information collected from the patient, patient's family, and case manager's reports (ICC=0.84); see our previous work for complete details (25).

2.4 Clinical Insight

Clinical insight was measured at each time point using a brief version of the Scale to Assess Unawareness of Mental Disorder (SUMD); items were rated from 1 (aware) to 5 (unaware) (20). For the purposes of this report, exploration was limited to the first three items: Q1 - awareness of a mental illness; Q2a - awareness of response to medication; and, Q2b - belief in the need for medication or would benefit from it; a sum of these three variables was also explored. Although ICCs for the SUMD were not available, our raters have achieved an ICC of 0.79 for the insight

item (G12) on the Positive and Negative Syndrome Scale for Schizophrenia (26) which shares similarities with the SUMD-Q1.

2.5 Other Variables of Interest

The type and dosage of antipsychotic prescribed were noted at each time point; dosage was converted into chlorpromazine equivalents (27, 28). Education level (years completed), Full Scale IQ (29, 30), and duration of untreated illness (DUI) were collected at baseline. The DUI was defined as the time period from the onset of any psychiatric symptoms (anxiety, depression, suicidal ideation, or social withdrawal) until 30 days of continuous treatment.

2.6 Statistical analyses

Generalized Estimating Equations (GEE) was used to explore the relationship between negative symptoms, medication adherence, and clinical insight over a 12 month period. Each variable of the SUMD (Q1, Q2a, Q2b, and Total) was individually modelled with factors of Time, NS-group (PNS, 2nd-NS, non-PNS), and ADH-group (Fully-Adherent, Partially-Adherent). Education were added as a covariate as significant differences were noted among the subgroups. All analyses were conducted using SPSS 22 (IBM Corp., Armonk, NY, USA) and were two-tailed with a critical *P*-value of 0.05. See supplementary material for complete description of this analysis and other analyses performed.

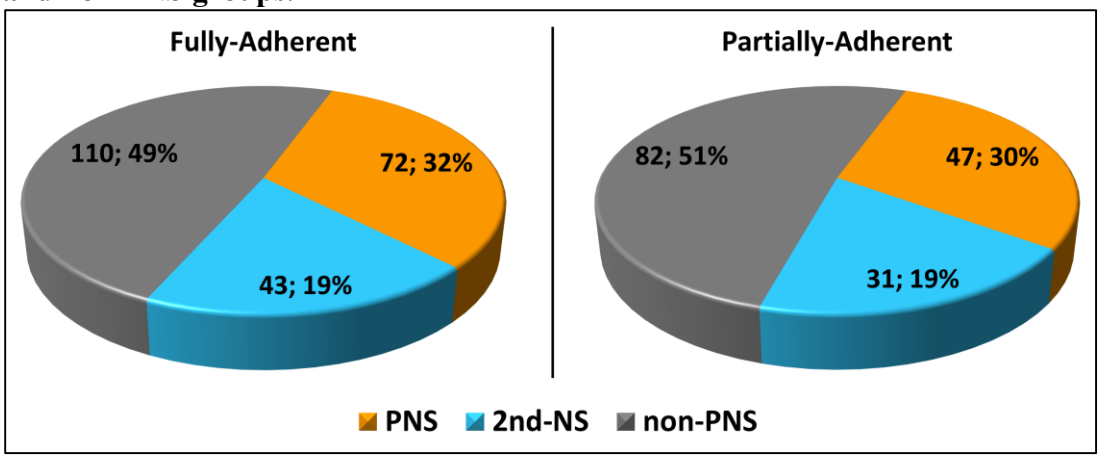
3. Results

3.1 Sample Description

Our sample included 385 FEP patients with longitudinal insight data, including 119 with PNS (60.5% were Fully-Adherent). Among the 266 non-PNS patients, 74 displayed secondary negative symptoms (2nd-NS) due to clinically relevant positive symptoms (n=44), depressive symptoms (n=14), extrapyramidal symptoms (n=4), positive and depressive symptoms (n=9), or positive and extrapyramidal symptoms (n=3); 58.1% were Fully-Adherent. Of the remaining 192 non-PNS patients, 57.3% were Fully-Adherent. Noteworthy, the ratio of Fully-Adherent to Partially-Adherent did not differ among the groups (χ^2=0.316, *P*=0.854); see **Figure 1** for pie-chart comparison. Although there was an effect of diagnosis (fewer affective diagnoses within 2nd-NS group; see **eTable 1** for data and result), we nevertheless included both affective and non-affective

diagnoses following the NIMH Research Domain Criteria (RDoC) initiative (http://www.nimh.nih.gov/research-priorities/rdoc/nimh-research-domain-criteria-rdoc.shtml).

Figure 1. Percentage of Fully-Adherent and Partially-Adherent patients within the PNS and non-PNS groups.



The data values represent the number of patients in each subgroup; the percentage is in relation to either the number of Fully-Adherent or Partially-Adherent patients.

3.2 General Characteristics

The subgroups did not significantly differ in age at entry, sex, Full IQ, or DUI. There was a significant effect regarding education with the 'non-PNS, Fully-Adherent' patients having completed the most years of school; **Table 1** presents data and statistical results.

Table 1 Sample Characteristics.

	PNS		2nd-NS		non-PNS				
	Fully Adherent [72]	Partially Adherent [47]	Fully Adherent [43]	Partially Adherent [31]	Fully Adherent [110]	Partially Adherent [82]	Statistic	df	*P*
Age of Entry (years), *M* (*SD*)	22.6 (4.3)	22.8 (4.3)	23.1 (4.9)	24.4 (4.2)	23.6 (4.8)	23.9 (4.3)	F=1.18	5,379	0.317
Male, n (%)	54 (75%)	36 (77%)	35 (81%)	22 (71%)	71 (65%)	53 (65%)	χ^2=7.21	5	0.205
Education (years), *M* (*SD*) [n]	10.9 (2.8) [70]	11.0 (2.2) [44]	11.2 (2.7) [42]	12.3 (3.5) [29]	12.3 (2.9) [102]	11.7 (2.9) [77]	F=3.06	5,358	**0.010** [b]
Full Scale IQ, *M* (*SD*) [n]	93.3 (15.3) [65]	95.6 (14.5) [39]	94.3 (18.5) [37]	98.3 (17.6) [30]	99.4 (14.2) [95]	94.9 (13.7) [58]	F=1.65	5,318	0.147
DUI (weeks), *Median* [n]	256 [66]	225 [42]	287	281 [28]	175 [106]	228 [71]	χ^2=5.95	5	0.311

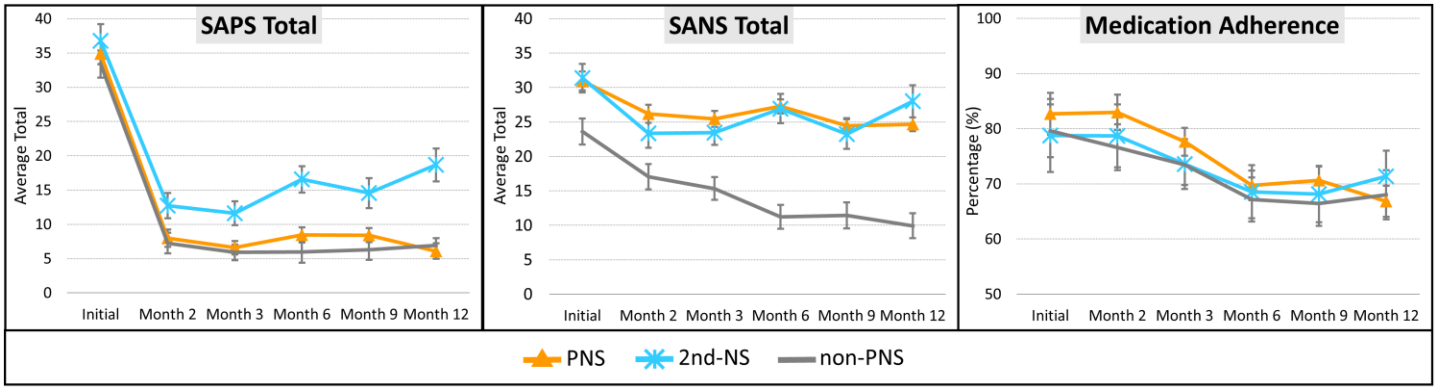
Abbreviations: *M* = mean; *SD* = standard deviation; *Mdn* = median; n = number of participants for whom data were available; PNS, primary negative symptoms; DUI, duration of untreated illness.
[a] Post-hoc: 'non-PNS, Fully-Adherent' > 'PNS, Fully-Adherent' (*P*=0.002), 'PNS, Partially-Adherent' (*P*=0.008), and '2nd-NS, Fully-Adherent' (*P*=0.026); '2nd-NS, Partially-Adherent' > PNS, Fully-Adherent' (*P*=0.034).

3.3 Symptom Totals and Medication Adherence

Figure 2 and **eFigure 1** present profiles for SAPS and SANS totals and medication adherence over the 12-month period; **eTable 2** presents the data values. Note, a complete description of results are in the supplementary material with a brief summary provided below, highlighting the more prominent results.

For the SAPS and SANS totals, all FEP patients showed a significant decrease from Baseline to Month 2, with quite a pronounced decrease for the SAPS total. The PNS and 2nd-NS groups had higher SANS totals at each timepoint over the 12-month period that did not significantly differ from one another. The 2nd-NS group had the highest SAPS totals at each timepoint over the 12-month period that significantly differed from both the PNS and non-PNS groups. The PNS, 2nd-NS, and non-PNS groups did not differ in medication adherence at any point; however, those who were Partially-Adherent displayed a significant decrease in adherence from Baseline to Month 6.

Figure 2. Symptom totals and medication adherence among subgroups.



Abbreviations: SAPS, Scale for the Assessment of Positive Symptoms; SANS, Scale for the Assessment of Negative Symptoms; CPZ, chlorpromazine. Note vertical titles for different scales among panels. Errors bars reflect the standard error.

3.4 Primary negative symptoms, clinical insight, and medication adherence

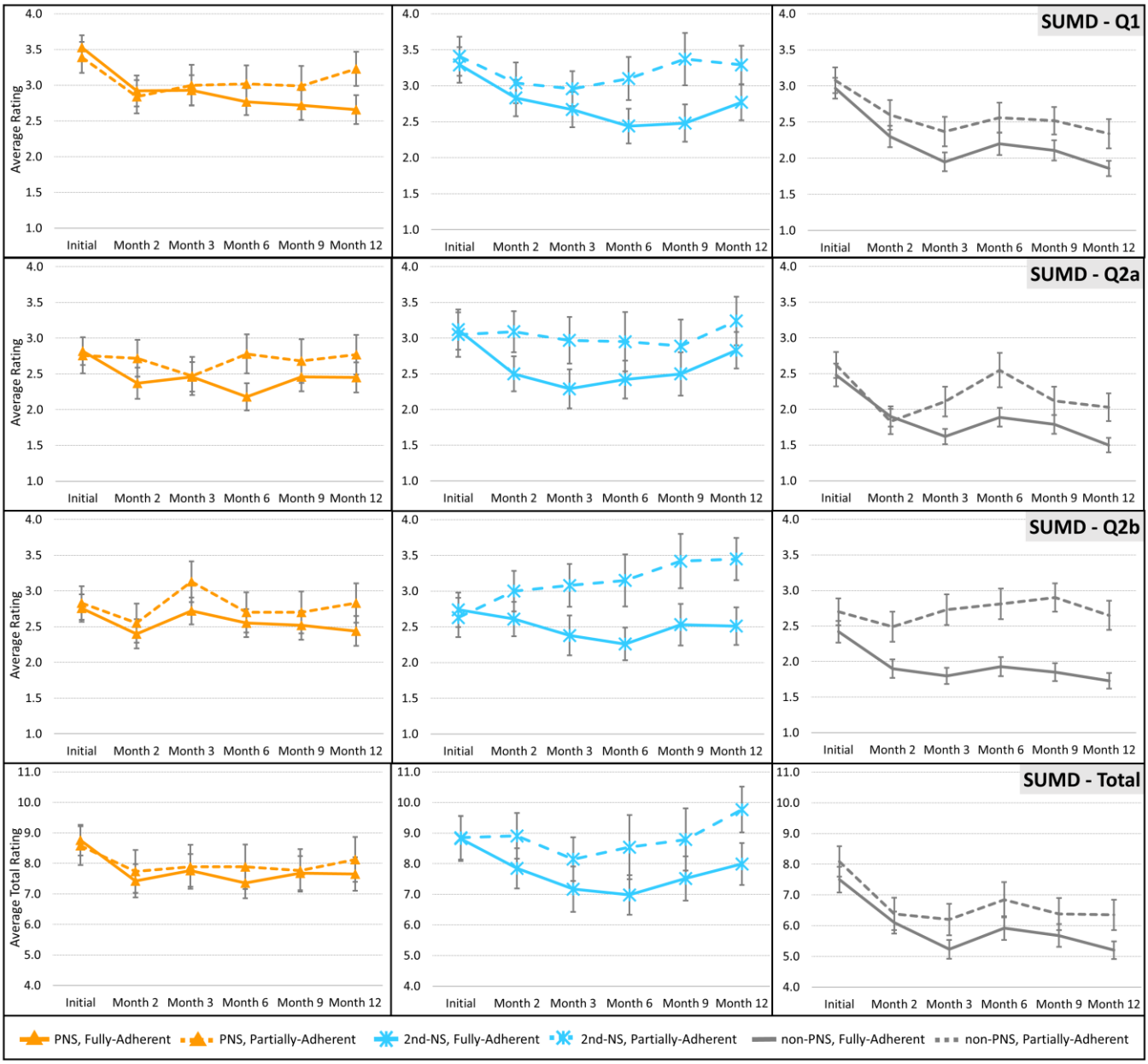
Figure 3 presents the profiles for SUMD Total, Q1, Q2a and Q2b over the 12-month time period for PNS, 2nd-NS, and non-PNS groups; **eTable 3** presents the data values. A complete description of the results are in the supplementary material with a brief summary below.

For SUMD-Q1 (awareness of mental illness), all FEP patients showed a significant decrease (better insight) from Baseline to Month 2; the non-PNS group showed further decreases from Month 2 to Month 3 and again from Month 9 to Month 12. In fact, the non-PNS group had lower ratings (better insight) at all timepoints compared to both the PNS and 2nd-NS groups which, in turn, did not significantly differ from one another at any point over the 12 months. Importantly, there was no significant group by medication adherence interaction. For SUMD-Q2a (belief in response to medication), only the non-PNS group showed a significant decrease from Baseline to Month 2. And, as with SUMD-Q1, the non-PNS had lower ratings at all timepoints compared to the PNS and 2nd-NS groups, which did not differ from one another. There was no interaction with medication adherence.

Finally, and most striking, for SUMD-Q2b (belief in need for treatment) there was a significant group by medication adherence interaction. Intriguingly, within the PNS group, there was no difference in ratings between Fully-Adherent and Partially-Adherent (a trend noticeable for the other two insight questions; see Figure 3). Conversely, within the 2nd-NS group, the Partially-Adherent patients showed a steady increase in ratings (worsening insight) over the 12-month period that significantly differed from the Fully-Adherent patients from Month 6 until

Month 12. In fact, by the end, the '2nd-NS, Partially-Adherent' subgroup showed the worst insight among all of the subgroups. Finally, within the non-PNS group, the Fully-Adherent patients showed a stable, low-level rating that significantly differed from the Partially-Adherent patients from Month 2 to Month 12.

Figure 3. Data profile for SUMD total among the subgroups.



Abbreviations: PNS, primary negative symptoms; 2nd-NS, secondary negative symptoms. The vertical axes represent the average estimated rating from the GEE analysis where a lower rating represents better insight. Errors bars reflect the standard error. The SUMD Total is the sum of Q1, Q2a, and Q2b.

4. Discussion

This study explored changes in clinical insight (awareness of mental illness, awareness of response to medication, and belief in the need for treatment) and medication adherence in relation to persistent (primary) negative symptoms (PNS) and secondary negative symptoms (2nd-NS) over a 12-month period following admission into an early intervention first-episode of psychosis (FEP) service. Several key results were revealed.

First, all FEP patients showed an initial improvement in overall insight over the first two months, driven by improvements in awareness of mental illness and awareness of response to medication. Second, compared to the non-PNS group, the PNS and 2nd-NS groups displayed poorer awareness of mental illness and awareness of response to medication that varied minimally over the 12-month period and did not interact with medication adherence.

The same result was found for 'belief in need for treatment', but there was a significant interaction with medication adherence. To begin, the 'non-PNS, Fully-Adherent' patients showed the best insight overall with a significant improvement over the first two months. Conversely, the '2nd-NS, Partially-Adherent' patients showed a steady decline over the 12-month period that resulted in this group having the poorest insight. Quite strikingly, the PNS group showed no interaction with medication adherence on this or any other measure of insight over the 12-month period. Relatedly, and of particular note, medication adherence profiles were the same among the PNS, 2nd-NS, or non-PNS groups.

4.1 Treatment Adherence and Insight in Patients with PNS

Primary negative symptoms continue to represent a significant, unmet therapeutic need among people with psychosis (3, 31) that crucially needs to be addressed as PNS has been linked to a worse functional outcome (32, 33). With pharmacotherapies likely to be available for PNS (4, 5), two critical factors related to implementation of such therapy need to be addressed: insight and adherence.

People with PNS have been shown to display decreased motivation in obtaining and taking medication (7, 19). Negative symptoms have also been identified as an initial barrier towards developing a good therapeutic alliance (34, 35). This has led to the idea that patients with PNS invariably display poor adherence. However, as shown by our results, and contrary to several studies (36, 37), adherence was the same among the PNS, 2nd-NS, and non-PNS groups.

Moreover, among the PNS patients, poorer insight had no overall effect on actual mediation adherence. This supported a recent review (38) that questioned the findings linking insight and adherence (39-44). As such, PNS patients may indeed be agreeable to treatment and those who displayed poorer adherence may have done so, simply because antipsychotics are not overly effective in treating negative symptoms (6).

Predictably, those with PNS displayed poorer insight which should be addressed during treatment. Although no current treatments are specifically designed to improve insight, a current meta-analysis confirmed that insight is a potential therapeutic target amenable to improvement (45). In two recent 1-year clinical trials, participants who received basic cognitive behavioral therapy (CBT) (46) or Guided Self-Determination (GSD) adapted for schizophrenia (47) significantly gained more insight. Further, the patients displayed significant reductions in negative symptom severity and improved social functioning (47). These clinical trials suggested interventions are available to help improve insight and negative symptoms; however, it is unknown if improvements would be similar in patients with PNS. With no known studies having addressed this idea, it would be beneficial to conduct a study employing PNS patients to receive basic CBT treatment to see if either insight or negative symptom severity could be improved. This would be key to seeing whether or not insight could be improved beyond we observed over the first two months and to the levels displayed by the non-PNS patients. A better appreciation of the association that insight may share with PNS in psychosis could improve our knowledge about etiology, prognosis, and treatment-related facets, such as implementation of newer medications, in those with psychosis, specifically among those with PNS (48).

4.2 Limitations

Unlike previous cross-sectional studies examining the inter-relationship among negative symptoms, insight, and treatment adherence at a single timepoint, reducing all findings to a regression, the longitudinal nature of our study made it possible to use statistical methods that captured the temporal dynamics among these factors. Nevertheless, several limitations of our study need to be considered. First, although we measured medication adherence using a reliable and validated method (25), it was not possible to monitor direct intake of medication or how "true" adherence may have affected the results. Future studies may consider employing long-acting injectables, to ensure adherence and improve our understanding of the relationship with insight

and negative symptoms. Second, as with any longitudinal study, there were multiple data points missing across the multiple timepoints. Although we used a powerful GEE analysis, designed to account for missing data, caution is still advised when interpreting data that has been inferred.

4.3 Summary

While receiving treatment from a specialized early intervention service, FEP patients identified with PNS and 2nd-NS displayed worse insight, compared to non-PNS patients, over a 12-month follow-up period. Further, poorer insight in the PNS group actually became stable after the first few months of treatment. We also found that medication adherence did not differ among the three groups. And, quite importantly, insight and adherence showed no interaction within the PNS group, unlike what was found for the 2nd-NS and non-PNS groups. In sum, PNS patients appear amenable to treatment but current treatments, even those provided early on by specialized intervention services, appear to be inadequate in treating PNS; newer, more efficacious treatments are needed.

5. References

1. Hovington CL, Bodnar M, Joober R, Malla AK, Lepage M. Identifying persistent negative symptoms in first episode psychosis. BMC psychiatry. 2012;12:224.
2. Chang WC, Hui CL, Tang JY, Wong GH, Lam MM, Chan SK, Chen EY. Persistent negative symptoms in first-episode schizophrenia: a prospective three-year follow-up study. Schizophrenia Research. 2011;133:22-28.
3. Kirkpatrick B, Fenton WS, Carpenter WT, Jr., Marder SR. The NIMH-MATRICS consensus statement on negative symptoms. Schizophrenia Bulletin. 2006;32:214-219.
4. Arango C, Garibaldi G, Marder SR. Pharmacological approaches to treating negative symptoms: a review of clinical trials. Schizophrenia Research. 2013;150:346-352.
5. Davis MC, Horan WP, Marder SR. Psychopharmacology of the negative symptoms: current status and prospects for progress. Eur Neuropsychopharmacol. 2014;24:788-799.
6. Moller HJ, Czobor P. Pharmacological treatment of negative symptoms in schizophrenia. European archives of psychiatry and clinical neuroscience. 2015.
7. Haddad PM, Brain C, Scott J. Nonadherence with antipsychotic medication in schizophrenia: challenges and management strategies. Patient related outcome measures. 2014;5:43-62.
8. Dantas CR, Barros BR, Fernandes PT, Li LM, Banzato CE. Insight controlled for cognition in deficit and nondeficit schizophrenia. Schizophrenia Research. 2011;128:124-126.
9. Kirkpatrick B, Castle D, Murray RM, Carpenter WT, Jr. Risk factors for the deficit syndrome of schizophrenia. Schizophrenia Bulletin. 2000;26:233-242.

10. Kosger F, Sahin H, Essizoglu A, Aksaray G. [Insight and social functioning in deficit and nondeficit schizophrenia]. Turk psikiyatri dergisi = Turkish journal of psychiatry. 2014;25:157-162.
11. Pegoraro LF, Dantas CR, Banzato CE, Fuentes D. Correlation between insight dimensions and cognitive functions in patients with deficit and nondeficit schizophrenia. Schizophrenia Research. 2013;147:91-94.
12. Trotman HD, Kirkpatrick B, Compton MT. Impaired insight in patients with newly diagnosed nonaffective psychotic disorders with and without deficit features. Schizophrenia Research. 2011;126:252-256.
13. Buchy L, Bodnar M, Malla A, Joober R, Lepage M. A 12-month outcome study of insight and symptom change in first-episode psychosis. Early intervention in psychiatry. 2010;4:79-88.
14. Mintz AR, Addington J, Addington D. Insight in early psychosis: a 1-year follow-up. Schizophrenia Research. 2004;67:213-217.
15. Konstantakopoulos G, Ploumpidis D, Oulis P, Soumani A, Nikitopoulou S, Pappa K, Papadimitriou GN, David AS. Is insight in schizophrenia multidimensional? Internal structure and associations of the Greek version of the Schedule for the Assessment of Insight-Expanded. Psychiatry Res. 2013;209:346-352.
16. Amador XF, Strauss DH, Yale SA, Flaum MM, Endicott J, Gorman JM. Assessment of insight in psychosis. Am J Psychiatry. 1993;150:873-879.
17. David AS. Insight and psychosis. Br J Psychiatry. 1990;156:798-808.
18. Ayesa-Arriola R, Rodriguez-Sanchez JM, Morelli C, Pelayo-Teran JM, Perez-Iglesias R, Mata I, Martinez-Garcia O, Pardo-Garcia G, Vazquez-Barquero JL, Crespo-Facorro B. Insight dimensions in first-episode psychosis patients: clinical, cognitive, pre-morbid and socio-demographic correlates. Early intervention in psychiatry. 2011;5:140-149.
19. Rettenbacher MA, Hofer A, Eder U, Hummer M, Kemmler G, Weiss EM, Fleischhacker WW. Compliance in schizophrenia: psychopathology, side effects, and patients' attitudes toward the illness and medication. The Journal of clinical psychiatry. 2004;65:1211-1218.
20. Amador XF, Flaum M, Andreasen NC, Strauss DH, Yale SA, Clark SC, Gorman JM. Awareness of illness in schizophrenia and schizoaffective and mood disorders. Arch Gen Psychiatry. 1994;51:826-836.
21. Andreasen NC: Modified Scale for the Assessment of Negative Symptoms (SANS). Iowa City, University of Iowa; 1984.
22. Andreasen NC: Scale for the Assessment of Positive Symptoms (SAPS). Iowa City, University of Iowa; 1984.
23. Addington D, Addington J, Schissel B. A depression rating scale for schizophrenics. Schizophrenia Research. 1990;3:247-251.
24. Velligan DI, Lam YW, Glahn DC, Barrett JA, Maples NJ, Ereshefsky L, Miller AL. Defining and assessing adherence to oral antipsychotics: a review of the literature. Schizophrenia Bulletin. 2006;32:724-742.
25. Cassidy CM, Rabinovitch M, Schmitz N, Joober R, Malla A. A comparison study of multiple measures of adherence to antipsychotic medication in first-episode psychosis. Journal of Clinical Psychopharmacology. 2010;30:64-67.
26. Kay S, Fiszbein, A., Opler, L. . The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophrenia Bulletin. 1987;13:261-276.
27. Woods SW. Chlorpromazine equivalent doses for the newer atypical antipsychotics. The Journal of clinical psychiatry. 2003;64:663-667.

28. Woods SW: Chlorpromazine Equivalent Doses for Atypical Antipsychotics: An Update 2003-2010. 2011. pp. 1-8.
29. Wechsler D: Wechsler Adult Intelligence Scale 3rd ed. San Antonio, TX, The Psychological Corporation; 1997.
30. Wechsler D: Wechsler Abbreviated Scale of Intelligence. San Antonio, TX, The Psychological Corporation; 1999.
31. Buchanan RW. Persistent negative symptoms in schizophrenia: an overview. Schizophrenia Bulletin. 2007;33:1013-1022.
32. Fervaha G, Foussias G, Agid O, Remington G. Impact of primary negative symptoms on functional outcomes in schizophrenia. European psychiatry : the journal of the Association of European Psychiatrists. 2014;29:449-455.
33. Foussias G, Agid O, Fervaha G, Remington G. Negative symptoms of schizophrenia: clinical features, relevance to real world functioning and specificity versus other CNS disorders. Eur Neuropsychopharmacol. 2014;24:693-709.
34. Jung E, Wiesjahn M, Lincoln TM. Negative, not positive symptoms predict the early therapeutic alliance in cognitive behavioral therapy for psychosis. Psychotherapy research : journal of the Society for Psychotherapy Research. 2014;24:171-183.
35. Lysaker PH, Davis LW, Buck KD, Outcalt S, Ringer JM. Negative symptoms and poor insight as predictors of the similarity between client and therapist ratings of therapeutic alliance in cognitive behavior therapy for patients with schizophrenia. Journal of Nervous and Mental Disease. 2011;199:191-195.
36. Subotnik KL, Ventura J, Gretchen-Doorly D, Hellemann GS, Agee ER, Casaus LR, Luo JS, Villa KF, Nuechterlein KH. The impact of second-generation antipsychotic adherence on positive and negative symptoms in recent-onset schizophrenia. Schizophrenia Research. 2014.
37. Tattan TM, Creed FH. Negative symptoms of schizophrenia and compliance with medication. Schizophrenia Bulletin. 2001;27:149-155.
38. Lincoln TM, Lullmann E, Rief W. Correlates and long-term consequences of poor insight in patients with schizophrenia. A systematic review. Schizophrenia Bulletin. 2007;33:1324-1342.
39. Chan KW, Hui LM, Wong HY, Lee HM, Chang WC, Chen YH. Medication adherence, knowledge about psychosis, and insight among patients with a schizophrenia-spectrum disorder. Journal of Nervous and Mental Disease. 2014;202:25-29.
40. Beck EM, Cavelti M, Kvrgic S, Kleim B, Vauth R. Are we addressing the 'right stuff' to enhance adherence in schizophrenia? Understanding the role of insight and attitudes towards medication. Schizophrenia Research. 2011;132:42-49.
41. Lepage M, Bodnar M, Buchy L, Joober R, Malla A. Early Medication Adherence and Insight Change in First-Episode Psychosis. Clinical schizophrenia & related psychoses. 2010;3:201 - 208.
42. Buckley PF, Wirshing DA, Bhushan P, Pierre JM, Resnick SA, Wirshing WC. Lack of insight in schizophrenia: impact on treatment adherence. CNS drugs. 2007;21:129-141.
43. Mutsatsa SH, Joyce EM, Hutton SB, Webb E, Gibbins H, Paul S, Barnes TR. Clinical correlates of early medication adherence: West London first episode schizophrenia study. Acta psychiatrica Scandinavica. 2003;108:439-446.
44. Lin IF, Spiga R, Fortsch W. Insight and adherence to medication in chronic schizophrenics. The Journal of clinical psychiatry. 1979;40:430-432.
45. Pijnenborg GH, van Donkersgoed RJ, David AS, Aleman A. Changes in insight during treatment for psychotic disorders: a meta-analysis. Schizophrenia Research. 2013;144:109-117.

46. Turkington D, Kingdon D, Rathod S, Hammond K, Pelton J, Mehta R. Outcomes of an effectiveness trial of cognitive-behavioural intervention by mental health nurses in schizophrenia. Br J Psychiatry. 2006;189:36-40.
47. Jorgensen R, Licht RW, Lysaker PH, Munk-Jorgensen P, Buck KD, Jensen SO, Hansson L, Zoffmann V. Effects on cognitive and clinical insight with the use of Guided Self-Determination in outpatients with schizophrenia: A randomized open trial. European psychiatry : the journal of the Association of European Psychiatrists. 2015.
48. Joseph B, Narayanaswamy JC, Venkatasubramanian G. Insight in schizophrenia: relationship to positive, negative and neurocognitive dimensions. Indian J Psychol Med. 2015;37:5-11.

Supplementary Material for Article 2

1. Supplementary Methods

Statistical analyses

The relationship between persistent negative symptoms, medication adherence, and clinical insight over a 12 month period was analyzed using Generalized Estimating Equations (GEE). The GEE analysis is considered a multivariate extension of the generalized linear model to analyze repeated measurements or other correlated observations. There are several advantages inherent to GEE for examining a large, longitudinal data set including its robust nature to accommodate violations of normality (homogeneity of variance) and incomplete data based on population quantities and data distributions (exclude missing observations within a subject and not entire subject).

Each variable of the SUMD (Q1, Q2a, Q2b, and Total) was individually modelled with these factors: time, NS-group (PNS, 2nd-NS, non-PNS), and ADH-group (Fully-Adherent vs. Partially-Adherent). Education were added as a covariate as significant differences were noted among the four subgroups. Estimated means and, where necessary, pairwise comparisons were computed for all factors and factor interactions. For each model, we specified the following: distribution as 'normal'; link function as 'log'; and working correlation matrix as 'unstructured'. The 'normal, log' models the data along a normal distribution after being log(x) transformed (SUMD data were not normally distributed). The 'unstructured' matrix models the actual correlations among the data and was chosen over 'independent' which assumes all correlations are zero and 'exchangeable' which assumes all correlations are equal.

Age, education level, Full Scale IQ, and overall medication adherence were compared with ANOVAs; sex (male vs. female) with cross-tabulations and a Chi-squared test; and DUI with a Median test. Symptom totals, antipsychotic dosage, and medication adherence at 4 time points were compared with GEE; using the same parameters as with the insight analysis. All analyses were conducted using SPSS 22 (IBM Corp., Armonk, NY, USA) and were two-tailed with a critical *P*-value of 0.05.

2. Supplementary Results

Symptom totals, medication adherence, and antipsychotic dosage

Negative Symptom (SANS) Totals

For SANS Totals, the GEE analysis revealed significant main effects of 'Time' (Wald χ^2=222.15, df=5, P<0.001) and 'NS-group' (Wald χ^2=192.47, df=2, P<0.001). There were significant 'Time x NS-group' (Wald χ^2=117.80, df=10, P<0.001) and 'Time x ADH-group' (Wald χ^2=13.42, df=5, P=0.020) interactions.

For the 'Time x ADH-group' interaction, the Fully-Adherent and Partially-Adherent decreased in SAPS total from Baseline to Month 2 (Ps<0.001); the Fully-Adherent showed a further decrease from Month 3 to Month 6 (P=0.004) and from Month 6 to Month 9 (P=0.003). In addition, the Fully-Adherent patients had higher totals compared to the Partially-Adherent patients at Baseline (P=0.040), Month 2 (P=0.009), and Month 3 (P=0.002).

For the 'Time x NS-group' interaction, the PNS group displayed a significant decrease from Baseline to Month 2 (P<0.001). The non-PNS group had significant decreases between all successive timepoints (Ps<0.034), except from Month 6 to Month 9 (P=0.691). The 2nd-NS group displayed a highly variable trend over the time period with deceases from Baseline to Month 2 (P<0.001) and from Month 6 to Month 9 (P<0.001), as well as increases from Month 3 to Month 6 (P=0.006) and from Month 9 to Month 12 (P=0.002). Finally, the PNS group did not significantly differ from the 2nd-NS group at any timepoint (Ps>0.230). The non-PNS group had significantly lower totals at all timepoints compared to the PNS and 2nd-NS groups (Ps<0.001).

Positive Symptom (SAPS) Totals

For SAPS Totals, the GEE analysis revealed significant main effects of 'Time' (Wald χ^2=1000.60, df=5, P<0.001), 'ADH-group' (Wald χ^2=5.65, df=1, P=0.018), and 'NS-group' (Wald χ^2=66.70, df=2, P<0.001). There were

significant 'Time x NS-group' (Wald χ^2=89.56, df=10, *P*<0.001) and 'Time x ADH-group' (Wald χ^2=33.42, df=5, *P*<0.001) interactions.

For the 'Time x ADH-group' interaction, the Fully-Adherent and Partially-Adherent decreased in SAPS total from Baseline to Month 2 (*P*s<0.001); the Fully-Adherent had a further decrease from Month 2 to Month 3 (*P*<0.001) whereas the Partially-Adherent had a significant increase from Month 3 to Month 6 (*P*=0.003). The Fully-Adherent patients had lower totals compared to the Partially-Adherent patients at Month 6 (*P*=0.007), Month 9 (*P*=0.001), and Month 12 (*P*=0.001).

For the 'Time x NS-group' interaction, the non-PNS group displayed a significant decrease from Baseline to Month 2 (*P*<0.001) and from Month 2 to Month 3 (*P*=0.001). The PNS group also displayed a significant decrease from Baseline to Month 2 (*P*<0.001) and from Month 2 to Month 3 (*P*=0.014); however, they showed an increase in total score from Month 3 to Month 6 (*P*=0.046) followed by a decrease from Month 9 to Month 12 (*P*=0.048). The 2nd-NS group displayed a highly variable trend over the time period with an initial decease from Baseline to Month 2 (*P*<0.001) followed by increases from Month 3 to Month 6 (*P*=0.002) and from Month 9 to Month 12 (*P*=0.036). Finally, the PNS group did not significantly differ from the non-PNS group at any timepoint (*P*s>0.129, except at Month 6 (*P*=0.030). The 2nd-NS group had significantly higher totals at all timepoints compared to the PNS (*P*s<0.014) and non-PNS (*P*s<0.001) groups, except at Baseline (*P*=0.398 vs. PNS; *P*=0.092 vs. non-PNS).

Depression Symptom (CDSS) Totals

For CDSS Totals, the GEE analysis revealed significant main effects of 'Time' (Wald χ^2=187.37, df=5, *P*<0.001) and 'NS-group' (Wald χ^2=27.72, df=2, *P*<0.001), and a significant 'Time x NS-group' interaction (Wald χ^2=31.94, df=10, *P*<0.001).

For the interaction, all groups displayed a significant decrease in total score from Baseline to Month 2 (*P*s<0.001) with no further changes over time for any group (*P*s>0.054), except for the PNS group from Month 9 to Month 12 (*P*=0.011). The PNS group did not significantly differ from the non-PNS group at any timepoint (*P*s>0.128). The 2nd-NS group had significantly higher totals at all timepoints compared to the PNS group (*P*s<0.035) and non-PNS group (*P*s<0.005), except at Baseline (*P*=0.0.085 vs. PNS; *P*=0.528 vs. non-PNS) and at Month 2 (*P*=0.342 vs. non-PNS).

Medication Adherence Percentage

For adherence, the GEE analysis revealed significant main effects of 'Time' (Wald χ^2=59.48, df=5, *P*<0.001) and 'ADH-group' (Wald χ^2=219.70, df=1, *P*<0.001), and a significant 'Time x ADH-group' interaction (Wald χ^2=119.91, df=5, *P*<0.001).

For the interaction, the Fully-Adherent patients significantly increased in adherence from Baseline to Month 2 (*P*=0.001), Month 2 to Month 3 (*P*=0.005), and Month 3 to Month 6 (*P*=0.031). In contrast, the Partially-Adherent patients significantly decreased in adherence from Baseline to Month 2 (*P*=0.011), Month 2 to Month 3 (*P*<0.001), and Month 3 to Month 6 (*P*=0.015). Finally, the Fully-Adherent and Partially-Adherent patients significantly differed at all timepoints (*P*s<0.013).

Antipsychotic Dosage (Chlorpromazine Equivalents)

For antipsychotic dosage, there were significant main effects of 'Time' (Wald χ^2=12.72, df=5, *P*=0.026), 'NS-group' (Wald χ^2=8.76, df=2, *P*=0.012), and 'ADH-group' (Wald χ^2=9.97, df=1, *P*=0.002). There was also a significant 'Time x NS-group' interaction (Wald χ^2=18.31, df=10, *P*=0.050).

For the main effect of 'ADH-group', further analyses revealed the Fully-Adherent patients were prescribed a higher dosage overall compared to the Partially-Adherent patients. For the interaction, the PNS and non-PNS groups did not significantly differ at any timepoint (*P*s<0.087). The 2nd-NS group was prescribed a higher dosage at all timepoints that significantly higher at Month 9 (*P*=0.013 vs. PNS; *P*=0.005 vs. non-PNS) and Month 12 (*P*=0.005 vs. PNS; *P*<0.001 vs. non-PNS).

Clinical Insight and Medication Adherence among Patient Groups

SUMD - Total

For the SUMD Total, there were significant main effects of 'Time' (Wald χ^2=39.29, df=5, *P*<0.001), 'NS-group' (Wald χ^2=35.25, df=2, *P*<0.001), and 'ADH-group' (Wald χ^2=6.99, df=1, *P*=0.008). There was also a significant 'Time x NS-group' interaction (Wald χ^2=22.53, df=10, *P*=0.013). The main effect of 'ADH-group' reflected that the Fully-Adherent patients showed a lower overall average rating (better insight) compared to the Partially-Adherent patients.

For the interaction, the non-PNS (*P*<0.001) and PNS (*P*=0.016) groups showed a significant decrease (improved insight) from Baseline to Month 2; however, the non-PNS group showed a worsening from Month 6 to Month 9 (*P*=0.023). For the between-group comparisons, the PNS and 2nd-NS subgroups did not significantly differ at any timepoint (*P*s>0.158). For the between-group differences, the non-PNS had a significantly lower overall average rating compared to the PNS (Ps<0.009) and 2nd-NS (Ps<0.019) subgroups at all timepoints, except at Baseline trend-level differences were apparent (*P*=0.062 vs. PNS; *P*=0.052 vs. 2nd-NS).

SUMD – Q1

For the SUMD-Q1, there were significant main effects of 'Time' (Wald χ^2=67.47, df=5, *P*<0.001), 'NS-group' (Wald χ^2=25.76, df=2, *P*<0.001), and 'ADH-group' (Wald χ^2=9.27, df=1, *P*=0.002). As well, there was a significant 'Time x NS-group' interaction (Wald χ^2=26.22, df=10, *P*=0.003). The main effect of 'ADH-group' reflected that the Fully-Adherent patients showed a lower average rating (better insight of mental illness) compared to the Partially-Adherent patients.

For the interaction, all groups showed a significant decrease (improved insight) from Baseline to Month 2 (*P*s<0.023); the non-PNS group also showed significant decreases from Month 2 to Month 3 (*P*=0.006) and from Month 9 to Month 12 (*P*=0.040). For between-group differences, the PNS and 2nd-NS groups did not significantly differ at any timepoint (*P*s>0.506). Moreover, the non-PNS group had a significantly lower average rating compared to the PNS (Ps<0.021) and 2nd-NS (Ps<0.018) groups at all timepoints, except versus the 2nd-NS group at Baseline (*P*=0.122) and at Month 6 (*P*=0.084).

SUMD – Q2a

For SUMD Q2a, there were significant main effects of 'Time' (Wald χ^2=27.73, df=5, *P*<0.001), 'NS-group' (Wald χ^2=32.39, df=2, *P*<0.001), and 'ADH-group' (Wald χ^2=9.94, df=1, *P*=0.002). As well, there was a significant 'Time x NS-group' interaction (Wald χ^2=18.64, df=10, *P*=0.045). Regarding the main effect of 'ADH-group', the Fully-Adherent patients showed a lower overall average rating (better insight of response to medications) compared to the Partially-Adherent patients.

For the interaction, only the non-PNS group showed a significant decrease (improved insight) from Baseline to Month 2 (*P*<0.001) followed by an increase (worsening) from Month 3 to Month 6 (*P*=0.007). The PNS and 2nd-NS groups did not show any significant change between successive timepoints (*P*s>0.189). For between-group differences, the PNS and 2nd-NS groups did not significantly differ at any timepoint (*P*s>0.113). Moreover, the non-PNS group had a significantly lower average rating compared to the PNS (Ps<0.002) and 2nd-NS (Ps<0.022) groups at all timepoints, except at Baseline vs. PNS (*P*=0.218) and at Month 6 vs. PNS (*P*=0.198) and 2nd-NS (*P*=0.072).

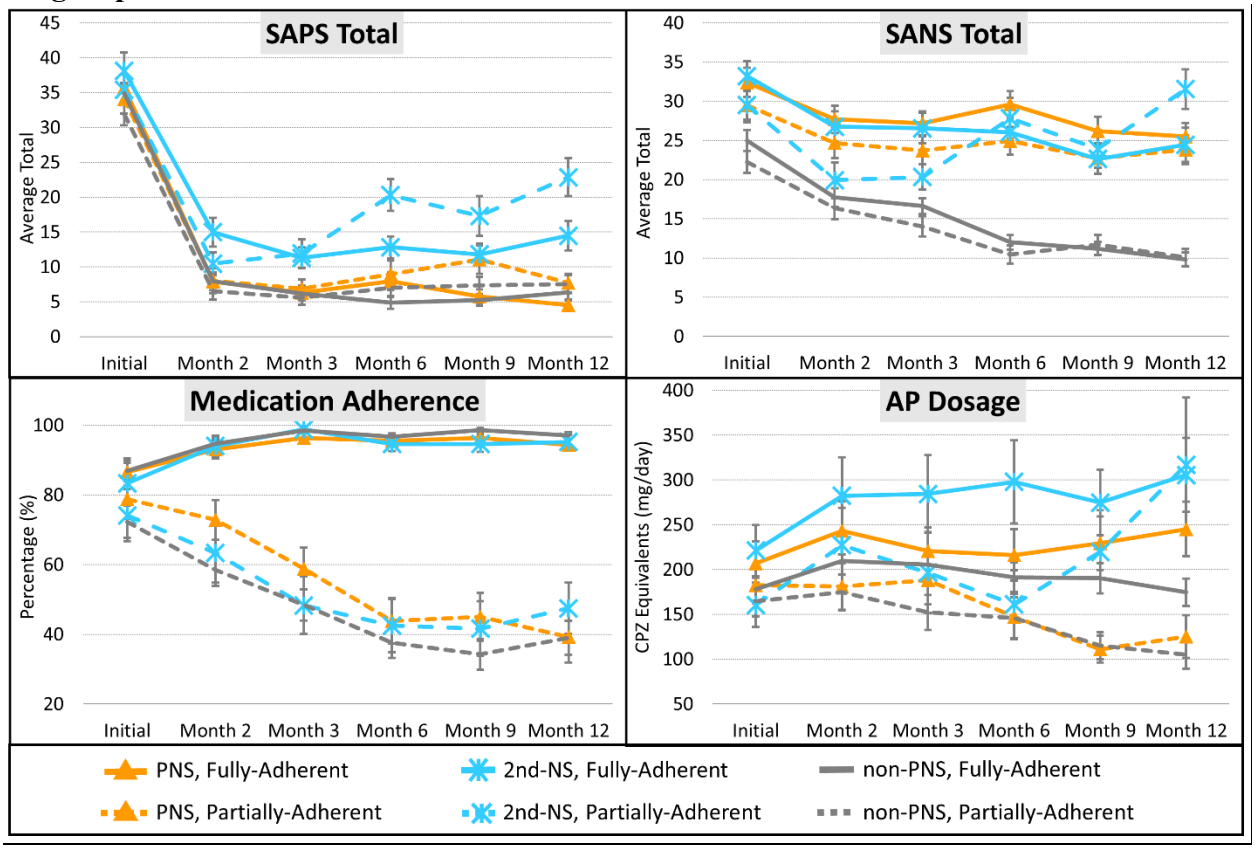
SUMD – Q2b

For SUMD Q2b, there was a main effects of NS-group' (Wald χ^2=15.46, df=2, *P*<0.001), and 'ADH-group' (Wald χ^2=23.73, df=1, *P*<0.001), as well as three significant interactions: 'ADH-group x NS=group' (Wald χ^2=6.67, df=2, *P*=0.036); 'Time x ADH-group' (Wald χ^2=15.76, df=5, *P*=0.008); and 'Time x NS-group' (Wald χ^2=19.68, df=10, *P*=0.032). Finally, there was a significant 'Time x ADH-group x NS-group' (Wald χ^2=48.21, df=25, *P*=0.004).

For the triple interaction, the 'PNS, Fully-Adherent' (*P*=0.049) and 'non-PNS, Fully-Adherent' (*P*=0.001) showed a significant decrease (better belief in treatment) from Baseline to Month 2. The 'PNS, Partially-Adherent' showed an increase (worsening of insight) from Month 2 to Month 3 (*P*=0.044). Within the PNS group, the Fully-Adherent and Partially-Adherent did not differ at any time point (*P*s>0.195). Within the 2nd-NS group, the Partially-Adherent patients showed a worsening of insight that significantly differed from the Fully-Adherent patients at Month 6 (*P*=0.031), Month 9 (*P*=0.042), and Month 12 (*P*=0.011). In contrast, within the non-PNS group, the Fully-Adherent patients showed an improvement in insight that significantly differed from the Partially-Adherent patients between

successive timepoints from Month 2 until Month 12 (*P*s<0.010). By Month 12, the '2nd-NS, Partially-Adherent' subgroup displayed the worst insight that significantly differed from all subgroups (*P*s<0.018) except the 'PNS, Partially-Adherent' subgroup (*P*=0.105).

eFigure 1. Symptom totals, medication adherence, and antipsychotic dosage among subgroups.



Abbreviations: SAPS, Scale for the Assessment of Positive Symptoms; SANS, Scale for the Assessment of Negative Symptoms; CPZ, chlorpromazine. Note vertical titles for different scales among panels. Errors bars reflect the standard error.

eTable 1. Diagnoses among subgroups.

	Non-Affective (n=271)	Affective (n=111)	Total (n=382)
PNS			
Fully Adherent	53 (19.6%)	18 (16.2%)	71
Partially Adherent	36 (13.3%)	11 (9.9%)	47
2nd-NS			
Fully Adherent	36 (13.7%)	6 (5.4%)	42
Partially Adherent	24 (8.9%)	7 (6.3%)	31
non-PNS			
Fully Adherent	71 (26.2%)	38 (34.2%)	109
Partially Adherent	50 (18.5%)	31 (27.9%)	81

Number and Percentage of Diagnosis. Full sample with data (n=382); three did not have final diagnoses. Crosstabs analysis: $\chi^2 = 11.71$, p=0.039.

eTable 2. Raw Data for Clinical Characteristics.

	PNS		2nd-PNS		non-PNS	
All data: *M* (*SD*) [n]	Fully Adherent	Partially Adherent	Fully Adherent	Partially Adherent	Fully Adherent	Partially Adherent
SANS Total						
Initial	32.2 (15.6)	29.5 (12.7) [46]	33.1 (12.3)	29.4 (12.0)	25.0 (14.6)	22.2 (12.9)
Month 2	27.2 (13.4) [60]	24.7 (12.2) [39]	27.0 (12.0) [39]	19.6 (11.7) [28]	17.6 (12.1) [102]	16.5 (11.2) [59]
Month 3	27.1 (12.7) [71]	23.7 (11.4) [41]	26.3 (12.1) [40]	20.0 (8.4) [28]	16.5 (10.4) [103]	14.1 (11.0) [72]
Month 6	29.4 (13.3) [67]	25.0 (10.9) [38]	26.1 (10.2) [40]	27.0 (13.1) [25]	11.6 (9.0) [103]	10.5 (9.9) [72]
Month 9	26.1 (14.9) [68]	22.7 (11.5) [35]	22.8 (11.6) [39]	23.2 (12.1) [25]	10.8 (8.5) [104]	11.8 (11.0) [72]
Month 12	25.1 (14.0) [64]	23.8 (11.2) [38]	24.9 (13.6) [39]	30.7 (14.1) [30]	9.5 (8.4) [104]	10.1 (9.0) [63]
SAPS Total						
Initial	35.7 (16.2)	34.3 (14.1) [46]	38.1 (17.3)	35.4 (12.4)	34.9 (15.3) [109]	31.9 (14.3)
Month 2	7.9 (9.6) [60]	8.2 (11.1) [38]	15.0 (12.8) [39]	10.5 (8.7) [28]	8.0 (13.0) [102]	6.6 (9.6) [59]
Month 3	6.3 (8.3) [71]	7.0 (8.6) [41]	11.3 (9.3) [40]	11.9 (10.7) [28]	6.2 (9.8) [104]	5.6 (8.7) [72]
Month 6	7.9 (10.0) [67]	9.1 (11.9) [37]	12.8 (9.9) [40]	20.3 (11.4) [25]	4.9 (9.2) [103]	7.0 (11.0) [72]
Month 9	5.8 (7.8) [68]	11.3 (12.2) [35]	11.8 (9.6) [39]	17.3 (14.3) [25]	5.2 (8.6) [104]	7.4 (10.3) [71]
Month 12	4.5 (5.7) [64]	7.7 (8.3) [38]	14.5 (13.1) [39]	22.9 (14.8) [30]	6.3 (9.8) [104]	7.5 (10.3) [63]
CDSS Total						
Initial	4.3 (4.2)	4.5 (4.9) [46]	6.1 (5.2)	5.3 (5.0)	5.4 (5.3) [109]	5.1 (4.9)
Month 2	2.3 (3.0) [61]	1.9 (2.2) [39]	3.9 (4.2) [39]	2.1 (2.5) [28]	2.7 (3.4) [102]	2.5 (3.5) [59]
Month 3	1.5 (2.7) [71]	2.3 (2.8) [41]	3.6 (3.9) [40]	3.3 (3.2) [28]	2.1 (2.8) [105]	2.1 (3.4) [72]
Month 6	2.4 (4.3) [67]	1.5 (2.8) [39]	4.2 (4.6) [40]	3.6 (4.5) [25]	1.7 (2.7) [103]	2.3 (3.8) [72]
Month 9	1.5 (2.5) [68]	2.5 (3.8) [36]	4.6 (4.9) [39]	3.6 (3.7) [25]	1.4 (2.5) [104]	1.9 (3.5) [73]
Month 12	1.0 (1.6) [64]	1.2 (2.0) [38]	4.4 (4.5) [39]	3.2 (4.2) [30]	1.2 (2.4) [104]	2.0 (3.4) [63]
Adherence						
Initial	87.0 (32.1) [71]	78.8 (38.4) [46]	83.7 (37.4)	75.0 (40.3)	86.6 (32.8)	72.0 (42.0)
Month 2	93.3 (21.5) [71]	72.8 (38.7) [46]	94.2 (18.8)	62.1 (46.0)	94.6 (19.6)	59.0 (42.3) [81]
Month 3	96.5 (11.4) [71]	58.9 (40.3) [45]	98.8 (5.3)	46.8 (45.1)	98.4 (6.1)	49.1 (30.9)
Month 6	95.7 (11.2) [70]	43.9 (44.0) [45]	94.8 (12.9)	41.1 (41.6)	96.6 (10.4)	38.1 (40.5)
Month 9	96.4 (10.7) [70]	45.0 (46.0) [45]	94.8 (15.0)	41.9 (42.5)	98.6 (5.7) [109]	34.1 (40.2) [80]

Month 12	94.2 (13.0) [69]	39.3 (47.5) [42]	95.4 (12.5)	47.6 (39.5)	97.2 (9.3) [108]	38.9 (41.8) [74]
AP dosage						
Initial	207 (206) [71]	179 (141) [46]	221 (186)	161 (139)	178 (135)	165 (154)
Month 2	243 (220) [71]	180 (179) [46]	282 (281)	227 (272)	210 (160)	175 (180) [81]
Month 3	221 (222) [71]	187 (184) [45]	284 (283)	197 (252)	206 (168)	152 (178)
Month 6	216 (241) [71]	147 (173) [44]	298 (305)	161 (207) [30]	191 (171) [109]	146 (210)
Month 9	229 (248) [70]	110 (101) [44]	275 (239) [42]	220 (257)	190 (173) [108]	115 (134) [78]
Month 12	245 (255) [69]	129 (152) [39]	305 (271)	317 (410) [30]	175 (157) [103]	105 (130) [71]

Abbreviations: *M* = mean; *SD* = standard deviation; n = number of participants for whom data were available; SANS = Scale for the Assessment of Negative Symptoms; SAPS = Scale for the Assessment of Positive Symptoms; CDSS = Calgary Depression Scale for Schizophrenia; AP dosage, antipsychotic dosage in chlorpromazine equivalents (mg/day).

eTable 3. Raw Data for SUMD Total, Q1, Q2a, and Q2b.

	PNS		2nd-NS		non-PNS	
All data: *M* (*SD*) [n]	Fully Adherent	Partially Adherent	Fully Adherent	Partially Adherent	Fully Adherent	Partially Adherent
SUMD – Total						
Initial	8.8 (3.6) [51]	8.7 (3.8) [35]	8.9 (3.7) [26]	9.0 (3.5) [24]	7.5 (3.6) [74]	8.0 (3.5) [50]
Month 2	7.4 (3.7) [46]	7.9 (3.7) [28]	7.9 (3.8) [33]	9.1 (3.5) [22]	6.2 (3.2) [78]	6.3 (3.3) [39]
Month 3	8.1 (4.0) [55]	8.2 (3.6) [25]	7.0 (4.0) [29]	8.3 (3.1) [19]	5.3 (2.9) [88]	6.2 (3.1) [35]
Month 6	7.7 (3.9) [59]	8.3 (3.6) [24]	7.0 (3.7) [33]	7.9 (4.1) [15]	6.1 (3.5) [81]	6.7 (3.3) [33]
Month 9	7.9 (4.3) [59]	6.7 (3.2) [21]	7.3 (3.9) [30]	9.3 (3.9) [15]	5.9 (3.3) [78]	6.7 (3.1) [35]
Month 12	7.5 (3.9) [51]	7.4 (3.4) [21]	8.3 (3.9) [33]	9.5 (3.6) [23]	5.1 (2.6) [80]	6.6 (2.9) [33]
SUMD - Q1						
Initial	3.5 (1.2) [56]	3.4 (1.3) [38]	3.2 (1.4) [30]	3.4 (1.4) [26]	2.9 (1.3) [84]	3.1 (1.3) [56]
Month 2	2.9 (1.5) [48]	3.0 (1.3) [31]	2.8 (1.4) [33]	3.0 (1.4) [26]	2.3 (1.3) [79]	2.6 (1.4) [45]
Month 3	3.0 (1.5) [56]	3.0 (1.5) [29]	2.5 (1.3) [30]	3.0 (1.2) [25]	1.9 (1.3) [89]	2.4 (1.4) [46]
Month 6	2.9 (1.4) [59]	2.9 (1.3) [26]	2.5 (1.4) [34]	2.9 (1.4) [21]	2.3 (1.4) [81]	2.4 (1.4) [43]
Month 9	2.8 (1.6) [59]	2.7 (1.5) [27]	2.4 (1.4) [30]	3.4 (1.5) [19]	2.1 (1.3) [78]	2.5 (1.4) [50]
Month 12	2.6 (1.4) [51]	3.0 (1.2) [27]	2.8 (1.4) [33]	3.3 (1.4) [27]	1.8 (1.0) [80]	2.4 (1.3) [39]
SUMD - Q2a						
Initial	2.7 (1.4) [51]	2.7 (1.5) [35]	3.1 (1.5) [27]	3.0 (1.5) [24]	2.4 (1.4) [75]	2.5 (1.3) [50]
Month 2	2.4 (1.5) [47]	2.5 (1.4) [30]	2.5 (1.4) [33]	3.1 (1.4) [22]	1.9 (1.3) [79]	1.8 (1.1) [40]
Month 3	2.5 (1.5) [57]	2.4 (1.4) [27]	2.3 (1.5) [31]	3.0 (1.4) [20]	1.6 (1.0) [89]	2.1 (1.3) [37]
Month 6	2.3 (1.4) [60]	2.8 (1.4) [25]	2.3 (1.5) [33]	2.8 (1.7) [16]	1.9 (1.2) [84]	2.4 (1.4) [36]
Month 9	2.5 (1.5) [59]	2.4 (1.4) [22]	2.4 (1.6) [30]	2.9 (1.4) [15]	1.8 (1.2) [81]	2.2 (1.3) [38]
Month 12	2.4 (1.5) [52]	2.3 (1.3) [21]	2.8 (1.5) [34]	3.1 (1.6) [23]	1.5 (0.9) [81]	2.2 (1.2) [35]
SUMD - Q2b						
Initial	2.8 (1.4) [55]	2.8 (1.4) [37]	2.7 (1.3) [29]	2.7 (1.4) [26]	2.4 (1.4) [82]	2.7 (1.4) [56]
Month 2	2.4 (1.4) [49]	2.6 (1.6) [33]	2.6 (1.4) [33]	3.0 (1.5) [26]	2.0 (1.2) [80]	2.4 (1.4) [46]
Month 3	2.7 (1.4) [58]	3.1 (1.6) [30]	2.4 (1.5) [30]	3.0 (1.5) [26]	1.8 (1.1) [90]	2.7 (1.4) [45]
Month 6	2.6 (1.5) [60]	2.7 (1.4) [26]	2.1 (1.3) [34]	3.0 (1.7) [21]	2.0 (1.3) [84]	2.6 (1.5) [60]
Month 9	2.5 (1.5) [26]	2.5 (1.5) [26]	2.5 (1.5) [30]	3.5 (1.6) [19]	1.9 (1.2) [81]	2.9 (1.4) [48]
Month 12	2.4 (1.5) [52]	2.7 (1.4) [27]	2.6 (1.5) [34]	3.4 (1.5) [27]	1.8 (1.0) [81]	2.7 (1.3) [41]

Abbreviations: *M* = mean; *SD* = standard deviation; n = number of participants for whom data were available; SUMD, Scale to Assess Unawareness of Mental Disorder

Chapter 4

Conclusions & Future Directions

4.1 Primary negative symptoms, achieving remission, and recovery

From being first wrote about in 1835 by Gogol (Gogol, 1835) to being systematically described in the early 1900s by Kraepelin and Blueler (Bleuler, 1952; Kraepelin, 1919), negative symptoms have always been considered a central feature of schizophrenia and to outcome. However, the discovery of antipsychotics in the 1950's provided an efficacious treatment for positive symptoms which propelled them into the spotlight and positive symptom reduction became the primary measure of outcome (Foussias et al., 2014; Foussias & Remington, 2010). Over the past 40 years, negative symptoms have reclaimed part of the spotlight, culminating with the 2005 consensus definition of remission in schizophrenia that equated the importance of both positive and negative symptoms in determining outcome (Andreasen et al., 2005).

Since then, a plethora of studies have employed the proposed consensus remission definition and found rates varied from 17% to 78%; the majority of the variance was linked to studies not employing the six-month time component (AlAqeel & Margolese, 2012). For example, Emsley and colleagues showed that 70% of patients met the cut-off criteria for symptom ratings, but only 24% met both the cut-off and time criteria (Emsley, Rabinowitz, Medori, & Early Psychosis Global Working Group, 2007). Similarly, Addington and Addington found 77% achieved remission at some point during follow-up but only 37% achieved remission when including the time criteria (J. Addington & Addington, 2008). Interestingly, reducing the time criterion from six months to three months did not alter the predictive power of achieving remission on functional outcome (Cassidy, Norman, Manchanda, Schmitz, & Malla, 2010). However, as argued by the consensus group and other research groups, the six-month time criterion is a necessary part of the definition to take into account the longitudinal nature of schizophrenia in terms of both illness progression and the lengthy treatment process involved (AlAqeel & Margolese, 2012; Andreasen et al., 2005; van Os et al., 2006).

Regardless if the time criterion is included or not, negative symptom severity at the start of treatment and throughout the treatment process has been found to be a strong predictor of not achieving full remission (AlAqeel & Margolese, 2012). This was strongly supported by our current findings that showed 19% achieved remission of both positive and negative symptoms whereas nearly 52% achieved sustained positive symptom remission. This finding was supported by a previous study that published results from our sister treatment centre (PEPP-London, Ontario)

where removing negative symptoms from the definition similarly increased remission rates by 30% (Cassidy, Norman, et al., 2010).

Unique to our current research was the fact that we were able to quantify the prevalence of those who did not achieve remission due to negative symptoms alone, and more importantly, to quantify primary negative symptoms vs. secondary negative symptoms. To begin, we found that 54% of clients did not achieve remission due to negative symptoms alone. Moreover, primary negative symptoms were prevalent in 40% of these clients; the other 14% included those who presented with moderate or worse negative symptom severity not maintained for six months. We also found that secondary negative symptoms represented 28% of those not achieving remission (i.e., moderate or worse negative symptom severity sustained for six months in the presence of clinically relevant positive, depressive, or extrapyramidal symptoms). From our findings, there appears to be an overwhelming number of clients not achieving remission due to negative symptoms, with primary negative symptoms at the core.

Considering that remission has been described as "a necessary but not sufficient step toward recovery" (Andreasen et al., 2005), it would be no surprise that negative symptoms largely account for many not achieving recovery. Of those with schizophrenia, it has been generally accepted that there are three possible outcomes: some will fully recover, some will partially recover, and some will never recover (Torrey, 2013). Interestingly, one review found recovery rates - as measured with the Bleuler symptom scale - varied from 28% to as high as 77% over a 12 to 26 year follow-up period (Warner, 2009). However, from more short term studies, a meta-analysis examining 8994 unique individuals found recovery rates varied from 8.1% to 20.0%; recovery was defined as improvements in both clinical and social domains with improvements in at least 1 domain persisting for at least 2 years (Jaaskelainen et al., 2013). Finally, the Schizophrenia Outpatients Health Outcomes (SOHO) study (n=6642), that stringently defined recovery as long-lasting symptomatic and functional remission and adequate quality of life for a minimum of 24 months, found 33% achieved symptomatic remission but only 4% were considered to be recovered (Novick et al., 2009). Importantly, the SOHO study along with several other large scale, longitudinal outcome studies identified negative symptoms, social functioning, medication adherence, and type of antipsychotic as predictors of recovery (Albert et al., 2011; Austin et al., 2013; Novick et al., 2009; Shrivastava et al., 2010). In fact, negative symptom severity has been consistently found as

the most robust marker of a poorer outcome in general (Leucht, 2014; Torrey, 2013), with the primary negative symptoms displaying an even stronger association (Fervaha et al., 2014; Foussias et al., 2014).

Thus, negative symptoms represent a core feature that remains largely untreatable. And this has been the case since Ewald Hecker stated in 1871, in relation to recovery from hebephrenia, "For the time being I have to regard it – not absolutely, however – as very unfavourable". This outlook has largely remained the same over the past 150 years as negative symptoms are still regarded as an unmet therapeutic need in schizophrenia (Foussias et al., 2015; Kirkpatrick et al., 2006).

4.2 Clinical Insight and Medication Adherence

Up until now, the majority of research findings underscored people with schizophrenia with primary negative symptoms display poorer medication adherence (Haddad, Brain, & Scott, 2014) and have poorer clinical insight (Chang et al., 2011; Dantas, Barros, Fernandes, Li, & Banzato, 2011; Kirkpatrick et al., 2000; Kosger et al., 2014; Pegoraro, Dantas, Banzato, & Fuentes, 2013; Trotman et al., 2011).

The first of that statement was refuted as our findings showed medication adherence was not any worse in those with primary negative symptoms. For all clients at the clinic, they were separable into fully-adherent or partially-adherent regardless of the presenting symptomatic profile. The second part was supported and extended in that clients with primary negative symptoms did display poor insight but not any poorer than those with secondary negative symptoms.

Finally, and most importantly, we found that insight had no effect on adherence in those with primary negative symptoms. Although these clients showed a somewhat neutral level of insight into the 'belief in need for treatment', the level of insight showed little change over the 12-month follow-up period. This suggested these clients may indeed be agreeable to treatment and those who displayed poorer adherence may have done so, simply because the antipsychotics provided had no noticeable effect in treating their negative symptoms.

In sum, our findings support the notion of an unmet therapeutic need but further suggest there is an absolute need to search for and develop newer, more effective treatments for primary negative

symptoms. When these treatments indeed become available, implementation may not be as prone to the extra barrier of non-compliance that has been, up to this point, so strongly associated with those who present with primary negative symptoms.

4.3 Currently available treatments for primary negative symptoms

As repeatedly stated, there are currently no efficacious, first-line pharmacotherapies available for treating primary negative symptoms (Arango et al., 2013; Davis et al., 2014; Moller & Czobor, 2015). Moreover, with the exception of amisulpride, in some European countries, there are no pharmacological agents approved for the treatment of negative symptoms (Marder et al., 2013). Nevertheless, there are multiple ongoing clinical trials exploring for newer pharmacotherapies (Arango et al., 2013; Davis et al., 2014), as well as, research exploring psychosocial interventions (Eack, Mesholam-Gately, Greenwald, Hogarty, & Keshavan, 2013; Staring, Ter Huurne, & van der Gaag, 2013) and non-invasive stimulation of the brain (Quan et al., 2015; Shi, Yu, Cheung, Shum, & Chan, 2014). The major problem faced with many of the results presented below is that most trials involve people presenting with a wide array of negative symptoms, not just primary negative symptoms. And, in 2013, the NEWMEDS consortium (Novel Methods leading to NeW MEdications in Depression and Schizophrenia), highlighted that future clinical trials should mainly contain those with predominant negative symptoms and remove those presenting with depression (Marder et al., 2013). This would essentially reduce the heterogeneity of the sample and allow for definitive results to emerge.

4.3.1 Antipsychotics

Among the trials that have explicitly examined primary negative symptoms, no first-generation or second-generation antipsychotic has been found to be efficacious (Davis et al., 2014; Moller & Czobor, 2015). However, newer second-generation antipsychotics, like aripiprazole, described as a dopamine/serotonin system stabilizer due to its D2, $5HT_{1A}$, and $5HT_7$ agonistic and D1, $5HT_{2A}$, and $5HT_6$ antagonistic nature (Russo et al., 2013), may show better efficacy compared to other antipsychotics tested thus far (Davis et al., 2014). In fact, improved memory function was found in people with schizophrenia treated with aripiprazole (Bervoets et al., 2012; Kern et al., 2006; Riedel et al., 2010). With previous work from our lab revealing verbal memory deficits among

those with primary negative symptoms (Hovington, Bodnar, Joober, Malla, & Lepage, 2013), aripiprazole may offer some benefit to these people, if not in the form of symptom relief but perhaps by helping to improve memory. According to the ClinicalTrials.gov website, there are no current trials explicitly exploring the effect of aripiprazole on primary negative symptoms.

Another newer second-generation antipsychotic with potential is asenapine. This antipsychotic was measured against olanzapine in two clinical trials employing patients with persistent negative symptoms. The pooled analysis of these trials revealed asenapine showed a therapeutic effect on negative symptoms as measured by reductions in total negative symptom scores (Potkin et al., 2013). Although somewhat promising, the overall results for the antipsychotics is bleak and a main reason why research has turned towards adjunctive treatments.

4.3.2 Add-on pharmacotherapies

There have been a plethora of add-on agents explored. These have included: antidepressants, psychostimulants (modafinil), NMDA glutamatergics (e.g., glycine, sarcosine, D-cycloserine, D-serine, N-acetylcysteine, and memantine), cholinergics (e.g., donepezil, galantamine, rivastigmine), hormones (e.g., estradiol, pregnenolone, and testosterone), and oxytocin.

Just to cover a few results, some of the first add-ons tested were the antidepressants. Meta-analyses of these results reported significant benefit with medium overall effect sizes (Rummel, Kissling, & Leucht, 2005; Singh, Singh, Kar, & Chan, 2010). There were, however, no differences in effects when comparing those with prominent negative symptoms to those without. There were also modest effects found particularly for D-serine, sarcosine, and N-acetylcysteine (Singh & Singh, 2011; Tuominen, Tihonen, & Wahlbeck, 2006). However, as highlighted by Davis and colleagues, these studies were smaller in number, generally short-term, and several agents required doses above recommended levels to show any level of efficacy (Davis et al., 2014). Although these results are promising, with moderate effect sizes, none of these agents were tested in those presenting with primary negative symptoms.

4.3.3 Non-invasive brain stimulation

Repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS) are non-invasive brain stimulation techniques that have also been explored. The basic concept of both techniques is to noninvasively alter cortical excitability; rTMS accomplishes this via a pulsed magnetic field provided by a 'figure-8 coil' placed above the scalp, whereas tDCS does so by delivering an electrical current through surface-placed electrodes. Repetitive transcranial magnetic stimulation has been used to treat various psychiatric disorders, such as obsessive-compulsive disorder, post-traumatic stress disorder, bipolar disorders, schizophrenia, and depression (Rossi, Hallett, Rossini, Pascual-Leone, & Safety of, 2009). In fact, the Food and Drug Administration (FDA) in the United States has approved the use of rTMS to treat refractory depression.

With rTMS as a viable option for those with severe depression, this was widely explored in the treatment of negative symptoms in people with schizophrenia. Positive results have been reported with a meta-analysis revealing a moderate effect size in sham-controlled trials (Shi et al., 2014). More impressively, a recently published study provided rTMS to 117 patients with prominent negative symptoms for a total of 20 minutes per day (Quan et al., 2015). After receiving treatment for six weeks, negative symptom severity was significantly reduced with an effect that still measured 24 weeks after the last session. Although very promising, not all people are able to tolerate this type of treatment as side-effects, such as headaches, for example, have been frequently reported (Rossi et al., 2009).

As an alternative to rTMS, tDCS was developed with the basic premise to make the treatment more affordable and more portable; the entire apparatus can literally be carried around in a backpack. There have been very few studies exploring the effects of tDCS in schizophrenia. One study, for example, found a significant decrease in negative symptom totals after 5 days of treatment with treatment lasting 40 minutes per day (Brunelin et al., 2012). However, the main goal of this study was to treat auditory hallucinations which were treated quite effectively. As a result, the reduction in negative symptoms may have been secondary to the reduction in severity of the auditory hallucinations. Moreover, the significant reduction in hallucinatory behaviour lasted one month and three months after the last session; however, the authors failed to report if the same effect was observed for the negative symptoms.

Both rTMS and tDCS show promise as alternatives to be explored in the treatment of negative symptoms. However, future studies should explicitly explore any effects in relation to primary negative symptoms.

4.3.4 CBT Therapy

Negative symptoms can broadly be defined as ‘‘missing’’ social skills; i.e., not smiling (flat affect), reduced content when talking (alogia), and lacking initiative (avolition) to engage in social activities (asociality). Thus, future pharmacotherapies could help alleviate many of the symptoms but just like depression and other mood disorders, a better, longer-term outcome is experienced when CBT is included in the treatment process (Hofmann, Asnaani, Vonk, Sawyer, & Fang, 2012).

A study recently published by the van der Gagg research group (Staring et al., 2013) explicitly examined the effects of CBT for negative symptoms (CBT-n). Although this study did not employ those with primary negative symptoms, the patients did display prominent levels (i.e. mild or higher on at least three negative items of the PANSS). Participants were treated with weekly sessions of 45 minutes to a maximum of 20 sessions. The therapy sessions, for example, focused on exploring for and restructuring negative expectations about performance, social skills, and ability to enjoy and experience positive emotions; it also addressed avoidance in terms of withdrawal, inactivity, emotion suppression, and so on. Although most patients benefited from therapy, the pace of progress varied considerably. Nevertheless, over an average of 18 sessions, there was a significant decrease in negative symptom totals and the number of dysfunctional beliefs. Although this was not a controlled trial and did not formally treat those with primary negative symptoms, the results were promising.

A large scale meta-analysis in 2008 found that regular CBT had a beneficial effect in treating negative symptoms; however, improvements were correlated with improvements in other domains such as positive symptoms (Wykes, Steel, Everitt, & Tarrier, 2008). A more recent meta-analysis of CBT trials specifically aimed at reducing negative symptom severity found only small effects (Velthorst et al., 2014). Thus, more trials and newer techniques like the abovementioned CBT-n are needed if we are to truly move forward and offer treatments specifically designed for those with primary negative symptoms.

4.4 The parahippocampal cortex – a distinct marker of primary negative symptoms

Reduced grey matter volume in the parahippocampal gyrus has been shown to be consistently found in people with schizophrenia compared to non-clinical healthy controls (Shenton, Dickey, Frumin, & McCarley, 2001; Shepherd, Laurens, Matheson, Carr, & Green, 2012; Williams, 2008). Interestingly, several studies have found a reduction in volume particular to those with prominent negative symptoms (Sigmundsson et al., 2001) and in those with primary negative symptoms (Benoit, Bodnar, Malla, Joober, & Lepage, 2012; Bodnar, Harvey, Malla, Joober, & Lepage, 2011; Bodnar et al., 2014); see Appendix A for the results from our lab using cortical thickness.

The findings from this thesis extended the above in several ways. First, a smaller (and decreasing) parahippocampal cortex (PHC) volume and smaller hippocampal tail volume were verified as neuroanatomical markers of not achieving remission (Bodnar et al., 2010; Bodnar, Malla, et al., 2012). Secondly, the PHC was identified as a specific marker of unremitting primary negative symptoms. That is, those with primary negative symptoms did not differ from other non-remitted clients in hippocampal tail volume but did significantly so in PHC volume.

The idea of those with primary negative symptoms representing a distinct subtype within schizophrenia was supported by the abovementioned result, in that, among the unremitted clients, those with primary negative symptoms displayed a clear neuroanatomical marker (i.e., smaller volume in the PHC). Of course, further investigations are warranted to confirm this finding but the PHC should be of particular interest in future studies exploring for and designing more target-specific interventions aimed at treating primary negative symptoms.

4.4.1 The role of the parahippocampus in primary negative symptoms

As stated above, negative symptoms can be broadly defined as ‘‘missing’’ social skills. As highlighted in our cortical thickness analysis, we found cortical thinning in the parahippocampal gyrus and the temporo-parietal junction (TPJ) in clients with PNS compared to those without (Appendix A; Bodnar et al., 2014). The discussion in this particular article was geared towards the TPJ as this structure represented the largest effect but more so because an article by Cynthia Wible pointed towards schizophrenia as a social communication disorder with the TPJ as a central structure of interest (Wible, 2012). Not to discount the parahippocampus in any way, but this

structure was discussed in depth in our previous articles that examined remission (Bodnar, Achim, et al., 2012; Bodnar et al., 2011; Bodnar, Malla, et al., 2012).

The premise of the previous discussions focused on how reduced parahippocampal gyrus volume was already present in people starting in the prodromal phase (Bangalore et al., 2009) and how social withdrawal, the one symptom strongly correlated with parahippocampal volume (Bodnar et al., 2011; Bodnar, Malla, et al., 2012), could be affected by and related to altered memory processes (Achim & Lepage, 2003; Ragland et al., 2009; Ramsey, Jansma, Jager, Van Raalten, & Kahn, 2004; Sanfilipo et al., 2002; Squire & Zola-Morgan, 1991). With the PHC identified as a neuroanatomical marker of primary negative symptoms, it was warranted to extend our previous discussions.

The PHC is considered part of a large network that connects regions of the temporal, parietal, and frontal cortices and is believed to be involved with both spatial and non-spatial memory with a central role in contextual associative processing (Aminoff, Kveraga, & Bar, 2013). As stated by Aminoff and colleagues (2013), "contexts are important for generating expectations about other objects, the spatial relations between objects, and associated behaviors to be found within the environment". The latter part of this statement particularly resonated with the idea of "missing" social skills. If the main function of the PHC is to process the binding of associations through repeated exposure to typical contexts, this could imply that a failure in this structure could result in an inability to learn proper social skills (smiling, adding context to speech, and being social) in a given environment. An interesting study to conduct would be to explore if abnormal activity in the PHC exists when people with primary negative symptoms are presented with various social environments; one could compare various appropriate and inappropriate behaviours presented in variable contexts to create and explore the different contrasts. Moreover, it would be more profound to see whether or not if abnormal activity in the PHC, if it were to exist, could be "restored to normal" with therapies like CBT-n aimed at improving social skills.

As a core structure identified in schizophrenia, further study of the PHC could provide an excellent opportunity to better our understanding of negative symptoms with research turning towards improving social skills verifying whether or not there is indeed a change in activity. This would help to show if the PHC is indeed a trait or state marker of primary negative symptoms.

4.4.2 Exercise and the parahippocampus

In 2010, Pajonk and colleagues released a seminal finding that demonstrated significant increases in hippocampal volume in male patients with schizophrenia (n=8) who took part in a three month exercise regime (30 minutes per day, 3 days per week) compared to those who did not (played table football or "foosball") (Pajonk et al., 2010). The authors also reported an increase in verbal memory performance, but no effect on positive or negative symptoms. In a follow-up analysis of the same sample, the authors reported no changes in the cortical regions of the brain (Falkai et al., 2013). Moreover, a follow-up study by this research group involving 20 people with enduring schizophrenia and 21 matched controls found no significant increase in hippocampal volume, or subfield hippocampal volume, in either group (Malchow et al., 2015). The lack of hippocampal growth in relation to exercise was supported by two other studies (Rosenbaum et al., 2015; Scheewe et al., 2013); the study by Scheewe and colleagues was particularly damning as their sample included 63 people with schizophrenia and 55 healthy controls who took part in in 2 hours of weekly exercise for six months. To top it off, a systematic review of the literature by Vancampfort and colleagues concluded the link between physical exercise and hippocampal growth was indeed ambiguous citing methodological differences as a potential confounder (Vancampfort et al., 2014)

One study of particular note, however, employed 29 participants considered as ultra-high-risk (UHR) for psychosis and 27 matched controls (Mittal et al., 2013). This study was unique as participants wore "The ActiGraph Monitor", a wrist-worn device that monitors daily activity, for a five day period which allowed the researchers to quantify "Total Physical Activity" based on how much time was spent being sedentary or in light, moderate, or vigorous activity. There were two key findings: 1) the UHR groups spent significantly more time in an inactive state and 2) after controlling for group, total physical activity did not correlate with hippocampal volume but a significant positive correlation was found with bilateral parahippocampal volume, indicating higher levels of activity were associated with larger volumes. Moreover, another study of 95 healthy participants found general activity level positively correlated with parahippocampal volume (Demirakca et al., 2014). Although no causal relationship can be inferred, there appears to be a strong relationship between activity level and parahippocampal volume.

Of course, the above finding requires confirmation but should be expanded to include samples involving those who are predominantly amotivated and living a more sedentary lifestyle, i.e., those with primary negative symptoms of avolition and asociality. This could provide support for our finding of reduced parahippocampal cortex volumes over a 1-year period in clients who presented with primary negative symptoms, and overwhelmingly so displayed avolition and asociality. A future study could follow those with primary negative symptoms using a device similar to "The ActiGraph Monitor" to not only gauge the amount of activity these people engage in on a daily basis but to also explore if the changes in parahippocampal cortex volume we observed are in line with a more sedentary lifestyle or a definitive neuroanatomical marker of primary negative symptoms. Of course, in the case of people with schizophrenia, one has to consider the effect of antipsychotics on brain grey matter volume as differential effects have been reported, as revealed by multiple meta-analyses (Olabi et al., 2011; Vita, De Peri, Deste, Barlati, & Sacchetti, in press) and a systematic review (Moncrieff & Leo, 2010). In addition, a recent analysis from our group revealed aripiprazole was identified as a positive factor in hippocampal growth over a 1-year period (see Appendix B). So, in studies exploring changes in grey matter in the brain, in any regard, the effect of antipsychotics should be taken into account.

4.5 Closing Statement

A statement by the NIMH consensus group on negative symptoms in 2006, which has continually been restated over the past decade, stressed that "persistent and clinically significant negative symptoms are an unmet therapeutic need in a large proportion of cases" (Kirkpatrick et al., 2006). Although this meeting marked the beginning of a better framework to follow in research and strong initiatives towards identifying better treatments, very few advances have been made since that time (Foussias et al., 2015). And this has remained a fact over the past 150 years since Hecker highlighted that no viable treatments were available to treat hebephrenia, a disorder marked by severe negative symptoms (Hecker & Kraam, 2009). Nevertheless, progress can only be made by a collective effort of all those involved. The findings offered in this thesis offer new insight into primary negative symptoms and will hopefully stir up some debate, but also guide future research towards developing viable treatments

In closing, I offer this quote from a recent case study of a person who received add-on tDCS treatment (Narayanaswamy et al., 2014):

> In the subsequent 2-weeks after tDCS, she showed remarkably rapid improvement in motivation level, speech output, affective response and interpersonal interactions; this was appreciated by her family members as well. Moreover, she reported that she could concentrate better and started going [to] classes regularly and was able to socialise well with her friends and peers. (p.4).

This provided me with profound happiness knowing that our collective efforts are making a difference, even if it is only in one person at a time.

Bibliography

Achim, A. M., & Lepage, M. (2003). Is associative recognition more impaired than item recognition memory in Schizophrenia? A meta-analysis. *Brain and Cognition, 53*(2), 121-124.

Addington, D., Addington, J., & Maticka-Tyndale, E. (1994). Specificity of the Calgary Depression Scale for schizophrenics. *Schizophrenia Research, 11*(3), 239-244.

Addington, D., Addington, J., & Schissel, B. (1990). A depression rating scale for schizophrenics. *Schizophrenia Research, 3*(4), 247-251.

Addington, J., & Addington, D. (2008). Symptom remission in first episode patients. *Schizophrenia Research, 106*(2-3), 281-285.

AlAqeel, B., & Margolese, H. C. (2012). Remission in schizophrenia: critical and systematic review. *Harv Rev Psychiatry, 20*(6), 281-297.

Albert, N., Bertelsen, M., Thorup, A., Petersen, L., Jeppesen, P., Le Quack, P., . . . Nordentoft, M. (2011). Predictors of recovery from psychosis Analyses of clinical and social factors associated with recovery among patients with first-episode psychosis after 5 years. *Schizophrenia Research, 125*(2-3), 257-266.

Amador, X. F., Strauss, D. H., Yale, S. A., Flaum, M. M., Endicott, J., & Gorman, J. M. (1993). Assessment of insight in psychosis. *American Journal of Psychiatry, 150*(6), 873-879.

Aminoff, E. M., Kveraga, K., & Bar, M. (2013). The role of the parahippocampal cortex in cognition. *Trends in Cognitive Sciences, 17*(8), 379-390.

Andreasen, N. C. (1982). Negative symptoms in schizophrenia. Definition and reliability. *Archives of General Psychiatry, 39*(7), 784-788.

Andreasen, N. C. (1984a). *Scale for the Assessment of Negative Symptoms (SANS)*. Iowa City: University of Iowa.

Andreasen, N. C. (1984b). *Scale for the Assessment of Positive Symptoms (SAPS)*. Iowa City: University of Iowa.

Andreasen, N. C., Carpenter, W. T., Jr., Kane, J. M., Lasser, R. A., Marder, S. R., & Weinberger, D. R. (2005). Remission in schizophrenia: proposed criteria and rationale for consensus. *American Journal of Psychiatry, 162*(3), 441-449.

Arango, C., Garibaldi, G., & Marder, S. R. (2013). Pharmacological approaches to treating negative symptoms: a review of clinical trials. *Schizophrenia Research, 150*(2-3), 346-352.

Austin, S. F., Mors, O., Secher, R. G., Hjorthoj, C. R., Albert, N., Bertelsen, M., . . . Nordentoft, M. (2013). Predictors of recovery in first episode psychosis: the OPUS cohort at 10 year follow-up. *Schizophrenia Research, 150*(1), 163-168.

Bangalore, S. S., Goradia, D. D., Nutche, J., Diwadkar, V. A., Prasad, K. M., & Keshavan, M. S. (2009). Untreated illness duration correlates with gray matter loss in first-episode psychoses. *Neuroreport, 20*(7), 729-734.

Bartels, S. J., Clark, R. E., Peacock, W. J., Dums, A. R., & Pratt, S. I. (2003). Medicare and medicaid costs for schizophrenia patients by age cohort compared with costs for depression, dementia, and medically ill patients. *American Journal of Geriatric Psychiatry, 11*(6), 648-657.

Benoit, A., Bodnar, M., Malla, A. K., Joober, R., & Lepage, M. (2012). The structural neural substrates of persistent negative symptoms in first-episode of non-affective psychosis: a voxel-based morphometry study. *Frontiers in Psychiatry, 3*, 42.

Berrios, G. E. (1985). Positive and negative symptoms and Jackson. A conceptual history. *Archives of General Psychiatry, 42*(1), 95-97.

Bervoets, C., Morrens, M., Vansteelandt, K., Kok, F., de Patoul, A., Halkin, V., . . . Sabbe, B. (2012). Effect of aripiprazole on verbal memory and fluency in schizophrenic patients : results from the ESCAPE study. *CNS Drugs, 26*(11), 975-982.

Bleuler, E. (1952). *Dementia praecox or the Group of the Schizophrenias*. Madison, CT: International Universities Press, Inc.

Bodnar, M., Achim, A. M., Malla, A. K., Joober, R., Benoit, A., & Lepage, M. (2012). Functional magnetic resonance imaging correlates of memory encoding in relation to achieving remission in first-episode schizophrenia. *British Journal of Psychiatry, 200*(4), 300-307.

Bodnar, M., Harvey, P.-O., Malla, A. K., Joober, R., & Lepage, M. (2011). The parahippocampal gyrus as a neural marker of early remission in first-episode psychosis: a voxel-based morphometry study. *Clinical Schizophrenia & Related Psychoses, 4*(4), 217-228.

Bodnar, M., Hovington, C. L., Buchy, L., Malla, A. K., Joober, R., & Lepage, M. (2014). Cortical thinning in temporo-parietal junction (TPJ) in non-affective first-episode of psychosis patients with persistent negative symptoms. *PLoS One, 9*(6), e101372.

Bodnar, M., Malla, A. K., Czechowska, Y., Benoit, A., Fathalli, F., Joober, R., . . . Lepage, M. (2010). Neural markers of remission in first-episode schizophrenia: a volumetric neuroimaging study of the hippocampus and amygdala. *Schizophrenia Research, 122*(1-3), 72-80.

Bodnar, M., Malla, A. K., Joober, R., Lord, C., Smith, E., Pruessner, J., & Lepage, M. (2012). Neural markers of early remission in first-episode schizophrenia: A volumetric neuroimaging study of the parahippocampus. *Psychiatry Research: Neuroimaging, 201*(1), 40-47.

Bollini, P., Pampallona, S., Nieddu, S., Bianco, M., Tibaldi, G., & Munizza, C. (2008). Indicators of conformance with guidelines of schizophrenia treatment in mental health services. *Psychiatr Serv, 59*(7), 782-791.

Brunelin, J., Mondino, M., Gassab, L., Haesebaert, F., Gaha, L., Suaud-Chagny, M. F., . . . Poulet, E. (2012). Examining transcranial direct-current stimulation (tDCS) as a treatment for hallucinations in schizophrenia. *American Journal of Psychiatry, 169*(7), 719-724.

Buchanan, R. W. (2007). Persistent negative symptoms in schizophrenia: an overview. *Schizophrenia Bulletin, 33*(4), 1013-1022.

Buchy, L., Bodnar, M., Malla, A., Joober, R., & Lepage, M. (2010). A 12-month outcome study of insight and symptom change in first-episode psychosis. *Early Intervention in Psychiatry, 4*(1), 79-88.

Carpenter, W. T., Jr., Heinrichs, D. W., & Wagman, A. M. (1988). Deficit and nondeficit forms of schizophrenia: the concept. *American Journal of Psychiatry, 145*(5), 578-583.

Cassidy, C. M., Norman, R., Manchanda, R., Schmitz, N., & Malla, A. (2010). Testing definitions of symptom remission in first-episode psychosis for prediction of functional outcome at 2 years. *Schizophrenia Bulletin, 36*(5), 1001-1008.

Cassidy, C. M., Rabinovitch, M., Schmitz, N., Joober, R., & Malla, A. (2010). A comparison study of multiple measures of adherence to antipsychotic medication in first-episode psychosis. *J Clin Psychopharmacol, 30*(1), 64-67.

Chang, W. C., Hui, C. L., Tang, J. Y., Wong, G. H., Lam, M. M., Chan, S. K., & Chen, E. Y. (2011). Persistent negative symptoms in first-episode schizophrenia: a prospective three-year follow-up study. *Schizophrenia Research, 133*(1-3), 22-28.

Cohen, C. I., Meesters, P. D., & Zhao, J. (2015). New perspectives on schizophrenia in later life: implications for treatment, policy, and research. *Lancet Psychiatry, 2*(4), 340-350.

Collie, A., Darby, D., & Maruff, P. (2001). Computerised cognitive assessment of athletes with sports related head injury. *Br J Sports Med, 35*(5), 297-302.

Dantas, C. R., Barros, B. R., Fernandes, P. T., Li, L. M., & Banzato, C. E. (2011). Insight controlled for cognition in deficit and nondeficit schizophrenia. *Schizophrenia Research, 128*(1-3), 124-126.

Davis, M. C., Horan, W. P., & Marder, S. R. (2014). Psychopharmacology of the negative symptoms: current status and prospects for progress. *European Neuropsychopharmacology, 24*(5), 788-799.

Demirakca, T., Brusniak, W., Tunc-Skarka, N., Wolf, I., Meier, S., Matthaus, F., . . . Diener, C. (2014). Does body shaping influence brain shape? Habitual physical activity is linked to

brain morphology independent of age. *World Journal of Biological Psychiatry, 15*(5), 387-396.

Eack, S. M., Mesholam-Gately, R. I., Greenwald, D. P., Hogarty, S. S., & Keshavan, M. S. (2013). Negative symptom improvement during cognitive rehabilitation: results from a 2-year trial of Cognitive Enhancement Therapy. *Psychiatry Res, 209*(1), 21-26.

Emsley, R., Rabinowitz, J., Medori, R., & Early Psychosis Global Working, G. (2007). Remission in early psychosis: Rates, predictors, and clinical and functional outcome correlates. *Schizophrenia Research, 89*(1-3), 129-139.

Falkai, P., Malchow, B., Wobrock, T., Gruber, O., Schmitt, A., Honer, W. G., . . . Cannon, T. D. (2013). The effect of aerobic exercise on cortical architecture in patients with chronic schizophrenia: a randomized controlled MRI study. *European Archives of Psychiatry and Clinical Neuroscience, 263*(6), 469-473.

Fervaha, G., Foussias, G., Agid, O., & Remington, G. (2014). Impact of primary negative symptoms on functional outcomes in schizophrenia. *European Psychiatry, 29*(7), 449-455.

First, M. B., Spitzer, R. L., Gibbon, M., & Williams, J. B. W. (1998). *Structured Clinical Interview for DSM-IV Axis I Disorders, Patient Edition (SCID-I/P & SCID-I/NP), Version 2*. New York: New York Psychiatric Institute, Biometrics Research.

Foussias, G., Agid, O., Fervaha, G., & Remington, G. (2014). Negative symptoms of schizophrenia: clinical features, relevance to real world functioning and specificity versus other CNS disorders. *European Neuropsychopharmacology, 24*(5), 693-709.

Foussias, G., & Remington, G. (2010). Negative symptoms in schizophrenia: avolition and Occam's razor. *Schizophrenia Bulletin, 36*(2), 359-369.

Foussias, G., Siddiqui, I., Fervaha, G., Agid, O., & Remington, G. (2015). Dissecting negative symptoms in schizophrenia: opportunities for translation into new treatments. *Journal of Psychopharmacology, 29*(2), 116-126.

Galderisi, S., Bucci, P., Mucci, A., Kirkpatrick, B., Pini, S., Rossi, A., . . . Maj, M. (2013). Categorical and dimensional approaches to negative symptoms of schizophrenia: focus on long-term stability and functional outcome. *Schizophrenia Research, 147*(1), 157-162.

Galderisi, S., Maj, M., Mucci, A., Cassano, G. B., Invernizzi, G., Rossi, A., . . . Pini, S. (2002). Historical, psychopathological, neurological, and neuropsychological aspects of deficit schizophrenia: a multicenter study. *American Journal of Psychiatry, 159*(6), 983-990.

Galderisi, S., Merlotti, E., & Mucci, A. (2015). Neurobiological background of negative symptoms. *European Archives of Psychiatry and Clinical Neuroscience*.

Goetz, R. R., Corcoran, C., Yale, S., Stanford, A. D., Kimhy, D., Amador, X., & Malaspina, D. (2007). Validity of a 'proxy' for the deficit syndrome derived from the Positive And Negative Syndrome Scale (PANSS). *Schizophrenia Research, 93*(1-3), 169-177.

Gogol, N. (1835). *Diary of a Madman* (C. Field, Trans.): Project Gutenberg.

Haddad, P. M., Brain, C., & Scott, J. (2014). Nonadherence with antipsychotic medication in schizophrenia: challenges and management strategies. *Patient Relat Outcome Meas, 5*, 43-62.

Hafner, H. (1998). Onset and course of the first schizophrenic episode. *The Kaohsiung Journal of Medical Sciences, 14*(7), 413-431.

Hecker, E., & Kraam, A. (2009). 'Hebephrenia. A contribution to clinical psychiatry' by Dr. Ewald Hecker in Gorlitz. 1871. *History of Psychiatry, 20*(77 Pt 1), 87-106.

Heering, H. D., Janssens, M., Boyette, L. L., van Haren, N. E., & investigators, G. R. O. U. P. (2015). Remission criteria and functional outcome in patients with schizophrenia, a longitudinal study. *Australian & New Zealand Journal of Psychiatry, 49*(3), 266-274.

Hofmann, S. G., Asnaani, A., Vonk, I. J., Sawyer, A. T., & Fang, A. (2012). The Efficacy of Cognitive Behavioral Therapy: A Review of Meta-analyses. *Cognitive Therapy and Research, 36*(5), 427-440.

Hollingshead, A. (1965). *Two-Factor Index of Social Position*. New Haven, CN: Yale University Press.

Hovington, C. L., Bodnar, M., Joober, R., Malla, A. K., & Lepage, M. (2012). Identifying persistent negative symptoms in first episode psychosis. *BMC Psychiatry, 12*(224), 1-11.

Hovington, C. L., Bodnar, M., Joober, R., Malla, A. K., & Lepage, M. (2013). Impairment in verbal memory observed in first episode psychosis patients with persistent negative symptoms. *Schizophrenia Research, 147*(2-3), 223-229.

Hovington, C. L., & Lepage, M. (2012). Neurocognition and neuroimaging of persistent negative symptoms of schizophrenia. *Expert Rev Neurother, 12*(1), 53-69.

Iyer, S., Jordan, G., MacDonald, K., Joober, R., & Malla, A. (2015). Early intervention for psychosis: a canadian perspective. *The Journal of Nervous and Mental Disease, 203*(5), 356-364.

Jaaskelainen, E., Juola, P., Hirvonen, N., McGrath, J. J., Saha, S., Isohanni, M., . . . Miettunen, J. (2013). A systematic review and meta-analysis of recovery in schizophrenia. *Schizophrenia Bulletin, 39*(6), 1296-1306.

Jensen, B., Regier, L.D. (Ed.). (2012). *Drug Comparison Charts* (9th Edition ed.). Saskatoon, Saskatchewan: RxFiles Academic Detailing Program.

Joober, R., Rouleau, G. A., Lal, S., Dixon, M., O'Driscoll, G., Palmour, R., . . . Benkelfat, C. (2002). Neuropsychological impairments in neuroleptic-responder vs. -nonresponder schizophrenic patients and healthy volunteers. *Schizophrenia Research, 53*(3), 229-238.

Kahlbaum, K., Berrios, G. E., & Kraam, A. (2002). On Heboidphrenia. *History of Psychiatry, 13*(50), 197-208.

Kay, S., Fiszbein, A., Opler, L. . (1987). The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophrenia Bulletin, 13*(2), 261-276.

Kern, R. S., Green, M. F., Cornblatt, B. A., Owen, J. R., McQuade, R. D., Carson, W. H., . . . Marcus, R. (2006). The neurocognitive effects of aripiprazole: an open-label comparison with olanzapine. *Psychopharmacology, 187*(3), 312-320.

Kirkpatrick, B. (2014). Progress in the study of negative symptoms. *Schizophrenia Bulletin, 40 Suppl 2*, S101-106.

Kirkpatrick, B., Buchanan, R. W., Breier, A., & Carpenter, W. T., Jr. (1993). Case identification and stability of the deficit syndrome of schizophrenia. *Psychiatry Res, 47*(1), 47-56.

Kirkpatrick, B., Buchanan, R. W., McKenney, P. D., Alphs, L. D., & Carpenter, W. T., Jr. (1989). The Schedule for the Deficit syndrome: an instrument for research in schizophrenia. *Psychiatry Res, 30*(2), 119-123.

Kirkpatrick, B., Buchanan, R. W., Ross, D. E., & Carpenter, W. T., Jr. (2001). A separate disease within the syndrome of schizophrenia. *Archives of General Psychiatry, 58*(2), 165-171.

Kirkpatrick, B., Castle, D., Murray, R. M., & Carpenter, W. T., Jr. (2000). Risk factors for the deficit syndrome of schizophrenia. *Schizophrenia Bulletin, 26*(1), 233-242.

Kirkpatrick, B., Fenton, W. S., Carpenter, W. T., Jr., & Marder, S. R. (2006). The NIMH-MATRICS consensus statement on negative symptoms. *Schizophrenia Bulletin, 32*(2), 214-219.

Kirkpatrick, B., Strauss, G. P., Nguyen, L., Fischer, B. A., Daniel, D. G., Cienfuegos, A., & Marder, S. R. (2011). The brief negative symptom scale: psychometric properties. *Schizophrenia Bulletin, 37*(2), 300-305.

Kosger, F., Sahin, H., Essizoglu, A., & Aksaray, G. (2014). [Insight and social functioning in deficit and nondeficit schizophrenia]. *Türk Psikiyatri Dergisi, 25*(3), 157-162.

Kraepelin, E. (1919). *Dementia praecox and paraphrenia.* (R. M. Barclay, Trans.). Edinburgh: Livingstone.

Kring, A. M., Gur, R. E., Blanchard, J. J., Horan, W. P., & Reise, S. P. (2013). The Clinical Assessment Interview for Negative Symptoms (CAINS): final development and validation. *American Journal of Psychiatry, 170*(2), 165-172.

Lambert, M., Karow, A., Leucht, S., Schimmelmann, B. G., & Naber, D. (2010). Remission in schizophrenia: validity, frequency, predictors, and patients' perspective 5 years later. *Dialogues Clin Neurosci, 12*(3), 393-407.

Lepage, M., Bodnar, M., Buchy, L., Joober, R., & Malla, A. K. (2010). Early medication adherence and insight change in first episode psychosis. *Clinical Schizophrenia & Related Psychoses, 3*(4), 201-208.

Leucht, S. (2014). Measurements of response, remission, and recovery in schizophrenia and examples for their clinical application. *Journal of Clinical Psychiatry, 75 Suppl 1*, 8-14.

Malchow, B., Keeser, D., Keller, K., Hasan, A., Rauchmann, B. S., Kimura, H., . . . Falkai, P. (2015). Effects of endurance training on brain structures in chronic schizophrenia patients and healthy controls. *Schizophrenia Research*.

Malla, A., Norman, R., Schmitz, N., Manchanda, R., Bechard-Evans, L., Takhar, J., & Haricharan, R. (2006). Predictors of rate and time to remission in first-episode psychosis: a two-year outcome study. *Psychological Medicine, 36*(5), 649-658.

Malla, A. K., Norman, R. M., Manchanda, R., Ahmed, M. R., Scholten, D., Harricharan, R., . . . Takhar, J. (2002). One year outcome in first episode psychosis: influence of DUP and other predictors. *Schizophrenia Research, 54*(3), 231-242.

Marder, S. R., Alphs, L., Anghelescu, I. G., Arango, C., Barnes, T. R., Caers, I., . . . Rabinowitz, J. (2013). Issues and perspectives in designing clinical trials for negative symptoms in schizophrenia. *Schizophrenia Research, 150*(2-3), 328-333.

Maruff, P., Thomas, E., Cysique, L., Brew, B., Collie, A., Snyder, P., & Pietrzak, R. H. (2009). Validity of the CogState brief battery: relationship to standardized tests and sensitivity to cognitive impairment in mild traumatic brain injury, schizophrenia, and AIDS dementia complex. *Archives of Clinical Neuropsychology 24*(2), 165-178.

Messinger, J. W., Tremeau, F., Antonius, D., Mendelsohn, E., Prudent, V., Stanford, A. D., & Malaspina, D. (2011). Avolition and expressive deficits capture negative symptom phenomenology: implications for DSM-5 and schizophrenia research. *Clinical Psychology Review, 31*(1), 161-168.

Mittal, V. A., Gupta, T., Orr, J. M., Pelletier-Baldelli, A., Dean, D. J., Lunsford-Avery, J. R., . . . Millman, Z. B. (2013). Physical activity level and medial temporal health in youth at ultra high-risk for psychosis. *Journal of Abnormal Psychology, 122*(4), 1101-1110.

Moller, H. J., & Czobor, P. (2015). Pharmacological treatment of negative symptoms in schizophrenia. *European Archives of Psychiatry and Clinical Neuroscience*.

Moncrieff, J., & Leo, J. (2010). A systematic review of the effects of antipsychotic drugs on brain volume. *Psychological Medicine, 40*(9), 1409-1422.

Muller, M. J., Muller, K. M., & Fellgiebel, A. (2006). Detection of depression in acute schizophrenia: sensitivity and specificity of 2 standard observer rating scales. *Canadian Journal of Psychiatry, 51*(6), 387-392.

Narayanaswamy, J. C., Shivakumar, V., Bose, A., Agarwal, S. M., Venkatasubramanian, G., & Gangadhar, B. N. (2014). Sustained improvement of negative symptoms in schizophrenia with add-on tDCS: a case report. *Clinical Schizophrenia & Related Psychoses, 8*(3), 135-136.

Novick, D., Haro, J. M., Suarez, D., Vieta, E., & Naber, D. (2009). Recovery in the outpatient setting: 36-month results from the Schizophrenia Outpatients Health Outcomes (SOHO) study. *Schizophrenia Research, 108*(1-3), 223-230.

Olabi, B., Ellison-Wright, I., McIntosh, A. M., Wood, S. J., Bullmore, E., & Lawrie, S. M. (2011). Are there progressive brain changes in schizophrenia? A meta-analysis of structural magnetic resonance imaging studies. *Biol Psychiatry, 70*(1), 88-96.

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia, 9*(1), 97-113.

Overall, J. E., & Gorham, D. R. (1962). The brief psychiatric rating scale. *Psychol Rep, 10*, 790-812.

Pajonk, F. G., Wobrock, T., Gruber, O., Scherk, H., Berner, D., Kaizl, I., . . . Falkai, P. (2010). Hippocampal plasticity in response to exercise in schizophrenia. *Archives of General Psychiatry, 67*(2), 133-143.

Pegoraro, L. F., Dantas, C. R., Banzato, C. E., & Fuentes, D. (2013). Correlation between insight dimensions and cognitive functions in patients with deficit and nondeficit schizophrenia. *Schizophrenia Research, 147*(1), 91-94.

Potkin, S. G., Phiri, P., Szegedi, A., Zhao, J., Alphs, L., & Cazorla, P. (2013). Long-term effects of asenapine or olanzapine in patients with persistent negative symptoms of schizophrenia: a pooled analysis. *Schizophrenia Research, 150*(2-3), 442-449.

Quan, W. X., Zhu, X. L., Qiao, H., Zhang, W. F., Tan, S. P., Zhou, D. F., & Wang, X. Q. (2015). The effects of high-frequency repetitive transcranial magnetic stimulation (rTMS) on negative symptoms of schizophrenia and the follow-up study. *Neuroscience Letters, 584*, 197-201.

Ragland, J. D., Laird, A. R., Ranganath, C., Blumenfeld, R. S., Gonzales, S. M., & Glahn, D. C. (2009). Prefrontal activation deficits during episodic memory in schizophrenia. *American Journal of Psychiatry, 166*(8), 863-874.

Ramsey, N. F., Jansma, J. M., Jager, G., Van Raalten, T., & Kahn, R. S. (2004). Neurophysiological factors in human information processing capacity. *Brain, 127*(Pt 3), 517-525.

Riedel, M., Spellmann, I., Schennach-Wolff, R., Musil, R., Dehning, S., Cerovecki, A., . . . Moller, H. J. (2010). Effect of aripiprazole on cognition in the treatment of patients with schizophrenia. *Pharmacopsychiatry, 43*(2), 50-57.

Rosenbaum, S., Lagopoulos, J., Curtis, J., Taylor, L., Watkins, A., Barry, B. K., & Ward, P. B. (2015). Aerobic exercise intervention in young people with schizophrenia spectrum disorders; improved fitness with no change in hippocampal volume. *Psychiatry Research: Neuroimaging, 232*(2), 200-201.

Rossi, S., Hallett, M., Rossini, P. M., Pascual-Leone, A., & Safety of, T. M. S. C. G. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clinical Neurophysiology, 120*(12), 2008-2039.

Rummel, C., Kissling, W., & Leucht, S. (2005). Antidepressants as add-on treatment to antipsychotics for people with schizophrenia and pronounced negative symptoms: a systematic review of randomized trials. *Schizophrenia Research, 80*(1), 85-97.

Russo, E., Citraro, R., Davoli, A., Gallelli, L., Di Paola, E. D., & De Sarro, G. (2013). Ameliorating effects of aripiprazole on cognitive functions and depressive-like behavior in a genetic rat model of absence epilepsy and mild-depression comorbidity. *Neuropharmacology, 64*, 371-379.

Sanfilipo, M., Lafargue, T., Rusinek, H., Arena, L., Loneragan, C., Lautin, A., . . . Wolkin, A. (2002). Cognitive performance in schizophrenia: relationship to regional brain volumes and psychiatric symptoms. *Psychiatry Research: Neuroimaging, 116*(1-2), 1-23.

Scheewe, T. W., van Haren, N. E., Sarkisyan, G., Schnack, H. G., Brouwer, R. M., de Glint, M., . . . Cahn, W. (2013). Exercise therapy, cardiorespiratory fitness and their effect on brain volumes: a randomised controlled trial in patients with schizophrenia and healthy controls. *European Neuropsychopharmacology, 23*(7), 675-685.

Shenton, M. E., Dickey, C. C., Frumin, M., & McCarley, R. W. (2001). A review of MRI findings in schizophrenia. *Schizophrenia Research, 49*(1-2), 1-52.

Shepherd, A. M., Laurens, K. R., Matheson, S. L., Carr, V. J., & Green, M. J. (2012). Systematic meta-review and quality assessment of the structural brain alterations in schizophrenia. *Neuroscience & Biobehavioral Reviews, 36*(4), 1342-1356.

Shi, C., Yu, X., Cheung, E. F., Shum, D. H., & Chan, R. C. (2014). Revisiting the therapeutic effect of rTMS on negative symptoms in schizophrenia: a meta-analysis. *Psychiatry Res, 215*(3), 505-513.

Shrivastava, A., Shah, N., Johnston, M., Stitt, L., & Thakar, M. (2010). Predictors of long-term outcome of first-episode schizophrenia: A ten-year follow-up study. *Indian Journal of Psychiatry, 52*(4), 320-326.

Sicras-Mainar, A., Maurino, J., Ruiz-Beato, E., & Navarro-Artieda, R. (2014). Impact of negative symptoms on healthcare resource utilization and associated costs in adult outpatients with schizophrenia: a population-based study. *BMC Psychiatry, 14*, 225.

Sicras-Mainar, A., Maurino, J., Ruiz-Beato, E., & Navarro-Artieda, R. (2015). Prevalence of metabolic syndrome according to the presence of negative symptoms in patients with schizophrenia. *Neuropsychiatric Disease and Treatment, 11*, 51-57.

Sigmundsson, T., Suckling, J., Maier, M., Williams, S., Bullmore, E., Greenwood, K., . . . Toone, B. (2001). Structural abnormalities in frontal, temporal, and limbic regions and interconnecting white matter tracts in schizophrenic patients with prominent negative symptoms. *American Journal of Psychiatry, 158*(2), 234-243.

Singh, S. P., & Singh, V. (2011). Meta-analysis of the efficacy of adjunctive NMDA receptor modulators in chronic schizophrenia. *CNS Drugs, 25*(10), 859-885.

Singh, S. P., Singh, V., Kar, N., & Chan, K. (2010). Efficacy of antidepressants in treating the negative symptoms of chronic schizophrenia: meta-analysis. *British Journal of Psychiatry, 197*(3), 174-179.

Squire, L. R., & Zola-Morgan, S. (1991). The medial temporal lobe memory system. *Science, 253*(5026), 1380-1386.

Staring, A. B., Ter Huurne, M. A., & van der Gaag, M. (2013). Cognitive Behavioral Therapy for negative symptoms (CBT-n) in psychotic disorders: a pilot study. *Journal of Behavior Therapy and Experimental Psychiatry, 44*(3), 300-306.

Tandon, R., Gaebel, W., Barch, D. M., Bustillo, J., Gur, R. E., Heckers, S., . . . Carpenter, W. (2013). Definition and description of schizophrenia in the DSM-5. *Schizophrenia Research, 150*(1), 3-10.

Torrey, E. F. (2013). *Surviving Schizophrenia: A Family Manual* (6th ed.). New York: HarperCollins Publishers Inc.

Trotman, H. D., Kirkpatrick, B., & Compton, M. T. (2011). Impaired insight in patients with newly diagnosed nonaffective psychotic disorders with and without deficit features. *Schizophrenia Research, 126*(1-3), 252-256.

Tuominen, H. J., Tihonen, J., & Wahlbeck, K. (2006). GlutamatergicDrugs for Schizophrenia. *Cochrane Database Systematic Review, CD003730*.

van Os, J., Burns, T., Cavallaro, R., Leucht, S., Peuskens, J., Helldin, L., . . . Kane, J. M. (2006). Standardized remission criteria in schizophrenia. *Acta Psychiatrica Scandinavica, 113*(2), 91-95.

van Os, J., & Kapur, S. (2009). Schizophrenia. *Lancet, 374*(9690), 635-645.

Vancampfort, D., Probst, M., De Hert, M., Soundy, A., Stubbs, B., Stroobants, M., & De Herdt, A. (2014). Neurobiological effects of physical exercise in schizophrenia: a systematic review. *Disability and Rehabilitation, 36*(21), 1749-1754.

Velthorst, E., Koeter, M., van der Gaag, M., Nieman, D. H., Fett, A. K., Smit, F., . . . de Haan, L. (2014). Adapted cognitive-behavioural therapy required for targeting negative symptoms in schizophrenia: meta-analysis and meta-regression. *Psychological Medicine*, 1-13.

Verdoux, H., Liraud, F., Assens, F., Abalan, F., & van Os, J. (2002). Social and clinical consequences of cognitive deficits in early psychosis: a two-year follow-up study of first-admitted patients. *Schizophrenia Research, 56*(1-2), 149-159.

Vita, A., De Peri, L., Deste, G., Barlati, S., & Sacchetti, E. (in press). The effect of antipsychotic treatment on cortical gray matter changes in schizophrenia: does the class matter? A meta-analysis and metaregression of longitudinal magnetic resonance imaging studies. *Biol Psychiatry*.

Warner, R. (2009). Recovery from schizophrenia and the recovery model. *Curr Opin Psychiatry, 22*(4), 374-380.

Wechsler, D. (1997a). *Wecshler Adult Intelligence Scale - 3rd Edition*. San Antonio, TX: The Psychological Corporation.

Wechsler, D. (1997b). *Weschler Memory Scale - 3rd Edition*. New York: The Psychological Corporation.

Wechsler, D. (1999). *Wechsler Abbreviated Scale of Intelligence*. San Antonio, TX: The Psychological Corporation.

Wible, C. G. (2012). Schizophrenia as a disorder of social communication. *Schizophrenia Research, 2012*, 920485.

Williams, L. M. (2008). Voxel-based morphometry in schizophrenia: implications for neurodevelopmental connectivity models, cognition and affect. *Expert Rev Neurother, 8*(7), 1049-1065.

Woods, S. W. (2003). Chlorpromazine equivalent doses for the newer atypical antipsychotics. *J Clin Psychiatry, 64*(6), 663-667.

Woods, S. W. (2011). Chlorpromazine Equivalent Doses for Atypical Antipsychotics: An Update 2003-2010. 1-8. Retrieved from: www.scottwilliamwoods.com/files/WoodsEquivUpdate.doc

Wykes, T., Steel, C., Everitt, B., & Tarrier, N. (2008). Cognitive behavior therapy for schizophrenia: effect sizes, clinical models, and methodological rigor. *Schizophrenia Bulletin, 34*(3), 523-537.

Appendix A

Article 3: Cortical Thinning in Temporo-Parietal Junction (TPJ) in Non-Affective First-Episode of Psychosis Patients with Persistent Negative Symptoms

OPEN ACCESS Freely available online

Cortical Thinning in Temporo-Parietal Junction (TPJ) in Non-Affective First-Episode of Psychosis Patients with Persistent Negative Symptoms

Michael Bodnar[1,2], Cindy L. Hovington[3], Lisa Buchy[3], Ashok K. Malla[1,4], Ridha Joober[1,4], Martin Lepage[1,2,3,4]*

1 Prevention and Early Intervention Program for Psychoses (PEPP – Montreal), Douglas Mental Health University Institute, Montreal, Canada, 2 Department of Psychology, McGill University, Montreal, Canada, 3 Department of Neurology & Neurosurgery, McGill University, Montreal, Canada, 4 Department of Psychiatry, McGill University, Montreal, Canada

Abstract

Background: Negative symptoms represent an unmet therapeutic need in many patients with schizophrenia. In an extension to our previous voxel-based morphometry findings, we employed a more specific, vertex-based approach to explore cortical thinning in relation to persistent negative symptoms (PNS) in non-affective first-episode of psychosis (FEP) patients to advance our understanding of the pathophysiology of primary negative symptoms.

Methods: This study included 62 non-affective FEP patients and 60 non-clinical controls; 16 patients were identified with PNS (i.e., at least 1 primary negative symptom at moderate or greater severity sustained for at least 6 consecutive months). Using cortical thickness analyses, we explored for differences between PNS and non-PNS patients as well as between each patient group and healthy controls; cut-off threshold was set at $p > 0.01$, corrected for multiple comparisons.

Results: A thinner cortex prominently in the right superior temporal gyrus extending into the temporo-parietal junction (TPJ), right parahippocampal gyrus, and left orbital frontal gyrus was identified in PNS patients vs. non-PNS patients. Compared with healthy controls, PNS patients showed a thinner cortex prominently in the right superior temporal gyrus, right parahippocampal gyrus, and right cingulate; non-PNS patients showed a thinner cortex prominently in the parahippocampal gyrus bi-laterally.

Conclusion: Cortical thinning in the early stages of non-affective psychosis is present in the frontal and temporo-parietal regions in patients with PNS. With these brain regions strongly related to social cognitive functioning, our finding suggests a potential link between primary negative symptoms and social cognitive deficits through common brain etiologies.

Citation: Bodnar M, Hovington CL, Buchy L, Malla AK, Joober R, et al. (2014) Cortical Thinning in Temporo-Parietal Junction (TPJ) in Non-Affective First-Episode of Psychosis Patients with Persistent Negative Symptoms. PLoS ONE 9(6): e101372. doi:10.1371/journal.pone.0101372

Editor: Tianzi Jiang, Institute of Automation, Chinese Academy of Sciences, China

Received February 28, 2014; Accepted May 29, 2014; Published June 30, 2014

Funding: The study was supported by operating grants from CIHR (#68961) and the Sackler Foundation to Drs. M. Lepage/A.K Malla; Fonds de la Recherche en Santé du Québec (FRSQ) (salary award to M.L.); Canada Research Chairs Program (to A.K.M.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: M. Lepage reports having received honorariums for educational events from Janssen-Ortho & Lilly. A.K. Malla reports having received financial assistance/compensation for research and educational activities from Pfizer, Janssen-Ortho, AstraZeneca, and Bristol-Myers-Squibb. R. Joober reports having received consultancy honorarium from Pfizer and Janssen-Ortho. This does not alter the authors' adherence to all the PLOS ONE policies on sharing data and materials. All other authors declare no competing conflicts of interest.

* Email: martin.lepage@mcgill.ca

Introduction

Cortical thinning in fronto-temporal regions has become a well-documented finding in schizophrenia [1,2]. However, with the pivotal confounds associated with illness chronicity more recent studies have turned to exploring for morphological abnormalities in first-episode of psychosis (FEP) samples [3–5]. Although studies have shown cortical thinning related to symptomology during these early stages of illness, the relationship with negative symptoms remains vague with some studies identifying an association [6–8] and others not [3,9]. The ambiguity of these findings may be due to the fact that not all studies explicitly explored primary negative symptoms.

Primary and enduring negative symptoms are symptoms intrinsic to schizophrenia [10] that are readily studied in people with either deficit syndrome (DS) or persistent negative symptoms (PNS). For DS, 2 out of 6 items on the Schedule for the Deficit Syndrome (SDS) [11] need to be present for a minimum of 12 months and can only be measured using the SDS [10,12]. In contrast, for PNS, only 1 item of the 6 on the needs to be present for a minimum of 6 months; symptoms that can be measured using ratings scales other than the SDS (e.g., SANS or PANSS). Furthermore, PNS can easily characterized in FEP samples, removing such potential confounds such as illness or medication chronicity. These key characteristics have lead to a recent increase

Table 1. Socio-demographic characteristics and whole-brain tissue volumes for PNS patients, non-PNS patients, and controls.

	PNS (n = 16)	non-PNS (n = 46)	Controls (n = 60)	p
Socio-demographic variable				
Age at scan (years)	24.264.3	23.763.4	24.863.3	0.285
Parental SES[a]	3.461.0	3.461.2	3.161.1	0.394
Education level[b]	11.262.0	12.162.6	14.462.5	,0.001
Full Scale IQ[c]	97.6618.2	95.5612.2	107.9614.9	,0.001
Handed, Right/Other	12/4	40/6	55/5	0.193
Sex, Male/Female	13/3	32/14	40/20	0.529
Whole-brain tissue volumes (ml)				
Grey matter	624656	643660	658671	0.161
White matter	605665	596664	618671	0.264
Cerebral-spinal fluid	201627	197627	203635	0.656
Total intracranial	14306127	14376121	14796151	0.220

Abbreviations: PNS, persistent negative symptoms.
[a]Hollingshead parental socioeconomic status: 1 = highest and 5 = lowest.
[b]Education level measured as number of years completed; post-hoc tests revealed: PNS = non-PNS (p = 0.285); PNS , controls (p , 0.001); non-PNS , controls (p , 0.001).
[c]Full Scale IQ measured with the WAIS-III (data were available for only 58 controls); post-hoc tests revealed: PNS = non-PNS (p = 0.870); PNS , controls (p = 0.034); non-PNS , controls (p , 0.001).
doi:10.1371/journal.pone.0101372.t001

in studies exploring primary negative symptoms in PNS [10,12–18].

As an extension to our VBM study exploring PNS in a FEP sample [15], we wanted to see if cortical thickness analyses would identify the same regions of interest (right frontal medial-orbital and right parahippocampal gyri). Moreover, since cortical thickness has not been used to examine PNS in FEP patients, we set out to determine if other regions of interest could be

Table 2. Clinical characteristics for PNS patients and non-PNS patients.

	PNS (n = 16)	non-PNS (n = 46)	p
Negative symptom total (SANS)			
First Assessment	31.2613.6	27.4612.5	0.308
Month 6	30.1611.3	16.6611.7	,0.001
Month 12	30.3614.7	14.9610.0	,0.001
Positive symptom total (SAPS)			
First Assessment	34.5610.7	35.0617.9	0.909
Month 6	10.969.2	9.7611.9	0.710
Month 12	14.7614.2	10.5617.9	0.399
Depressive symptom total (CDSS)			
First Assessment	4.164.3	4.965.2	0.552
Month 6	3.463.7	1.863.3	0.105
Month 12	1.962.5	1.963.2	0.920
Antipsychotic dosage (mg/day)[a]			
First Assessment	151.56116.1	170.76161.8	0.665
Month 6	178.46164.9	198.76199.6	0.717
Month 12	104.7665.7	206.46253.4	0.119
Medication adherence[b]			
First Assessment	3.361.5	3.261.5	0.591
Month 6	3.161.2	3.061.4	0.951
Month 12	2.361.9	3.261.5	0.126

Abbreviations: PNS, persistent negative symptoms; SANS, Scale for the Assessment of Negative Symptoms; SAPS, Scale for the Assessment of Positive Symptoms; CDSS, Calgary Depression Scale for Schizophrenia.
[a]Antipsychotic totals presented in chlorpromazine equivalents.
[b]Medication adherence: 0 (never adherent) to 4 (fully adherent).
doi:10.1371/journal.pone.0101372.t002

identified using this more precise technique [19]. At the methodological level, VBM analyses capture the volume of structures by the totality of voxels it encompasses or by examining gray matter density; in contrast, cortical thickness analyses examine MRIs at a subvoxel level to provide a direct measurement in millimeters of gray matter morphology, an anatomically more meaningful measure reflecting cortical laminar structure and integrity. With VBM and cortical thickness becoming easily accessible imaging techniques, comparability of results between the two methods is a topic of great interest [19–22] and has been cited as a necessary step when investigating the pathophysiology of disorders such as schizophrenia [23].

Materials and Methods

2.1 Participants & Treatment Setting

All patients were recruited and treated through the Prevention and Early Intervention Program for Psychoses (PEPP-Montreal), a specialized early intervention service at the Douglas Mental Health University Institute serving a local catchment area in Montreal, Canada. People aged 14 to 35 years experiencing an affective or non-affective first-episode of psychosis who had not previously taken antipsychotic medication for more than one month with an IQ higher than 70 were consecutively admitted to the program as either in- or out-patients. For complete program details see [24] or http://www.douglas.qc.ca/pages/view?section_id = 165&locale = en. Only those with a non-affective diagnosis who were over the age of 18 years were included in the analysis.

Patients were identified as having 'persistent negative symptoms' if they had a global rating of moderate (value of 3) or more on at least one negative symptom (affective flattening, alogia, avolition-apathy, or anhedonia-asociality) as measured with the Scale for the Assessment of Negative Symptoms (SANS) [25]. Of note, if a global score of 3 or more was given on affective flattening and alogia entirely as a result of inappropriate affect and poverty of content of speech, respectively, these symptoms were not used in classifying PNS. Next, to ensure PNS were indeed primary negative symptoms, PNS patients had to have a global rating of mild (value of 2) or less on all positive symptoms as measured with the Scale for the Assessment of Positive Symptoms (SAPS) [26], a total score of 4 or less on the Calgary Depression Scale for Schizophrenia (CDSS) [27], and extrapyramidal symptoms that were absent or too mild to require treatment with anticholinergic medications. Finally, all scores had to be maintained for a period of at least 6 consecutive months (between month 6 and 12 after admission, in our case). See Hovington et al [12] for further details regarding the adapted criteria used for identifying PNS.

In all, 62 non-affective FEP patients were subsequently separated into two groups: PNS (n = 16, 25.8%) and non-PNS (n = 46, 74.2%). Among the 46 non-PNS patients, eight displayed PNS but were excluded from the PNS group because of clinically relevant positive (n = 6) and depressive symptoms (n = 2); none were excluded due to extrapyramidal symptoms. Diagnoses included: schizophrenia (PNS = 11; non-PNS = 33), schizoaffective disorder (PNS = 4; non-PNS = 7), schizophreniform disorder (non-PNS = 1), and psychosis NOS (PNS = 1; non-PNS = 5) according to the Structured Clinical Interview for DSM-IV [28] confirmed between two senior research psychiatrists (A.M. & R.J.).

Sixty non-clinical controls were recruited through advertisements in local newspapers and were included only if they had no current or past history of 1) any Axis I disorders, 2) any neurological diseases, 3) head trauma causing loss of consciousness, and 4) a first-degree family member suffering from schizophrenia or related schizophrenia spectrum psychosis.

2.2 Ethics Statement

All research was conducted according to the guidelines laid out by the Declaration of Helsinki, and was approved by the Research Ethics Board of the Douglas Mental Health University Institute

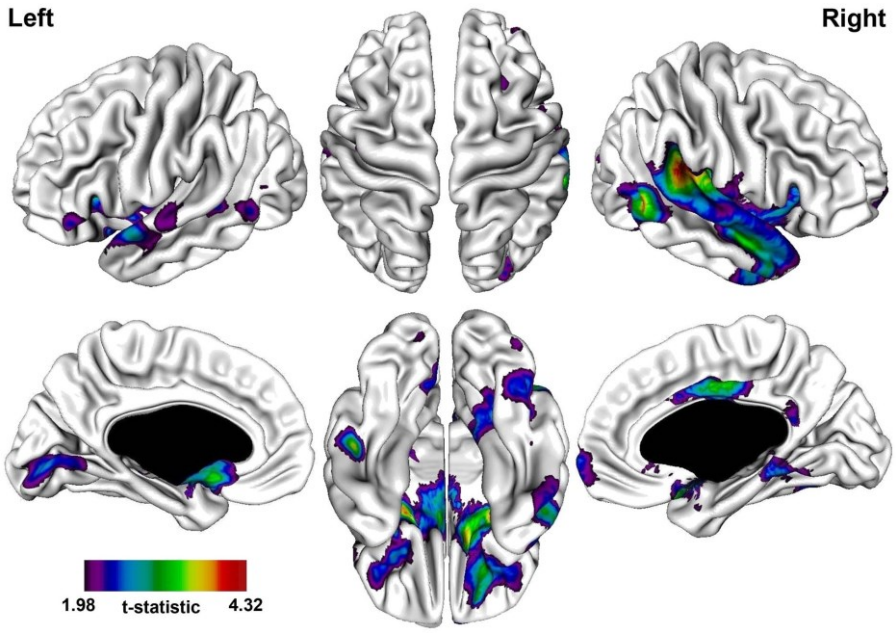


Figure 1. t-statistical brain maps showing cortical thinning in patients with persistent negative symptoms compared to patients without persistent negative symptoms. Most pronounced differences in the right temporo-parietal junction, right superior temporal gyrus, right parahippocampal gyrus, and left inferior frontal gyrus. The colour bar indicates the t-value. All areas shown exceed a FDR corrected statistical threshold of P ,, 0.01.
doi:10.1371/journal.pone.0101372.g001

Table 3. Areas of cortical thinning in PNS patients compared to non-PNS patients.

Region (Brodmann Area)	Coordinates in MNI space			
	x	y	z	t-value
Right hemisphere				
Medial frontal gyrus (10)	6	66	24	2.49
Orbital frontal gyrus (47)	20	32	223	3.25
Anterior cingulate (24)	3	30	27	2.13
Parahippocampal gyrus (34)	26	7	217	4.19
Inferior temporal gyrus (20)	50	0	234	3.09
Anterior/middle cingulate (24/23)	3	23	38	3.34
Middle temporal gyrus (21)	50	25	220	3.30
Middle temporal gyrus (39)	56	255	5	4.22
Superior temporal gyrus (41)	41	234	17	4.32
Posterior cingulate (30)	3	247	19	2.38
Fusiform gyrus (37)	42	265	216	2.54
Middle occipital gyrus (19)	30	285	18	2.38
Left hemisphere				
Inferior frontal gyrus (47)	254	35	21	2.36
Middle frontal gyrus (11)	223	27	217	2.69
Subgenual cingulate (25)	23	11	210	3.39
Inferior frontal gyrus (47)	219	8	219	3.97
Superior temporal gyrus (22)	259	3	27	2.89
Fusiform gyrus (20)	248	228	225	3.74
Middle temporal gyrus (22)	251	241	2	2.44
Middle temporal gyrus (21)	256	258	1	2.41
Middle temporal gyrus (39)	249	269	11	2.07
Cuneus (17)	27	283	2	2.80
Lingual gyrus (18)	213	288	212	2.30

Abbreviations: PNS, persistent negative symptoms.
doi:10.1371/journal.pone.0101372.t003

and the McGill University Faculty of Medicine Research Ethics Board. All participants provided written informed consent prior to engaging in any research-related activity, and were free to withdraw from the study at any time; verbal consent was not considered adequate. Particular to the patients, for the collection and disposition of clinical-based data, if a client was under 18 years of age or deemed incapable to properly represent themselves, written informed consent was obtained from the next of kin, caretaker, or legal guardian. The capacity for individual clients to provide consent was determined by the individual treating team (psychiatrist, case manager, and clinical evaluator) and confirmed by either of the two senior staff psychiatrists (A.K.M. & R.J.). For the collection and disposition of the neuroimaging data, only those aged 18 years and over were recruited from the PEPP clinic, and only after obtaining written informed consent for the collection and disposition of clinical-based data. Finally, after a comprehensive description of the neuroimaging study was provided and the patient displayed a complete understanding, written informed consent was obtained.

2.3 Data Collection

2.3.1 Symptom, Medication, and Socio-demographic Data. The SANS, SAPS, CDSS, and anticholinergic data were obtained at first assessment and at months 1, 2, 3, 6, 9, and 12 after first assessment; first assessment was conducted, on average, within one month after admission (in days; mean = 25.5, s.d. = 9.3, range = 4.8–51.0). Evaluators at PEPP have established an ICC of 0.74 on the SAPS and 0.71 on the SANS; all raters participated in inter-rater reliability sessions at least once a year to avoid rater drift. The type and dosage of antipsychotic taken were also recorded and subsequently converted into chlorpromazine equivalents [29–31]. Medication adherence, based on a 5-point scale ranging from 0 (never) to 4 (fully), was obtained from patients or, when possible, from family members; method was validated elsewhere [32]. Additionally, the following data were acquired at first assessment: education level (number of school years completed), Full Scale IQ with the Wechsler Adult Intelligence Scale [33], parental socio-economic status (SES) with the Hollingshead two-factor index [34], and handedness with the Edinburgh Handedness Inventory [35].

2.3.2 MRI Data Acquisition. Scanning was carried out at the Montreal Neurological Institute on a 1.5 T Siemens whole body MRI system. Structural T1 volumes were acquired for each participant using a three-dimensional (3D) gradient echo pulse sequence with sagittal volume excitation (repetition time = 22 ms, echo time = 9.2 ms, flip angle = 30u, 180 1 mm contiguous sagittal slices). The rectangular field-of-view for the images was 256 mm (SI)6204 mm (AP). Patient groups did not differ as to when sessions took place past entry (weeks; PNS mean = 15.9, s.d. = 5.8; non-PNS mean = 19.9, s.d. = 7.8; t = 1.82, df = 60, p = 0.07).

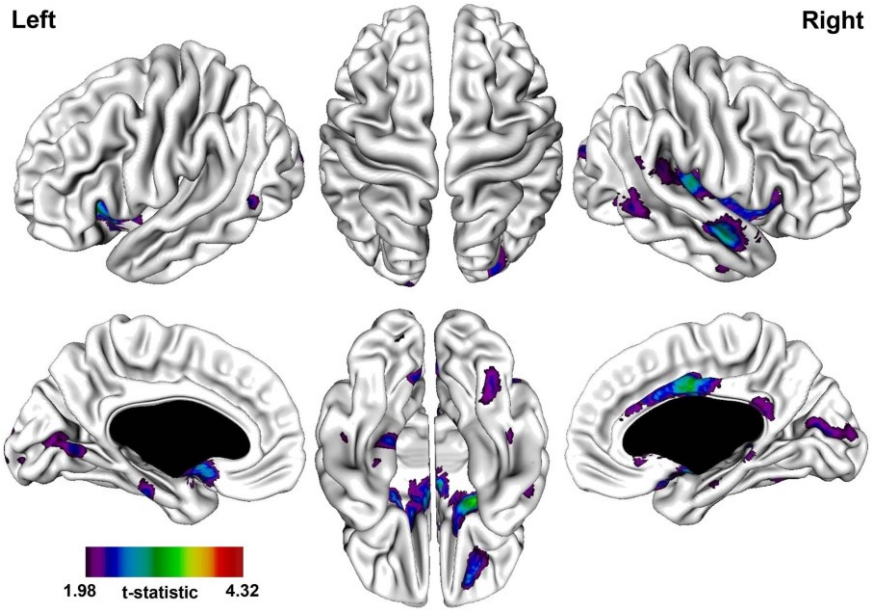


Figure 2. t-statistical brain maps showing cortical thinning in patients with persistent negative symptoms compared to healthy controls. Most pronounced differences in the right temporal gyrus, right parahippocampal gyrus, and right anterior/middle cingulate. The colour bar indicates the t-value. All areas shown exceed a FDR corrected statistical threshold of $P > 0.01$.
doi:10.1371/journal.pone.0101372.g002

2.4 Statistical Analyses

2.4.1. Measurement of Cortical Thickness. MRIs were submitted to the CIVET processing pipeline (Version 1.1.9) (http://wiki.bic.mni.mcgill.ca/index.php/CIVET) [36,37]. Native T1-weighted images were first registered to the ICBM152 template using linear transformation [38,39] and simultaneously corrected for non-uniformity artifacts using N3 [40]. The transformed images were then segmented into grey matter, white matter, cerebral spinal fluid and background using a neural net classifier (INSECT) [37]. Grey matter and white matter surfaces were extracted using CLASP algorithm [41–43]. A spherical-mesh deformation algorithm was used to produce a surface mesh of 81 920 polygons (40 962 nodes or vertices) for each hemisphere. Nonlinear registration of both cortical surfaces to a high resolution average surface template generated from the ICBM152 data set was performed to establish inter-subject correspondence of vertices [44,45]. Reverse linear transformation of volumes was performed to allow vertex-based corticometric (VBC) measurements in native space for each subject's MRI [46]. The deformation algorithm first fits the white matter surface and then expands to the outer GM and cerebral spinal fluid intersection. From these surfaces, cortical thickness was computed in native space using the t-link method [47], which determines the linked distance between the inner and outer cortical surfaces at each of 40 962 vertices. Each participant's cortical thickness map was subsequently blurred using a 20-mm full-width at half-maximum surface-based diffusion smoothing kernel [48].

Statistics were performed at all 40 962 vertices using three difference contrasts: PNS vs. non-PNS, PNS vs. Controls, and non-PNS vs. Controls. Total intracranial volume was not included as a covariate as cortical thickness and brain volume are poorly correlated [46,49]. Statistical maps were thresholded and multiple comparisons were taken in to account using the false discovery rate procedure, with $q = 0.05$ [50]; results were considered significant at $t = 2.64$ ($p > 0.01$).

2.4.2 Whole-brain Tissue Volumes. Finally, whole-brain GM, WM, and CSF volumes were estimated using VBM8 (http://dbm.neuro.uni-jena.de/vbm/download/) for each participant and were summed for an estimation of total intracranial volume (TIV); the four volumes were compared among the three groups using an ANOVA (post-hoc Tukey's HSD test).

2.4.3 Behavioral Analyses. Among the three groups, age at scan, education level, and Full Scale IQ were compared using a one-way ANOVA (post-hoc Tukey's HSD test), parental SES with a Kruskall-Wallis H-test (post-hoc Mann-Whitney U-test), and sex (male vs. female) and handedness (right vs. other) with cross tabulation and Chi-square tests. Between patient groups, independent t-tests were used to compare antipsychotic dosage and symptom totals and Mann-Whitney U-tests to compare medication adherence at first assessment, month 6, and month 12. CDSS ratings were log-transformed while SAPS ratings and antipsychotic total dosage were square-root transformed to achieve normal distribution; all other variables were normally distributed. All analyses were conducted using PASW Statistics 18 (SPSS Inc., 2009, Chicago, IL, USA) and were two-tailed with a critical p-value of 0.05.

Results

3.1 Socio-demographic and Clinical Characteristics

The groups did not significantly differ in age, parental SES, sex, or handedness. PNS and non-PNS patients had fewer years of education and a lower Full Scale IQ compared to controls; the patient groups did not significantly differ (Table 1). Patient groups did not significantly differ in negative symptoms at first assessment but the PNS patients showed significantly higher totals at month 6 and 12, as expected. The two groups did not significantly differ in positive or depressive symptoms, total antipsychotic dosage (in chlorpromazine equivalents), and medication adherence at any time point (Table 2).

Table 4. Areas of cortical thinning in PNS patients compared to controls.

Region (Brodmann Area)	Coordinates in MNI space			
	x	y	z	t-value
Right hemisphere				
Middle frontal gyrus (11)	18	48	220	2.66
Anterior cingulate (32)	3	23	28	2.25
Parahippocampal gyrus (34)	22	5	217	3.45
Inferior temporal gyrus (20)	49	23	232	2.38
Anterior/middle cingulate (24/23)	2	24	35	3.23
Superior temporal gyrus (21)	52	25	215	2.98
Parahippocampal gyrus (28)	24	218	220	2.38
Fusiform gyrus (20)	43	230	220	2.19
Parahippocampal gyrus (27)	16	236	23	2.37
Posterior cingulate (30)	3	245	22	2.34
Middle temporal gyrus (21)	58	256	1	2.48
Fusiform gyrus (37)	37	258	216	2.36
Middle temporal gyrus (37)	45	263	22	2.64
Cuneus (18)	5	278	12	2.41
Cuneus (19)	26	291	21	2.47
Left hemisphere				
Anterior cingulate (25)	24	10	210	2.79
Inferior frontal gyrus (47)	218	9	219	2.79
Parahippocampal gyrus (36)	229	213	230	2.25
Parahippocampal gyrus (35)	222	224	226	2.52
Fusiform gyrus (20)	248	228	226	2.11
Middle temporal gyrus (21)	255	256	2	2.39
Cuneus (30)	26	266	4	2.42
Lingual gyrus (18)	215	284	215	2.01
Middle occipital gyrus (18)	216	2102	11	2.51

Abbreviations: PNS, persistent negative symptoms.
doi:10.1371/journal.pone.0101372.t004

3.2 Cortical Thickness

3.2.1 PNS patients vs. non-PNS patients. A significantly thinner cortex in the PNS patients was observed in the following regions: bilateral frontal, temporal, fusiform, and occipital gyri, right parahippocampal gyrus, bi-lateral anterior cingulate, and right middle and posterior cingulate (Table 3; Figure 1). The most prominent difference was observed in the right superior temporal gyrus extending into the temporo-parietal junction (near the angular gyrus).

3.2.2 PNS patients vs. Controls. A significantly thinner cortex in the PNS patients was observed in the following regions: bi-lateral frontal, temporal, fusiform, parahippocampal, and occipital gyri, bi-lateral anterior cingulate, and right middle and posterior cingulate (Table 4; Figure 2). The most prominent difference was observed in the right parahippocampal gyrus.

3.2.3 non-PNS patients vs. Controls. A significantly thinner cortex in the non-PNS patients was observed in the following regions: bi-lateral parahippocampal gyrus, left superior temporal gyrus, and left inferior occipital gyrus (Table 5; Figure 3). The most prominent differences were observed in the bi-lateral parahippocampal gyrus.

Discussion

The present study used cortical thickness - a more precise method that directly measures gray matter morphology in millimeters reflecting cortical laminar structure and integrity - to explore the neural correlates of persistent negative symptoms (PNS) in non-affective first-episode of psychosis (FEP) patients using a well-established criteria for PNS.

We found a thinner cortex (less grey matter) in the right medial-orbital gyrus and right parahippocampal gyrus in the PNS patients compared to non-PNS patients, supporting of our previous VBM findings [15]. However, we were also able to identify cortical thinning in the PNS patients in additional frontal (cingulate cortex bilaterally) and temporal (temporal gyrus and fusiform gyrus bilaterally) regions, with the largest area of thinning extending into the temporo-parietal junction (TPJ). As well, when compared to controls, PNS patients showed greater cortical thinning overall, more notably in the anterior cingulate bilaterally, temporal gyrus bilaterally, and left parahippocampal gyrus. Given these findings, it is clear that the neural correlates of PNS involve multiple cortical and subcortical regions.

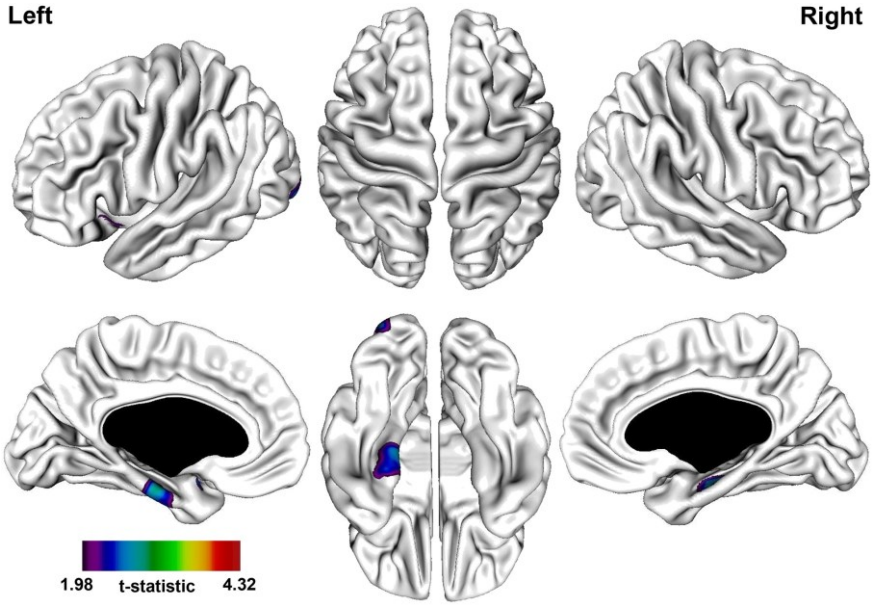


Figure 3. t-statistical brain maps showing cortical thinning in patients without persistent negative symptoms compared to healthy controls. Most pronounced difference in the parahippocampal gyrus bi-laterally. The colour bar indicates the t-value. All areas shown exceed a FDR corrected statistical threshold of $P > 0.01$.
doi:10.1371/journal.pone.0101372.g003

4.1 Negative Symptoms and the Temporal Lobe

4.1.1 Superior Temporal Gyrus and Temporo-parietal Junction. To date, this is the first study, to the best of our knowledge, to identify a thinner cortex in the superior temporal gyrus (STG) extending into the temporo-parietal junction (TPJ) in FEP patients with PNS.

Previous imaging studies have identified significantly less grey matter in the STG in relation to primary and enduring negative symptoms [6,8,51–53]. However, other studies [54,55] including our previous VBM analysis [15] did not demonstrate this relationship. Moreover, other studies have shown a correlation with positive symptoms [56,57], supporting its significant role in auditory and language processing [58,59]. The involvement of the STG as related to PNS appears somewhat unclear [60]. Alternatively, cortical thinning in the STG extending into the TPJ could be related to the social cognitive deficits that define non-affective psychoses, such as schizophrenia [61].

People with PNS are defined by a lack of social skills from not smiling (flat affect) to not talking (poverty of speech) with many withdrawing from society (asociality) or choosing not to engage in everyday activities (avolition). These ''missing'' social skills are innate to their presentation of psychosis. Our strongest finding was in the right TPJ and this region has received increasing attention concerning its role in social cognition, empathy, and social salience. The TPJ has been associated with various social cognitive processes [62–65] with the right TPJ specifically related to the attribution of thoughts in others compared to the attribution of appearance or bodily-sensations about a person [66,67]. The TPJ has also been implicated in identification and reorientation towards salient events in the sensory environment [68,69]. However, one study demonstrated that TPJ activation is limited to choice deliberation about the nature of the upcoming decision in a social context rather than external social stimuli itself [70]. Nevertheless, the TPJ is believed to play a critical role in coordinating behavior in a dynamic, social environment. We as humans must be able to make adaptive socially-correct decisions in a social context and the TPJ is central to this ability [61,70].

Of course, the TPJ has been generally associated with the positive symptoms of schizophrenia based on the plethora of studies that either evoke [71,72] or disrupt [73–76] activation of

Table 5. Areas of cortical thinning in non-PNS patients compared to controls.

Region (Brodmann Area)	Coordinates in MNI space			
	x	y	z	t-value
Right hemisphere				
Parahippocampal gyrus (34)	30	219	219	2.92
Left hemisphere				
Superior temporal gyrus (38)	234	7	221	2.64
Parahippocampal gyrus (28)	219	219	223	2.92
Inferior occipital gyrus (18)	228	296	213	2.72

Abbreviations: PNS, persistent negative symptoms.
doi:10.1371/journal.pone.0101372.t005

the TPJ leading to the induction or alleviation of auditory-related symptoms, respectively. However, there is a new direction that suggests schizophrenia as a social communication disorder with the TPJ as a central structure of interest [61]. Our findings support this idea if we equate primary negative symptoms to a diminished social cognitive ability (flat affect, poverty of speech, asociality, or avolition). Needless to say, we know that structure size does not correlate with function, but a lack of grey matter in the STG and TPJ could help us better understand the relationship among negative symptoms, socializing, and psychosis. Moreover, it could point to a region of interest in developing new treatments for this with primary negative symptoms.

4.1.2 Parahippocampus. Reduced grey matter in the parahippocampus has been identified as a consistent finding in schizophrenia [77–79] with these reductions associated with negative symptom severity [51,52,80,81]. However, these associations have been left-lateralized [52] or bilateral [51,80,81]. In contrast, our analyses examining PNS patients vs. non-PNS patients identified a thinner cortex as well as a reduced volume specific to the right side [15]. Yet, PNS patients showed a thinner cortex bilaterally compared to controls. As such, the association of negative symptoms with laterality is unclear or perhaps associations exist related to specific negative symptoms. For example, our group identified in FEP patients a significant correlation between higher social withdrawal ratings and reduced parahippocampal grey matter consistently on the right side [80,81] whereas, in people with chronic schizophrenia, we revealed a significant positive correlation between flat affect ratings and parahippocampal activity bilaterally [82]. Although these studies did not explicitly investigate primary negative symptoms, a neurobiological association may exist with specific negative symptoms that was not explored in the abovementioned studies. Further investigations are needed to explore the neurobiological basis of individual negative symptoms.

4.2 Cortical Thickness Vs. Voxel-Based Morphometry (VBM)

Recent studies have emerged using both cortical thickness and VBM to study various populations including healthy-aging controls [19], Alzheimer's [20], late-life depression [21], and schizophrenia [23]. In the study examining late-life depression, results revealed cortical thickness was more sensitive in detecting group differences than VBM [21]. Similarly, in the healthy-aging sample, the authors revealed both methods yield similar results but with cortical thickness more sensitive to grey matter decline [19]. Hutton et al elaborated on this by mentioning ''[cortical thickness] is expected to be more sensitive than [VBM]… if there is a prior hypothesis that grey matter changes are mainly due to changes in cortical thickness and also if there is any correlation between the effect of interest and the total brain volume'' [19]. Interestingly, for the Alzheimer's study comparing posterior cortical atrophy, similar results were found using both techniques [20]. Finally, for the schizophrenia study, Palaniyappan and Liddle concluded, ''while VBM may be more sensitive in identifying the regions with gray matter abnormalities, studies investigating the pathophysiology of illnesses such as schizophrenia are better informed when both [cortical thickness] and VBM analyses are performed concurrently'' [23]. In fact, Hutton et al drew a similar conclusion stating that both techniques should be used together to better separate and understand the underlying grey matter changes.

From our analyses, the cortical thickness analysis appeared more sensitive than our VBM analysis [15] in detecting group differences regarding negative symptoms in non-affective FEP patients. What could count for these difference considering that the same sample was examined? First, we must reiterate the fact that cortical thickness specifically measures the cortex thickness in millimeters while VBM measures grey matter differences in local surface area and cortical folding [19]. This leads to the possible reasons why VBM fails to detect more grey matter differences related to: (1) the changes in the shape or displacement of structures during spatial normalization [83–86] or (2) the variability of gyrification [87] that has been shown to be present in schizophrenia [88–92]. Furthermore, the blurring of cortical thickness data takes place in a topologically correct manner along the cortical surface, whereas VBM blurring is 3-dimensional, meaning it does not respect boundaries between tissue classes, leading to an increased likelihood of diluting existing signal or misinterpreting boundary shift as signal [22].

Importantly, we must highlight that our previous VBM analysis used a statistical threshold of $p \gtrsim 0.05$, family-wise error (FWE) corrected for multiple comparisons [15] while the current cortical thickness analysis used a statistical threshold of $p \gtrsim 0.01$, false-discovery rate (FDR) corrected. Although FWE is prone to more false negatives and FDR (considered a less stringent correction than FWE) is prone to more false positives [93], studies examining these corrections (using VBM) have shown results to be similar [94,95]. So, any observed differences in sensitivity between the techniques should not be solely attributed to the correction method employed. In addition, by using a cut-off of $p \gtrsim 0.01$ in the cortical thickness analysis we reduced the number of possible false positives and, effectively, the number of identifiable regions. Yet, more grey matter differences were still identified using this technique.

Taken together, it would appear that cortical thickness may be more sensitive in detecting grey matter anomalies in schizophrenia or related psychoses compared to VBM. This may help to explain why the cortical thickness analysis was able to detect more differences between all of the contrasts investigated and was able to detect more regions of interest related to primary negative symptoms. But for a more complete understanding of group differences in grey matter both techniques should be used to complement each other.

4.3 Conclusions

Our results along with previous studies investigating primary negative symptoms highlight neural abnormalities in two key regions: the frontal and temporal areas [6,8,15,51,52,54] supporting the proposed prefronto-temporolimbic model of negative symptoms [60,96]. Moreover, in the PNS patients, the largest area of cortical thinning was found in the right superior temporal gyrus extending into the temporo-parietal junction-core structures related to social cognitive functioning [61,62–65]. With both social cognitive deficits and negative symptoms characterizing schizophrenia, this area could be explored further in the development of more effective treatments. In fact, treatments utilizing transcranial magnetic stimulation have targeted both the frontal and temporal areas, but the temporal region has been generally targeted in the hope of alleviating positive symptoms [76]. So, perhaps the temporo-parietal junction could be targeted in the hope of alleviating negative symptoms or even the social cognitive difficulties expressed in people with schizophrenia [61]. Finally, it must be stressed when investigating between-group grey matter differences in disorders like schizophrenia, multiple fully-automated techniques should be employed to provide a better understanding of the results [23].

4.4 Limitations

There are several limitations in our study. First, although we employed a first- episode sample in an attempt to reduce the effect of antipsychotic exposure on brain morphology [97,98], the majority of patients were still treated with antipsychotics possibly affecting our results. Second, avolition and anhedonia were more prevalent than alogia and blunted affect in our sample and, as such, our results may not be generalizable to all primary negative symptoms. Furthermore, the categorical approach of this study did not allow us to specify which negative symptoms contributed the most to the structural differences identified; future studies need to examine these symptoms separately. Third, at the time of analysis, clinical data was only available for the first 12 months of treatment in our sample as it has been shown that PNS categorization is more consistent after the first year of treatment [17]. Fourth, our PNS patient group was relatively small, limiting the generalization (interpretation) of our results. Additionally, we could not examine whether the structural differences related to PNS were specific to one diagnosis or not because we were limited by the small number of patients with PNS that did not allow for any meaningful diagnosis specific between-group comparisons. Lastly, the non-PNS group were prescribed, on average, almost double the dosage of antipsychotics [in chlorpromazine equivalents (mg/day)] by month 12. Because treatment is determined on an individual basis at our clinic, we cannot provide any particular reason as to why the individuals of the PNS group were prescribed such a lower dosage. This is noteworthy as antipsychotics have been shown to affect brain morphology [97,99]. However, with scanning completed 18 weeks, on average, after the start of antipsychotic treatment only minimal effects, if any at all, were expected regarding the frontal and temporal regions [100].

Acknowledgments

We thank PEPP-Montreal research staff for their help with recruitment and for conducting the clinical assessments and the M. Lepage staff for acquiring the MRI scans. Finally, we are grateful to all the people who participated in the study.

Author Contributions

Conceived and designed the experiments: MB CLH AKM ML. Analyzed the data: MB LB. Wrote the paper: MB ML. Managed all patient recruitment and clinical assessments: AKM RJ.

References

1. Schultz CC, Koch K, Wagner G, Nenadic I, Schachtzabel C, et al. (2012) Reduced anterior cingulate cognitive activation is associated with prefrontal-temporal cortical thinning in schizophrenia. Biol Psychiatry 71: 146–153.
2. Nesvag R, Bergmann O, Rimol LM, Lange EH, Haukvik UK, et al. (2012) A 5-year follow-up study of brain cortical and subcortical abnormalities in a schizophrenia cohort. Schizophr Res 142: 209–216.
3. Crespo-Facorro B, Roiz-Santianez R, Perez-Iglesias R, Rodriguez-Sanchez JM, Mata I, et al. (2011) Global and regional cortical thinning in first-episode psychosis patients: relationships with clinical and cognitive features. Psychol Med 41: 1449–1460.
4. Schultz CC, Koch K, Wagner G, Roebel M, Schachtzabel C, et al. (2010) Reduced cortical thickness in first episode schizophrenia. Schizophr Res 116: 204–209.
5. Narr KL, Bilder RM, Toga AW, Woods RP, Rex DE, et al. (2005) Mapping cortical thickness and gray matter concentration in first episode schizophrenia. Cereb Cortex 15: 708–719.
6. Cascella NG, Fieldstone SC, Rao VA, Pearlson GD, Sawa A, et al. (2010) Gray-matter abnormalities in deficit schizophrenia. Schizophr Res 120: 63–70.
7. Venkatasubramanian G, Jayakumar PN, Gangadhar BN, Keshavan MS (2008) Automated MRI parcellation study of regional volume and thickness of prefrontal cortex (PFC) in antipsychotic-naive schizophrenia. Acta Psychiatr Scand 117: 420–431.
8. Sanfilipo M, Lafargue T, Rusinek H, Arena L, Loneragan C, et al. (2000) Volumetric measure of the frontal and temporal lobe regions in schizophrenia: relationship to negative symptoms. Arch Gen Psychiatry 57: 471–480.
9. Roiz-Santianez R, Perez-Iglesias R, Quintero C, Tordesillas-Gutierrez D, Mata I, et al. (2010) Insular cortex thinning in first episode schizophrenia patients. Psychiatry Res 182: 216–222.
10. Buchanan RW (2007) Persistent negative symptoms in schizophrenia: an overview. Schizophr Bull 33: 1013–1022.
11. Kirkpatrick B, Buchanan RW, McKenney PD, Alphs LD, Carpenter WT Jr (1989) The Schedule for the Deficit syndrome: an instrument for research in schizophrenia. Psychiatry Res 30: 119–123.
12. Hovington CL, Bodnar M, Joober R, Malla AK, Lepage M (2012) Identifying persistent negative symptoms in first episode psychosis. BMC Psychiatry 12: 224.
13. Jhamnani K, Shivakumar V, Kalmady S, Rao NP, Venkatasubramanian G (2013) Successful Use of Add-on Minocycline for Treatment of Persistent Negative Symptoms in Schizophrenia. J Neuropsychiatry Clin Neurosci 25: E06–07.
14. Galderisi S, Mucci A, Bitter I, Libiger J, Bucci P, et al. (2013) Persistent negative symptoms in first episode patients with schizophrenia: Results from the European First Episode Schizophrenia Trial. Eur Neuropsychopharmacol 23: 196–204.
15. Benoit A, Bodnar M, Malla AK, Joober R, Lepage M (2012) The structural neural substrates of persistent negative symptoms in first-episode of non-affective psychosis: a voxel-based morphometry study. Front Psychiatry 3: 42.
16. Buchanan RW, Panagides J, Zhao J, Phiri P, den Hollander W, et al. (2012) Asenapine versus olanzapine in people with persistent negative symptoms of schizophrenia. J Clin Psychopharmacol 32: 36–45.
17. Chang WC, Hui CL, Tang JY, Wong GH, Lam MM, et al. (2011) Persistent negative symptoms in first-episode schizophrenia: a prospective three-year follow-up study. Schizophr Res 133: 22–28.
18. Malla AK, Norman RM, Takhar J, Manchanda R, Townsend L, et al. (2004) Can patients at risk for persistent negative symptoms be identified during their first episode of psychosis? J Nerv Ment Dis 192: 455–463.
19. Hutton C, Draganski B, Ashburner J, Weiskopf N (2009) A comparison between voxel-based cortical thickness and voxel-based morphometry in normal aging. Neuroimage 48: 371–380.
20. Lehmann M, Crutch SJ, Ridgway GR, Ridha BH, Barnes J, et al. (2011) Cortical thickness and voxel-based morphometry in posterior cortical atrophy and typical Alzheimer's disease. Neurobiol Aging 32: 1466–1476.
21. Colloby SJ, Firbank MJ, Vasudev A, Parry SW, Thomas AJ, et al. (2011) Cortical thickness and VBM-DARTEL in late-life depression. J Affect Disord 133: 158–164.
22. Buchy L, Ad-Dab'bagh Y, Malla A, Lepage C, Bodnar M, et al. (2010) Cortical thickness is associated with poor insight in first-episode psychosis. J Psychiatr Res Epub ahead of print.
23. Palaniyappan L, Liddle PF (2012) Differential effects of surface area, gyrification and cortical thickness on voxel based morphometric deficits in schizophrenia. Neuroimage 60: 693–699.
24. Malla A, Norman R, McLean T, Scholten D, Townsend L (2003) A Canadian programme for early intervention in non-affective psychotic disorders. Aust N Z J Psychiatry 37: 407–413.
25. Andreasen NC (1984) Modified Scale for the Assessment of Negative Symptoms (SANS). Iowa City: University of Iowa.
26. Andreasen NC (1984) Scale for the Assessment of Positive Symptoms (SAPS). Iowa City: University of Iowa.
27. Addington D, Addington J, Maticka-Tyndale E (1993) Assessing depression in schizophrenia: the Calgary Depression Scale. Br J Psychiatry Suppl: 39–44.
28. First MB, Spitzer RL, Gibbon M, Williams JBW (1998) Structured Clinical Interview for DSM-IV Axis I Disorders, Patient Edition (SCID-I/P & SCID-I/NP), Version 2. New York: New York Psychiatric Institute, Biometrics Research.
29. Jensen B, Regier LD, editor (2012) Drug Comparison Charts. 9th Edition ed. Saskatoon, Saskatchewan: RxFiles Academic Detailing Program. 118 p.
30. Woods SW (2011) Chlorpromazine Equivalent Doses for Atypical Antipsychotics: An Update 2003–2010. 1–8.
31. Woods SW (2003) Chlorpromazine equivalent doses for the newer atypical antipsychotics. J Clin Psychiatry 64: 663–667.
32. Cassidy CM, Rabinovitch M, Schmitz N, Joober R, Malla A (2010) A comparison study of multiple measures of adherence to antipsychotic medication in first-episode psychosis. J Clin Psychopharmacol 30: 64–67.
33. Wechsler D (1997) Wechsler Adult Intelligence Scale San Antonio, TX: The Psychological Corporation.
34. Hollingshead A (1965) Two-Factor Index of Social Position. New Haven, CN: Yale University Press.
35. Oldfield RC (1971) The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 9: 97–113.
36. Ad-Dab'bagh Y, Einarson D, Lyttelton O, Muehlboeck J-S, Mok K, et al. (2006) The CIVET Image-Processing Environment: A Fully Automated Comprehen-

sive Pipeline for Anatomical Neuroimaging Research. The 12th Annual meeting of the Organization for Human Brain Mapping (OHBM). Florence, Italy.
37. Zijdenbos AP, Forghani R, Evans AC (2002) Automatic ''pipeline'' analysis of 3-D MRI data for clinical trials: application to multiple sclerosis. IEEE Trans Med Imaging 21: 1280–1291.
38. Collins DL, Neelin P, Peters TM, Evans AC (1994) Automatic 3D intersubject registration of MR volumetric data in standardized Talairach space. J Comput Assist Tomogr 18: 192–205.
39. Grabner G, Janke AL, Budge MM, Smith D, Pruessner J, et al. (2006) Symmetric atlasing and model based segmentation: an application to the hippocampus in older adults. Med Image Comput Comput Assist Interv 9: 58–66.
40. Sled JG, Zijdenbos AP, Evans AC (1998) A nonparametric method for automatic correction of intensity nonuniformity in MRI data. IEEE Trans Med Imaging 17: 87–97.
41. Kabani N, Le Goualher G, MacDonald D, Evans AC (2001) Measurement of cortical thickness using an automated 3-D algorithm: a validation study. Neuroimage 13: 375–380.
42. Kim JS, Singh V, Lee JK, Lerch J, Ad-Dab'bagh Y, et al. (2005) Automated 3-D extraction and evaluation of the inner and outer cortical surfaces using a Laplacian map and partial volume effect classification. Neuroimage 27: 210–221.
43. MacDonald D, Kabani N, Avis D, Evans AC (2000) Automated 3-D extraction of inner and outer surfaces of cerebral cortex from MRI. Neuroimage 12: 340–356.
44. Lyttelton O, Boucher M, Robbins S, Evans A (2007) An unbiased iterative group registration template for cortical surface analysis. Neuroimage 34: 1535–1544.
45. Robbins SM (2004) Anatomical standardization of the human brain in euclidean 3-space and on the cortical 2-manifold.: Monteal: School of Computer Science, McGill University, p. 315.
46. Ad-Dab'bagh Y, Singh V, Robbins S, Lerch J, Lyttelton O, et al. (2005) Native-Space Cortical Thickness Measurement And The Absence of Correlation to Cerebral Volume. The 11th Annual meeting of the Organization for Human Brain Mapping (OHBM). Toronto, Canada.
47. Lerch JP, Evans AC (2005) Cortical thickness analysis examined through power analysis and a population simulation. Neuroimage 24: 163–173.
48. Chung MK, Worsley KJ, Robbins S, Paus T, Taylor J, et al. (2003) Deformation-based surface morphometry applied to gray matter deformation. Neuroimage 18: 198–213.
49. Sowell ER, Peterson BS, Kan E, Woods RP, Yoshii J, et al. (2007) Sex differences in cortical thickness mapped in 176 healthy individuals between 7 and 87 years of age. Cereb Cortex 17: 1550–1560.
50. Genovese CR, Lazar NA, Nichols T (2002) Thresholding of statistical maps in functional neuroimaging using the false discovery rate. Neuroimage 15: 870–878.
51. Koutsouleris N, Gaser C, Jager M, Bottlender R, Frodl T, et al. (2008) Structural correlates of psychopathological symptom dimensions in schizophrenia: a voxel-based morphometric study. Neuroimage 39: 1600–1612.
52. Sigmundsson T, Suckling J, Maier M, Williams S, Bullmore E, et al. (2001) Structural abnormalities in frontal, temporal, and limbic regions and interconnecting white matter tracts in schizophrenic patients with prominent negative symptoms. Am J Psychiatry 158: 234–243.
53. Turetsky B, Cowell PE, Gur RC, Grossman RI, Shtasel DL, et al. (1995) Frontal and temporal lobe brain volumes in schizophrenia. Relationship to symptoms and clinical subtype. Arch Gen Psychiatry 52: 1061–1070.
54. Galderisi S, Quarantelli M, Volpe U, Mucci A, Cassano GB, et al. (2008) Patterns of structural MRI abnormalities in deficit and nondeficit schizophrenia. Schizophr Bull 34: 393–401.
55. Quarantelli M, Larobina M, Volpe U, Amati G, Tedeschi E, et al. (2002) Stereotaxy-based regional brain volumetry applied to segmented MRI: validation and results in deficit and nondeficit schizophrenia. Neuroimage 17: 373–384.
56. Tang J, Liao Y, Zhou B, Tan C, Liu W, et al. (2012) Decrease in temporal gyrus gray matter volume in first-episode, early onset schizophrenia: an MRI study. PLoS One 7: e40247.
57. Palaniyappan L, Balain V, Radua J, Liddle PF (2012) Structural correlates of auditory hallucinations in schizophrenia: a meta-analysis. Schizophr Res 137: 169–173.
58. Gernsbacher MA, Kaschak MP (2003) Neuroimaging studies of language production and comprehension. Annu Rev Psychol 54: 91–114.
59. Martin RC (2003) Language processing: functional organization and neuroanatomical basis. Annu Rev Psychol 54: 55–89.
60. Hovington CL, Lepage M (2012) Neurocognition and neuroimaging of persistent negative symptoms of schizophrenia. Expert Rev Neurother 12: 53–69.
61. Wible CG (2012) Schizophrenia as a disorder of social communication. Schizophr Res Treatment 2012: 920485.
62. Saxe R, Kanwisher N (2003) People thinking about thinking people. The role of the temporo-parietal junction in ''theory of mind''. Neuroimage 19: 1835–1842.
63. Hampton AN, Bossaerts P, O'Doherty JP (2008) Neural correlates of mentalizing-related computations during strategic interactions in humans. Proc Natl Acad Sci U S A 105: 6741–6746.
64. Zink CF, Tong Y, Chen Q, Bassett DS, Stein JL, et al. (2008) Know your place: neural processing of social hierarchy in humans. Neuron 58: 273–283.
65. Chiao JY, Harada T, Oby ER, Li Z, Parrish T, et al. (2009) Neural representations of social status hierarchy in human inferior parietal cortex. Neuropsychologia 47: 354–363.
66. Saxe R, Wexler A (2005) Making sense of another mind: the role of the right temporo-parietal junction. Neuropsychologia 43: 1391–1399.
67. Saxe R, Powell LJ (2006) It's the thought that counts: specific brain regions for one component of theory of mind. Psychol Sci 17: 692–699.
68. Corbetta M, Patel G, Shulman GL (2008) The reorienting system of the human brain: from environment to theory of mind. Neuron 58: 306–324.
69. Chechlacz M, Rotshtein P, Hansen PC, Deb S, Riddoch MJ, et al. (2013) The central role of the temporo-parietal junction and the superior longitudinal fasciculus in supporting multi-item competition: Evidence from lesion-symptom mapping of extinction. Cortex 49: 487–506.
70. Carter RM, Bowling DL, Reeck C, Huettel SA (2012) A distinct role of the temporal-parietal junction in predicting socially guided decisions. Science 337: 109–111.
71. Arzy S, Seeck M, Ortigue S, Spinelli L, Blanke O (2006) Induction of an illusory shadow person. Nature 443: 287.
72. Blanke O, Ortigue S, Landis T, Seeck M (2002) Stimulating illusory own-body perceptions. Nature 419: 269–270.
73. Hoffman RE, Gueorguieva R, Hawkins KA, Varanko M, Boutros NN, et al. (2005) Temporoparietal transcranial magnetic stimulation for auditory hallucinations: safety, efficacy and moderators in a fifty patient sample. Biol Psychiatry 58: 97–104.
74. Jardri R, Lucas B, Delevoye-Turrell Y, Delmaire C, Delion P, et al. (2007) An 11-year-old boy with drug-resistant schizophrenia treated with temporo-parietal rTMS. Mol Psychiatry 12: 320.
75. Poulet E, Brunelin J, Bediou B, Bation R, Forgeard L, et al. (2005) Slow transcranial magnetic stimulation can rapidly reduce resistant auditory hallucinations in schizophrenia. Biol Psychiatry 57: 188–191.
76. Freitas C, Fregni F, Pascual-Leone A (2009) Meta-analysis of the effects of repetitive transcranial magnetic stimulation (rTMS) on negative and positive symptoms in schizophrenia. Schizophr Res 108: 11–24.
77. Williams LM (2008) Voxel-based morphometry in schizophrenia: implications for neurodevelopmental connectivity models, cognition and affect. Expert Rev Neurother 8: 1049–1065.
78. Wright IC, Rabe-Hesketh S, Woodruff PW, David AS, Murray RM, et al. (2000) Meta-analysis of regional brain volumes in schizophrenia. Am J Psychiatry 157: 16–25.
79. Shenton ME, Dickey CC, Frumin M, McCarley RW (2001) A review of MRI findings in schizophrenia. Schizophr Res 49: 1–52.
80. Bodnar M, Malla AK, Joober R, Lord C, Smith E, et al. (2012) Neural markers of early remission in first-episode schizophrenia: A volumetric neuroimaging study of the parahippocampus. Psychiatry Res 201: 40–47.
81. Bodnar M, Harvey P-O, Malla AK, Joober R, Lepage M (2011) The parahippocampal gyrus as a neural marker of early remission in first-episode psychosis: a voxel-based morphometry study. Clin Schizophr Relat Psychoses 4: 217–228.
82. Lepage M, Sergerie K, Benoit A, Czechowska Y, Dickie E, et al. (2011) Emotional face processing and flat affect in schizophrenia: functional and structural neural correlates. Psychol Med 41: 1833–1844.
83. Bookstein FL (2001) ''Voxel-based morphometry'' should not be used with imperfectly registered images. Neuroimage 14: 1454–1462.
84. Davatzikos C (2004) Why voxel-based morphometric analysis should be used with great caution when characterizing group differences. Neuroimage 23: 17–20.
85. Pletson JE, editor (2007) Psychology and Schizophrenia. 1st ed. New York: Nova Publishers, Inc.
86. Thacker NA (2008) Tutorial: A Critical Analysis of Voxel Based Morphometry (VBM).
87. Park HJ, Levitt J, Shenton ME, Salisbury DF, Kubicki M, et al. (2004) An MRI study of spatial probability brain map differences between first-episode schizophrenia and normal controls. Neuroimage 22: 1231–1246.
88. Harris JM, Yates S, Miller P, Best JJ, Johnstone EC, et al. (2004) Gyrification in first-episode schizophrenia: a morphometric study. Biol Psychiatry 55: 141–147.
89. Kulynych JJ, Luevano LF, Jones DW, Weinberger DR (1997) Cortical abnormality in schizophrenia: an in vivo application of the gyrification index. Biol Psychiatry 41: 995–999.
90. Schultz CC, Koch K, Wagner G, Roebel M, Nenadic I, et al. (2010) Increased parahippocampal and lingual gyrification in first-episode schizophrenia. Schizophr Res 123: 137–144.
91. Wheeler DG, Harper CG (2007) Localised reductions in gyrification in the posterior cingulate: schizophrenia and controls. Prog Neuropsychopharmacol Biol Psychiatry 31: 319–327.
92. Palaniyappan L, Liddle PF (2012) Aberrant cortical gyrification in schizophrenia: a surface-based morphometry study. J Psychiatry Neurosci 37: 399–406.
93. Hobbs N, Novak M (2008) VBM: Voxel-based morphometry 37.
94. Silver M, Montana G, Nichols TE (2011) False positives in neuroimaging genetics using voxel-based morphometry data. Neuroimage 54: 992–1000.
95. Meyer-Lindenberg A, Nicodemus KK, Egan MF, Callicott JH, Mattay V, et al. (2008) False positives in imaging genetics. Neuroimage 40: 655–661.
96. Voineskos AN, Foussias G, Lerch J, Felsky D, Remington G, et al. (2013) Neuroimaging Evidence for the Deficit Subtype of Schizophrenia. JAMA Psychiatry: 1–9.

97. Moncrieff J, Leo J (2010) A systematic review of the effects of antipsychotic drugs on brain volume. Psychol Med 40: 1409–1422.
98. Ho BC, Andreasen NC, Ziebell S, Pierson R, Magnotta V (2011) Long-term antipsychotic treatment and brain volumes: a longitudinal study of first-episode schizophrenia. Arch Gen Psychiatry 68: 128–137.99. Puri BK (2011) Brain tissue changes and antipsychotic medication. Expert Rev Neurother 11: 943–946.
100. Leung M, Cheung C, Yu K, Yip B, Sham P, et al. (2011) Gray matter in first- episode schizophrenia before and after antipsychotic drug treatment. Anatomical likelihood estimation meta-analyses with sample size weighting.
Schizophr Bull 37: 199–211.

Appendix B

Article 4: The effect of second-generation antipsychotics on hippocampal volume and verbal memory performance in first episode psychosis: a longitudinal neuroimaging study

Abstract

Objectives: A poorer outcome following a first-episode of psychosis (FEP) has been related to both impaired verbal memory function and reduced hippocampal grey matter volume. With the current neuroscience literature reporting treatment with aripiprazole related to improved memory performance and subcellular changes in the hippocampus, we set out to explore changes in hippocampus grey matter volume and verbal memory performance over a one year treatment period with aripiprazole compared to other antipsychotics.

Methods: Baseline and follow-up MR images were obtained in 90 FEP patients and 46 healthy controls. Seventy-six patients were included in the final analyses and separated into five subgroups: aripiprazole (n=16), olanzapine (n=12), risperidone/paliperidone (n=21), mix (n=14), and refusal (n=13). Hippocampal-subfields were longitudinally processed using FreeSurfer v5.3. Verbal memory performance was evaluated in 49 of these patients.

Results: Compared to the other groups, the aripiprazole subgroup showed a significantly larger increase in bilateral hippocampal volume (all p-values < 0.017), with the most significant change localized to the CA4/Dentate Gyrus subfield (M=24.4mm^3, SD=36.3, Cohen's d=0.22). In addition, the aripiprazole group displayed an increase in verbal memory performance (z-score: M=0.20, SD=1.05, Cohen's d=0.06) that did not significantly differ from the other subgroups.

Conclusions: Aripiprazole is a first-line second-generation treatment option that may provide an added benefit of pro-hippocampal growth. The effect of this growth may be key towards improving memory functions and achieving a better clinical outcome.

1. Introduction

Second-generation antipsychotics (SGAs) represent an efficacious, first-line intervention for treating positive symptoms, especially during a first-episode of psychosis (FEP) (1). Unfortunately, no comparable medications currently exist for treating negative symptoms (2) or cognitive deficits (3). The latter is of keen interest as verbal memory deficits have been consistently reported in people with psychosis (4) with a greater deficit related to a poorer outcome (5). Moreover, reduced grey matter (GM) volume in the hippocampus, a structure vital to memory, has also been linked to a poorer clinical outcome (6).

In psychosis, the most consistent neuroimaging findings have been the progressive brain changes including marked decreases in whole-brain volume, whole-brain GM, and frontal grey and white matter (7-9). However, a recent meta-analysis found whole-brain GM loss was less evident and, in some cases, actually increased in patients taking SGAs; the largest positive effect was found with clozapine (9). Thus, identifying a molecule that could be protective of brain structure and improve memory function could alter the course of treatment moving more people towards a better outcome.

Aripiprazole has been described as a dopamine/serotonin system stabilizer due to its D2, $5HT_{1A}$, and $5HT_7$ agonistic and D1, $5HT_{2A}$, and $5HT_6$ antagonistic nature (10). Following administration of aripiprazole in animal models of depression and schizophrenia, better memory function (10-12) along with molecularly-based changes in the hippocampus (12, 13) have been reported. Moreover, chronic exposure has led to increased proliferation of newly generated cells in a mouse model involving neuronal loss in the dentate gyrus (14). Critically, improved memory function was identified in people with schizophrenia treated with aripiprazole (15-17). Additionally, a functional MRI study found improved working memory ability along with normalized activity in the anterior cingulate cortex (i.e., activation no longer differed from healthy controls) in people with schizophrenia who switched to aripiprazole (18). This suggested a potential memory-related alteration at the neural level related to aripiprazole.

While aripiprazole may help improve memory performance in humans, it is uncertain if there are any structural or functional brain alterations, particularly in the hippocampus. As part of a longitudinal neuroimaging study investigating remission in a naturalistic-outcome setting, we explored verbal memory and hippocampal GM changes over a one year period. We compared FEP patients taking aripiprazole to FEP patients taking other SGAs and to healthy controls

(hippocampal volumes only). We hypothesized that FEP patients taking aripiprazole would show improved verbal memory performance and an increase in hippocampal volume, with the largest effect in the dentate gyrus.

2. Materials and Methods

2.1 Participants & Treatment Setting

All patients were treated in the Prevention and Early Intervention Program for Psychoses (PEPP-Montreal), a specialized early intervention service with integrated clinical, research, and teaching modules, at the Douglas Mental Health University Institute in Montreal, Canada. People aged 14 to 35 years from a defined catchment area suffering from either schizophrenia-spectrum or affective psychosis who had not taken antipsychotic medication for more than one month with an IQ higher than 70 were consecutively admitted to the program as either in- or out-patients. Diagnoses were determined using the Structured Clinical Interview for DSM-IV and validated through consensus with a senior research psychiatrist (A.K.M. or R.J.).

Treatment involves a comprehensive approach towards recovery with intensive medical and psychosocial management provided primarily through modified assertive case management. Pharmacotherapy for all patients, regardless of initial diagnosis, begins with a SGA (olanzapine, risperidone, paliperidone, quetiapine, or aripiprazole) within the recommended doses. If therapeutic response is not optimal within 4-6 weeks or significant side effects emerge, a different SGA is prescribed. While treatment for psychosis begins with an antipsychotic, patients who refuse drug therapy are still provided with all available psychosocial interventions, especially case management and family intervention. For program details see http://www.douglas.qc.ca/page/programme-pepp?locale=en.

For the neuroimaging study, only patients over 18 years of age were considered. A control group was also recruited through advertisements in local newspapers; exclusion criteria included a current or past history of any Axis I disorder, any neurological disease, head trauma causing loss of consciousness, or a first-degree relative diagnosed with schizophrenia or a related spectrum disorder.

After a comprehensive description of the study was provided, written informed consent was obtained from all participants. All clients were free to withdraw from research-based activities at any point without compromising treatment. Research protocols were approved by by the

Research Ethics Boards of the Douglas Mental Health University Institute and the McGill University Faculty of Medicine.

2.2 Longitudinal Structural MRI Data Acquisition & Processing

Scanning was completed at the Montreal Neurological Institute on a 1.5T Siemens whole-body MRI system. For each participant, T1 MR images were acquired using a 3D gradient-echo pulse sequence (TR=22ms; TE=9.2ms; flip angle=30º; FOV=256mm SI x 204mm AP; 180 sagittal slices; voxel size=1mm^3). The same scanner and identical parameters were used at both Scan1 (baseline) and Scan2 (1-year follow-up); 90 FEP patients (treated between January 2004 and June 2014) and 46 controls completed both scans. Fourteen patients were removed due to: missing key clinical data (n=1), technical/processing errors (n=4), and formed insufficiently sized subgroups (n=9); see below and Table S1 for details.

To obtain reliable hippocampal volumes, T1 images were automatically processed in FreeSurfer v5.3 (http://surfer.nmr.mgh.harvard.edu) using the hippocampal-subfields (19) and longitudinal (20, 21)streams. For each participant, Scan1 and Scan2 were: 1) cross-sectionally processed ("recon-all…-all -hippo-subfields"); 2) used to create a within-subject template ("recon-all…-base -hippo-subfields"); and 3) longitudinally processed ("recon-all…-long -hippo-subfields"). A post-processing visual inspection of each scan was conducted for quality control.

The hippo-subfields stream resampled voxels to 0.25mm^3 to better estimate each subfield (CA1, CA2/3, CA4/DG, Presubiculum, Subiculum, and Tail), therefore extracted values were multiplied by 0.125 to obtain volumes in mm^3. Subfield volumes were summed to obtain whole hippocampal volume. Finally, volumes from Scan1 were subtracted from Scan2 to present results as a function of change over time. See SF1 to view an example of the hippocampal subfield segmentation performed by FreeSurfer with specific overlays highlighting the CA4/DG from Scan1 and Scan2.

2.3 Defining Antipsychotic Treatment Subgroups

Patients were separated into subgroups based on the antipsychotic taken during the interscan interval. To be considered for a subgroup, a patient had to take an antipsychotic for a minimum of 5 consecutive months with an average adherence above 50%. Some patients were switched or took another antipsychotic concurrently. In these case, patients were categorized into the "Mix"

subgroup if the second antipsychotic was taken for 40% or more of the time of the initial one. Finally, those who refused antipsychotic treatment or had less than 50% adherence were categorized into the "Refusal" subgroup.

Final groupings included: Risperidone/Paliperidone (Risp/Palp, n=21); Olanzapine (n=12); Aripiprazole (n=16); Mix (n=14); and Refusal (n=13). Those in the Quetiapine (n=4), Ziprasidone (n=3), Haloperidol (n=1), and Asenapine (n=1) subgroups were excluded as any results would have been unreliable due to small sample sizes. Patients taking paliperidone (n=5) were included as part of the Risp/Palp subgroup since paliperidone is the active metabolite of risperidone and has been shown to have a similar efficacy and treatment profile (22). See ST1 for complete pharmacotherapy information from Entry until Scan 2 to provide full disclosure and to aid with understanding the antipsychotic categorizations.

2.4 Clinical and Socio-demographic Data & Verbal Memory Measurement

Clinical data were collected near entry and at months 1, 2, 3, 6, 9, 12, and 18 thereafter; baseline assessment occurred, on average, 9.4 days after entry (SD=8.8, Range:-18-36). At each assessment, the type and dosage of antipsychotic prescribed were noted and converted into chlorpromazine equivalents (23, 24); medication adherence was determined via information obtained from the patient, family members, and treating team (25). Data on education level (years completed), parental socio-economic status (26), handedness (27), and duration of untreated psychosis were obtained at baseline.

Over the 18-month period, outcome was examined as changes in the Global Assessment of Functioning (GAF) and total positive symptoms from the Scale for the Assessment of Positive Symptoms (SAPS) (28). Also, the percentage of time spent in positive symptom remission during the interscan interval was calculated; positive remission was defined as mild or less on all four global scores of the SAPS (29). Evaluators have established inter-class correlations of 0.89 and 0.97 on the SAPS and GAF, respectively.

All patients completed a baseline neuropsychological evaluation, on average, 2.1 months after entry (SD=1.5, Range:0-6.1); 47 patients completed a follow-up evaluation, on average, 14.6 months later (SD=2.4, Range:11.4-22.7). Verbal memory was assessed using either the WMS-III (Logical Memory) (30) or CogState (Shopping List) (31) as our neuropsychological battery was updated in December 2010. A z-score score for verbal memory was calculated for each patient

based on the whole group mean and standard deviation; z-scores from baseline were subtracted from follow-up to present results as a function of change over time. Full-scale IQ (32, 33) was estimated during the baseline evaluation.

2.5 Statistical Analyses

Whole hippocampal volumes were analyzed using a MANOVA with 'Group' (risp/palp, olanzapine, aripiprazole, mix, refusal, controls) as the between-group factor and 'Side' (left, right) as the within-group factor; this analysis was one-tailed. Hippocampal-subfield volumes were analyzed using a MANOVA with 'Group' as the between-group factor and 'Side' and 'Subfield' (CA1, CA2/3, CA4/DG, presubiculum, subiculum, tail) as the within-group factors; analysis was one-tailed with a critical *p*-value set at 0.005 (0.05/10). Secondary analyses were conducted that included a matching covariate that was estimated using Propensity Score Matching (34); FEP patients and controls were matched on age at Scan1, interscan interval, education, sex, handedness, usage of antidepressants or mood stabilizers, antipsychotic dosage per month, and time spent in remission.

Demographic and clinical variables were analyzed with one-way ANOVAs for continuous variables or Kruskall-Wallis H tests for nominal variables; DUP with the Median test; and GAF and SAPS Total with Generalized Estimating Equations. Verbal memory performance was analyzed using an ANOVA with 'battery type' (old vs. new) entered as a random factor. Partial correlations controlling for 'Group' were used to explore associations between changes in verbal memory and hippocampal and CA4/DG volumes. All analyses were conducted using SPSS 22 (IBM Corp., Armonk, NY, USA) and were two-tailed with a critical *p*-value of 0.05, except where noted.

3. Results

3.1 Socio-demographic and clinical characteristics

There were no significant differences on age at Scan1, interscan interval, parental SES, sex, handedness, or Full-Scale IQ; however, there were significant differences regarding education with controls completing the most years and the Mix subgroup the fewest. Among the FEP subgroups, there were significant differences on antipsychotic dosage and adherence. As expected, the Mix subgroup was prescribed the highest dosage per month; the Refusal subgroup was prescribed the

lowest dosage and also showed the lowest adherence. There were no significant differences on DUP. See Table 1 for data and results.

3.2 Outcome

For SAPS Total, there were significant main effects of 'Time' (Wald χ^2=216.03, df=3, p<0.001) and 'Group' (Wald χ^2=10.91, df=4, p=0.028); see Figure 2, Panel A. All FEP patients displayed a significant decrease from Baseline to Month 6 (p<0.001) that remained steady thereafter (all p>0.952). The Mix subgroup displayed a higher total overall that significantly differed from the Risp/Palp (p=0.003) and Aripiprazole (p=0.004) subgroups; all other differences were not significant (all p>0.125). There was a significant main effect of 'Group' ($F_{4,71}$=4.91, p=0.001) for positive symptom remission; see Figure 2, Panel B. The Mix subgroup spent the lowest percentage of time in remission compared to the other subgroups (all p<0.027); no other differences were significant (all p>0.142). Finally, there were significant main effects of 'Time' (Wald χ^2=242.82, df=3, p<0.001) and 'Group' (Wald χ^2=31.69, df=4, p<0.001) on GAF scores; see Figure 2, Panel C. All FEP patients displayed a significant increase from Baseline to Month 6 (p<0.001) and from Month 12 to Month 18 (p=0.039); there was trend-level improvement from Month 6 to Month 12 (p=0.064). The Mix subgroup had the lowest overall GAF score compared to the other subgroups (all p<0.001); no other differences were significant (all p>0.134).

Table 1 Characteristics of the FEP Patients and Controls.

Variable	Risp/Palp (n=21)		Olanzapine (n=12)		Aripiprazole (n=16)		Mix (n=14)		Refusal (n=13)		Controls (n=46)		Statistic	df	*p*
	M	SD	M	SD	M	SD	M	SD	M	SD	M	SD			
Age at Scan1	23.8	4.3	23.9	3.8	24.0	4.7	23.7	3.4	23.8	3.3	25.4	3.3	F=0.42	5,116	0.834
Entry to Scan1 (months)	4.0	1.9	3.5	2.2	4.1	1.7	4.6	2.0	3.7	1.4	---	---	F=0.64	4,71	0.635
Interscan Interval [a]	13.7	1.4	13.0	1.4	12.7	1.2	12.8	1.0	13.2	1.3	12.7	1.3	F=3.53	5,116	0.062
Parental SES [b]	3.2	1.1	3.0	1.0	2.8	0.9	3.8	1.1	3.1	0.9	3.3	0.8	χ^2=7.43	5	0.191
Education [c]	11.4	2.5	13.3	2.1	12.8	2.1	10.9	2.4	12.5	2.6	**14.4**	2.6	F=42.52	5,116	**0.001**
Full Scale IQ	97.0	14.6	98.3	17.0	105.1	12.7	92.9	15.0	104.8	13.3	111.2	14.4	F=5.14	5,113	**0.001**
CPZ/month [d]	149.1	106.6	168.1	98.5	172.7	177.1	**397.6**	393.7	74.4	97.0	---	---	F=6.31	4,71	**0.001**
Adherence [e]	82.9	20.1	94.7	10.7	90.6	15.4	**75.9**	18.2	**37.8**	28.8	---	---	F=14.67	4,67	**0.001**
DUP (weeks) [f]	46.5	65.5	68.5	100.0	40.5	81.2	42.0	75.1	98.5	160.5	---	---			
median	22.6		18.1		12.8		15.6		20.6		---		χ^2=9.06	4	0.060
	N	%	N	%	N	%	N	%	N	%	N	%			
Right Handed	17	81.0	10	83.3	15	93.8	13	92.9	9	69.2	40	87.0	χ^2=3.47	5	0.628
Male	14	67.7	7	58.3	12	75.0	10	71.4	10	76.9	26	56.5	χ^2=4.67	5	0.458
Non-Affective Diagnosis	19	90.5	8	66.7	12	75.0	12	85.7	11	84.6	---	---	χ^2=3.58	4	0.466
Taking Anti-Depressant	3	14.3	4	33.3	3	18.8	5	35.7	1	7.7	---	---	χ^2=4.93	4	0.295
Taking Mood Stabilizer	0	0.0	2	16.7	0	0.0	0	0.0	2	15.4	---	---	χ^2=8.63	4	0.071

Abbreviations: Risp, Risperidone; Palp, Paliperidone; CPZ, chlorpromazine; DUP, duration of untreated psychosis.

[a] Time in months from Scan1 until Scan2.

[b] Hollingshead parental socioeconomic status: 1=highest and 5=lowest.

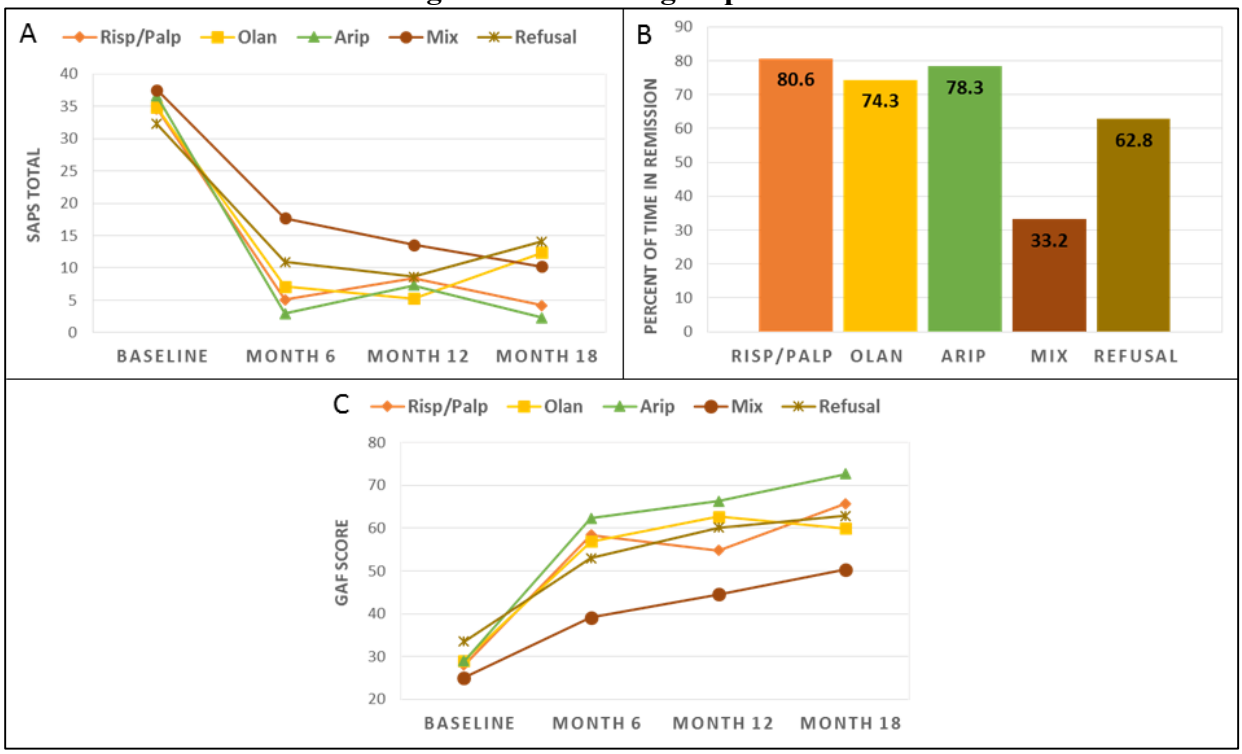
[c] Number of school years completed. Controls > all subgroups (all p<0.026) except Olanzapine; Mix < Aripiprazole (p=0.031) and Olanzapine (p=0.012); Risp/Palp < Olanzapine (p=0.034).

[d] Average prescribed dosage in CPZ equivalents (mg/day) per month during the interscan interval. Mix > Risp/Palp (p=0.001), Olanzapine (p=0.005), Aripiprazole (p=0.003), and Refusal (p<0.001).

[e] Average overall medication adherence during the interscan interval. Refusal < all subgroups (all p<0.001); Mix < Olanzapine (p=0.013) and Aripiprazole (p=0.036).

[f] DUP was defined as the time from the onset of any psychiatric symptoms to adequate treatment (30 days of continuous treatment or until positive symptoms remitted), plus any previous periods of psychosis that resolved spontaneously.

Figure 1 Positive Symptom Total, Time Spent in Positive Symptom Remission, and Functional Outcome and among the Patient Subgroups.



Abbreviations: Risp/Palp, risperidone/paliperidone; Olan, olanzapine; Arip, aripiprazole. **Panel A** present SAPS Total over 18 months. All FEP patients showed a decrease over the first six months. By Month 18 the Aripiprazole subgroup showed significant differences from the Refusal and Mix subgroups along with a strong trend-level difference from the Olanzapine subgroup. The Mix subgroup displayed higher totals compared to the other subgroups at Month 6 and at Month 12. **Panel B** presents the time spent in positive symptom remission during the interscan interval. The Mix subgroup spent the lowest amount of time in remission compared to the other subgroups; nominal differences were apparent among the other four subgroups. **Panel C** presents the GAF scores over 18 months. All FEP patients showed an increase in functioning over the time period; however, the Mix subgroup showed significantly lower scores compared to the other subgroups. Nominal differences were apparent among the other four subgroups.

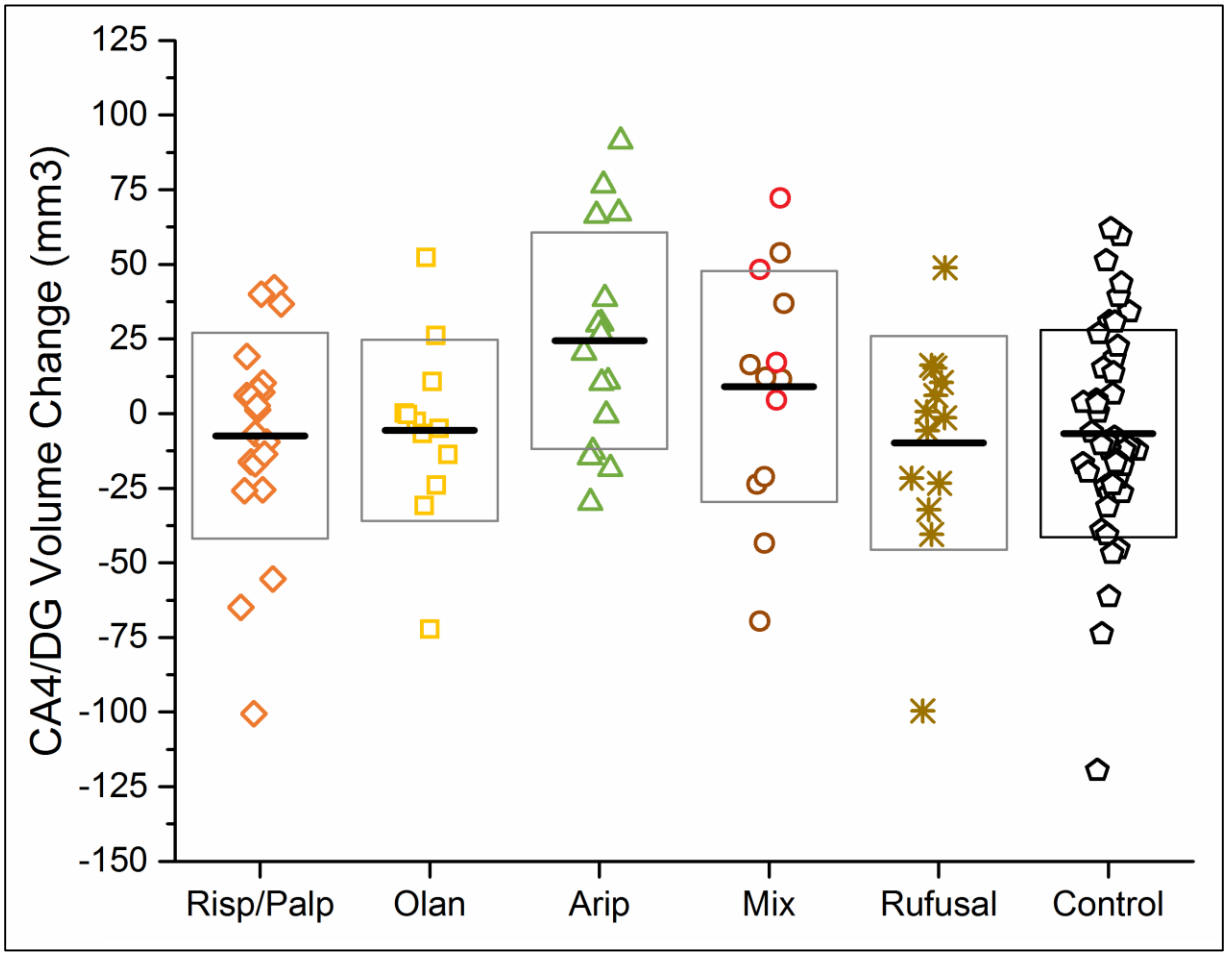
3.3 Change in Hippocampal and Subfield Volumes

The hippocampus analysis revealed a nearly significant 'Group' effect ($F_{5,116}$=1.82, p=0.058). The Aripiprazole subgroup had a significantly larger increase in volume compared to all FEP subgroups (all ps<0.028) and controls (p=0.006). Adding the matching covariate had no effect; the 'Group' effect remained nearly significant ($F_{5,115}$=1.95, p=0.053) with the Aripiprazole subgroup still significantly differing (all p<0.035).

The hippocampal-subfield analysis revealed a significant 'Group x Subfield' interaction ($F_{5,116}$=6.00, p<0.001). The Aripiprazole subgroup showed a larger increase in CA4/DG volume that significantly differed from controls (p=0.002) and Risp/Palp (p=0.004) and Refusal (p=0.005)

subgroups. The increase was nearly significant compared to the Olanzapine subgroup (p=0.013) but did not differ from the Mix subgroup (p=0.117). See Table 2 for subfield data and Figure 3 for a scatterplot of the CA4/DG data. Adding the matching covariate had minimal effect. The interaction remained significant ($F_{5,115}$=6.19, p<0.001); the Aripiprazole subgroup still differed from controls (p=0.002) and FEP subgroups (all p<0.015) except for the Mix subgroup (p=0.117). See ST2 for raw volumetric data at Scan1 and Scan2.

Figure 2 Scatterplot of Change in Grey Matter Volume in the CA4/DG for Patient Subgroups and Controls.



Abbreviations: Risp/Palp, risperidone/paliperidone; Olan, olanzapine; Arip, aripiprazole. Within the Mix group, those taking Aripiprazole are colored in red (n=4). The thick black bar represents the mean for each group while the grey box represents the standard deviation.

Table 2 Changes in Hippocampal Subfield Volumes in Patient Subgroups and Controls.

Group	CA1			CA2/3			CA4/DG			Presubiculum			Subiculum			Hippocampus Tail		
	M	SD	ES	M	SD	ES	M	SD	ES	M	SD	ES	M	SD	ES	M	SD	ES
Risp/Palp (n=21)	-0.3	24.3	0.01	-1.5	54.6	0.01	-7.4	34.5	0.06	-10.4	41.4	0.09	-3.7	48.1	0.03	5.7	27.9	0.05
Olan (n=12)	-10.0	28.7	0.15	2.4	52.7	0.01	-5.6	30.4	0.04	-3.2	29.4	0.04	-4.9	48.8	0.04	9.2	26.6	0.14
Arip (n=16)	15.0	38.7	0.22	21.4	50.6	0.11	24.4	36.3	0.22	9.5	49.1	0.09	28.5	50.0	0.18	0.0	31.6	0.01
Mix (n=14)	-8.8	38.9	0.15	-21.3	87.4	0.10	9.1	38.6	0.08	-2.0	34.9	0.04	-9.4	53.3	0.10	-11.7	34.7	0.15
Mix: no Arip (n=10)	*-14.1*	*36.7*	*0.23*	*-38.0*	*88.6*	*0.17*	*-1.5*	*37.5*	*0.02*	*-6.5*	*38.6*	*0.08*	*-19.9*	*58.7*	*0.20*	*-14.2*	*40.4*	*0.17*
Refusal (n=13)	5.6	16.5	0.05	-12.0	52.9	0.04	-9.8	35.9	0.06	-4.9	35.2	0.03	-3.1	37.1	0.02	-4.6	27.6	0.03
Controls (n=46)	3.5	28.3	0.06	0.1	54.9	0.00	-6.7	34.7	0.06	-3.5	51.0	0.03	-3.3	38.7	0.03	-2.9	35.6	0.03

Abbreviations: Risp, risperidone; Palp, paliperidone; Olan, olanzapine; Arip, aripiprazole; ES, Effect Size (Cohen's d).
The Effect Size represents the strength of the change within each group over the follow-up period. Data were provided for a Mix subgroup with those patients who were taking Aripiprazole as part of their medications.

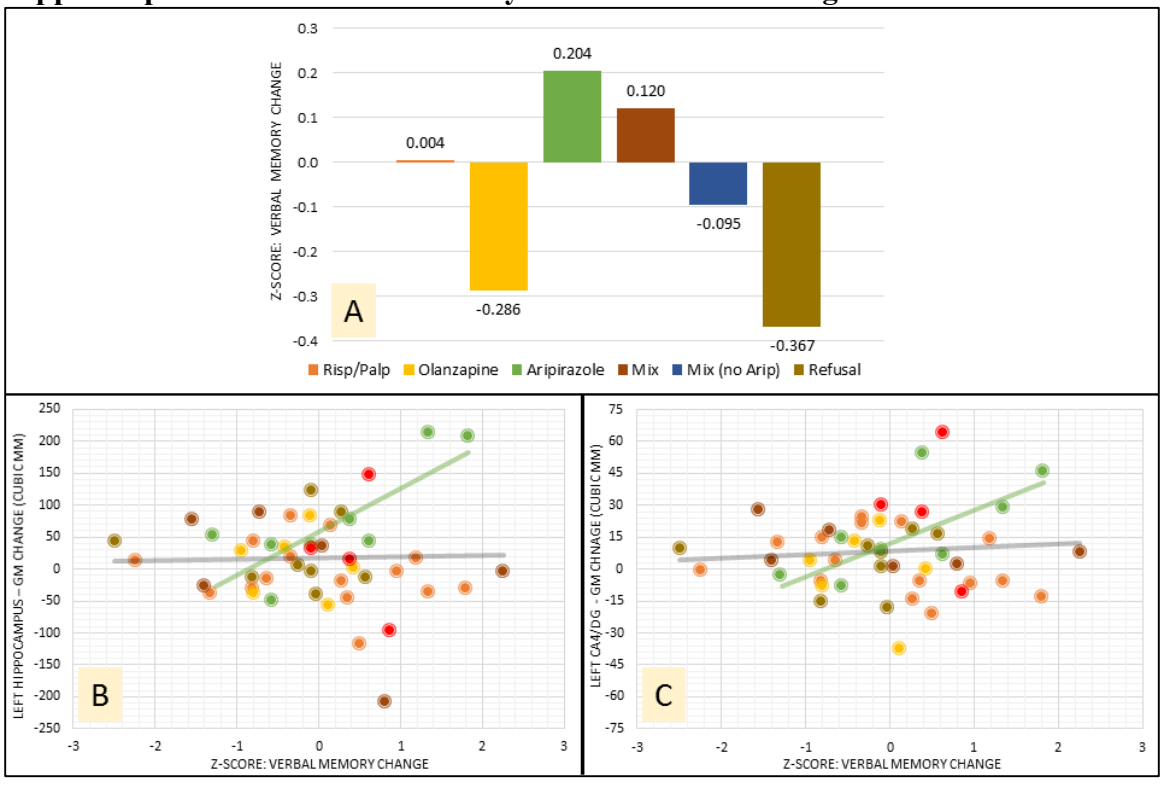
3.4 Change in Verbal Memory Performance among FEP Subgroups

There was no significant difference among the subgroups ($F_{4,39}$=0.38, p=0.815) but the Aripiprazole and Mix subgroups displayed a larger positive increase compared to the other subgroups. See Figure 3, Panel A for verbal memory comparisons and ST3 for data from baseline and follow-up evaluations.

3.5 Correlations between Verbal Memory and Hippocampal Volume

No partial correlations were significant (all p>0.612). However, among the Aripiprazole patients (n=8), a significant correlation was found with the left hippocampus (r=0.804, p=0.016) and a nearly significant association with the left CA4/DG subfield (r=0.705, p=0.051). See Figure 3, Panels B and C for correlational plots.

Figure 3 Verbal Memory Change among FEP Subgroups and Correlations with Left Hippocampus and Left CA4/DG Grey Matter Volume Change.



Abbreviations: Risp/Palp, risperidone/paliperidone; Olan, olanzapine; Arip, aripiprazole; GM, Grey Matter. **Panel A** presents verbal memory performance as a z-score calculated from the mean and standard deviation of FEP patients who completed both evaluations. The 'Mix (no Arip)' in blue is the Mix subgroup with four patients who took aripiprazole removed. **Panel B** (Left Hippocampus) and **Panel C** (Left CA4/DG) present each data point colored according to subgroup; the Mix/Aripiprazole patients are colored in red (n=4). The grey trendline is for the entire sample; the green trendline is for the Aripiprazole subgroup only.

3.6 Supplementary

To explore if results were specific to Aripiprazole, 4 patients from the Mix subgroup who had taken aripiprazole were removed and the hippocampal-subfield data re-analyzed; the critical *p*-value was set to 0.01 (0.05/5) as 'Side' was not entered. A significant 'Group' effect remained ($F_{5,111}$=2.91, p=0.008) with differences specific to the CA4/DG. The Aripiprazole subgroup still significantly differed from the controls (p=0.002) and the Risp/Palp (p=0.004) and Refusal (p=0.006) subgroups, but was now nearly significant compared to both the Olanzapine (p=0.014) and Mix (p=0.038) subgroups. See Table 2 for data ('Mix: no Arip'); Figure 2 highlights the Aripiprazole/Mix patients in the color red. For verbal memory, after removing the 4 patients, the Mix subgroup no longer showed an increase in performance but between-group differences remained non-significant ($F_{4,36}$=0.36, p=0.835). See ST3 for data and Figure 3, Panel B highlights the Aripiprazole/Mix patients in the color red.

4. Discussion

In this naturalistic outcome, longitudinal neuroimaging study, we observed that FEP patients taking aripiprazole displayed improved verbal memory performance as well as a significant increase in bilateral hippocampal volume over a one-year follow-up period compared to FEP patients taking other SGAs and healthy controls. Of importance, the most robust increase in hippocampal volume was found within the CA4/DG subfield.

Treatment with aripiprazole was equally efficacious compared to the other antipsychotics in terms of level of functioning achieved, total positive symptom reduction, and time spent in positive symptom remission. Altogether, these results suggest aripiprazole may be a good treatment option for people experiencing a first-episode of psychosis with an added benefit of enhanced hippocampal plasticity and improved verbal memory capacity. These results may have an important clinical impact since both the hippocampal volume and verbal memory performance vary as a function of future clinical status (5, 6, 35).

4.1 Improving Memory and Augmenting Hippocampal Growth

Aripiprazole has been shown to restore phencyclidine (PCP)-induced recognition memory deficits in mice (11) and to enhance spatial memory abilities in rats (10, 12). Moreover, multiple open-label studies involving schizophrenia have found a verbal memory enhancing effect in humans

(15-17). Additionally, improved memory performance has been associated with a normalization in brain activity in people with schizophrenia (18). Hence, as supported by our present results, aripiprazole may not only help improve memory function but may also support brain plasticity underpinning memory functions that could include the hippocampus.

The exact molecular mechanism underlying the link between hippocampal volume and memory functioning is unclear, but there is evidence to suggest brain-derived neurotrophic factor (BDNF) may play an important role. A recent study showed participants with a 'val66met' polymorphism displayed poorer memory performance along with abnormal hippocampal functioning, as a function of reduced BDNF secretion (36). Intriguingly, aripiprazole has been shown to enhance BDNF levels in the rat hippocampus (12) as well as in human neuroblastoma cells (37). Thus, the memory-enhancing effects of aripiprazole may be related to BDNF; an area that future studies will need to address.

Aripiprazole may also enhance adult neurogenesis through BDNF as the latter is known to play an important role in the survival and development of neurons throughout life (38). The adult human brain is capable of producing new functional neurons with growth limited to the dentate gyrus (39). Adult neurogenesis has attracted even more attention, since hippocampal-dependent learning and memory, such as spatial and object recognition memory, has been shown to contribute above and beyond normal daily growth (40). Importantly, exposure to aripiprazole has shown enhanced proliferation but not survival of newly generated neurons in the dentate gyrus of mice (14). So, aripiprazole appears to display enhancing effects on adult neurogenesis, but future studies need to explore the underlying mechanisms and the possible relationship with BDNF.

4.2 Limitations

Our results are strengthened by the fact our patients are largely previously untreated with antipsychotic medications, who are from a defined catchment area and treated in an early intervention service, not exclusively in-patients, and, therefore, are truly representative of FEP patients with varying severity. However, there are a number of limitations to consider. First, this study was not designed to explicitly explore the effects of antipsychotics on the brain. Pharmacotherapy began with a SGA, but some patients required additional medications such as a mood stabilizer, an antidepressant, or an additional antipsychotic. We co-varied for those taking mood stabilizers or antidepressants. And created a separate Mix subgroup. Nevertheless, it proved

difficult to gage the actual effect of the additional medications. Second, our study had two timepoints separated by one year making it problematic to fully characterize the temporal characteristics of the hippocampal volume change. Finally, although our patients demonstrated an overall medication adherence above 80%, using a reliable and validated method (25), it was not possible to monitor direct intake of medication or how adherence may have affected the results. Future studies may consider employing the long-acting injectable forms to ensure adherence and to improve our understanding of the effects of antipsychotics on cognition and the brain.

4.3 Summary

Aripiprazole is believed to have a pro-cognitive benefit in people with schizophrenia and the related psychoses. Our study extended this result by showing prolonged treatment with aripiprazole increased hippocampal volume with a particular effect localized to the CA4/DG subfield in patients with limited or no previous exposure to antipsychotic medications. With the added benefit of stimulating adult neurogenesis above and beyond normal daily growth, aripiprazole could represent not only a pharmacological treatment for symptomatic management in psychosis but could potentially help repair (in part) a putatively dysfunctional brain circuit in schizophrenia and related psychoses.

5. References

1. Agid O, Schulze L, Arenovich T, Sajeev G, McDonald K, Foussias G, Fervaha G, Remington G. Antipsychotic response in first-episode schizophrenia: efficacy of high doses and switching. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology. 2013;23:1017-1022.
2. Fusar-Poli P, Papanastasiou E, Stahl D, Rocchetti M, Carpenter W, Shergill S, McGuire P. Treatments of Negative Symptoms in Schizophrenia: Meta-Analysis of 168 Randomized Placebo-Controlled Trials. Schizophrenia bulletin. 2014.
3. Opler LA, Medalia A, Opler MG, Stahl SM. Pharmacotherapy of cognitive deficits in schizophrenia. CNS spectrums. 2014;19:142-156.
4. Bora E, Pantelis C. Meta-analysis of Cognitive Impairment in First-episode Bipolar Disorder: Comparison with First-episode Schizophrenia and Healthy Controls. Schizophrenia bulletin. 2015.
5. Lepage M, Bodnar M, Bowie CR. Neurocognition: clinical and functional outcomes in schizophrenia. Canadian journal of psychiatry Revue canadienne de psychiatrie. 2014;59:5-12.
6. Bodnar M, Malla AK, Czechowska Y, Benoit A, Fathalli F, Joober R, Pruessner M, Pruessner J, Lepage M. Neural markers of remission in first-episode schizophrenia: a volumetric neuroimaging study of the hippocampus and amygdala. Schizophrenia research. 2010;122:72-80.

7. Moncrieff J, Leo J. A systematic review of the effects of antipsychotic drugs on brain volume. Psychol Med. 2010;40:1409-1422.
8. Olabi B, Ellison-Wright I, McIntosh AM, Wood SJ, Bullmore E, Lawrie SM. Are there progressive brain changes in schizophrenia? A meta-analysis of structural magnetic resonance imaging studies. Biological psychiatry. 2011;70:88-96.
9. Vita A, De Peri L, Deste G, Barlati S, Sacchetti E. The effect of antipsychotic treatment on cortical gray matter changes in schizophrenia: does the class matter? A meta-analysis and metaregression of longitudinal magnetic resonance imaging studies. Biological psychiatry. in press.
10. Russo E, Citraro R, Davoli A, Gallelli L, Di Paola ED, De Sarro G. Ameliorating effects of aripiprazole on cognitive functions and depressive-like behavior in a genetic rat model of absence epilepsy and mild-depression comorbidity. Neuropharmacology. 2013;64:371-379.
11. Nagai T, Murai R, Matsui K, Kamei H, Noda Y, Furukawa H, Nabeshima T. Aripiprazole ameliorates phencyclidine-induced impairment of recognition memory through dopamine D1 and serotonin 5-HT1A receptors. Psychopharmacology. 2009;202:315-328.
12. Nowakowska E, Kus K, Ratajczak P, Cichocki M, Wozniak A. The influence of aripiprazole, olanzapine and enriched environment on depressant-like behavior, spatial memory dysfunction and hippocampal level of BDNF in prenatally stressed rats. Pharmacological reports : PR. 2014;66:404-411.
13. Li Z, Ichikawa J, Dai J, Meltzer HY. Aripiprazole, a novel antipsychotic drug, preferentially increases dopamine release in the prefrontal cortex and hippocampus in rat brain. European journal of pharmacology. 2004;493:75-83.
14. Yoneyama M, Hasebe S, Kawamoto N, Shiba T, Yamaguchi T, Kikuta M, Shuto M, Ogita K. Beneficial in vivo effect of aripiprazole on neuronal regeneration following neuronal loss in the dentate gyrus: evaluation using a mouse model of trimethyltin-induced neuronal loss/self-repair in the dentate gyrus. Journal of pharmacological sciences. 2014;124:99-111.
15. Bervoets C, Morrens M, Vansteelandt K, Kok F, de Patoul A, Halkin V, Pitsi D, Constant E, Peuskens J, Sabbe B. Effect of aripiprazole on verbal memory and fluency in schizophrenic patients : results from the ESCAPE study. CNS drugs. 2012;26:975-982.
16. Kern RS, Green MF, Cornblatt BA, Owen JR, McQuade RD, Carson WH, Ali M, Marcus R. The neurocognitive effects of aripiprazole: an open-label comparison with olanzapine. Psychopharmacology. 2006;187:312-320.
17. Riedel M, Spellmann I, Schennach-Wolff R, Musil R, Dehning S, Cerovecki A, Opgen-Rhein M, Matz J, Seemuller F, Obermeier M, Severus E, Engel RR, Muller N, Moller HJ. Effect of aripiprazole on cognition in the treatment of patients with schizophrenia. Pharmacopsychiatry. 2010;43:50-57.
18. Schlagenhauf F, Dinges M, Beck A, Wustenberg T, Friedel E, Dembler T, Sarkar R, Wrase J, Gallinat J, Juckel G, Heinz A. Switching schizophrenia patients from typical neuroleptics to aripiprazole: effects on working memory dependent functional activation. Schizophrenia research. 2010;118:189-200.
19. Van Leemput K, Bakkour A, Benner T, Wiggins G, Wald LL, Augustinack J, Dickerson BC, Golland P, Fischl B. Automated segmentation of hippocampal subfields from ultra-high resolution in vivo MRI. Hippocampus. 2009;19:549-557.
20. Reuter M, Fischl B. Avoiding asymmetry-induced bias in longitudinal image processing. NeuroImage. 2011;57:19-21.

21. Reuter M, Schmansky NJ, Rosas HD, Fischl B. Within-subject template estimation for unbiased longitudinal image analysis. NeuroImage. 2012;61:1402-1418.
22. Fu DJ, Bossie CA, Sliwa JK, Ma YW, Alphs L. Paliperidone palmitate versus oral risperidone and risperidone long-acting injection in patients with recently diagnosed schizophrenia: a tolerability and efficacy comparison. International clinical psychopharmacology. 2014;29:45-55.
23. Woods SW. Chlorpromazine equivalent doses for the newer atypical antipsychotics. The Journal of clinical psychiatry. 2003;64:663-667.
24. Woods SW: Chlorpromazine Equivalent Doses for Atypical Antipsychotics: An Update 2003-2010. 2011. pp. 1-8.
25. Cassidy CM, Rabinovitch M, Schmitz N, Joober R, Malla A. A comparison study of multiple measures of adherence to antipsychotic medication in first-episode psychosis. Journal of clinical psychopharmacology. 2010;30:64-67.
26. Hollingshead A: Two-Factor Index of Social Position. New Haven, CN, Yale University Press; 1965.
27. Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia. 1971;9:97-113.
28. Andreasen NC: Scale for the Assessment of Positive Symptoms (SAPS). Iowa City, University of Iowa; 1984.
29. Andreasen NC, Carpenter WT, Jr., Kane JM, Lasser RA, Marder SR, Weinberger DR. Remission in schizophrenia: proposed criteria and rationale for consensus. Am J Psychiatry. 2005;162:441-449.
30. Wechsler D: Weschler Memory Scale - 3rd Edition. New York, The Psychological Corporation; 1997.
31. Maruff P, Thomas E, Cysique L, Brew B, Collie A, Snyder P, Pietrzak RH. Validity of the CogState brief battery: relationship to standardized tests and sensitivity to cognitive impairment in mild traumatic brain injury, schizophrenia, and AIDS dementia complex. Archives of clinical neuropsychology : the official journal of the National Academy of Neuropsychologists. 2009;24:165-178.
32. Wechsler D: Wecshler Adult Intelligence Scale - 3rd Edition. San Antonio, TX, The Psychological Corporation; 1997.
33. Wechsler D: Wechsler Abbreviated Scale of Intelligence. San Antonio, TX, The Psychological Corporation; 1999.
34. Rubin DB. Estimating causal effects from large data sets using propensity scores. Annals of internal medicine. 1997;127:757-763.
35. Bodnar M, Malla A, Joober R, Lepage M. Cognitive markers of short-term clinical outcome in first-episode psychosis. The British journal of psychiatry : the journal of mental science. 2008;193:297-304.
36. Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. Cell. 2003;112:257-269.
37. Park SW, Lee JG, Ha EK, Choi SM, Cho HY, Seo MK, Kim YH. Differential effects of aripiprazole and haloperidol on BDNF-mediated signal changes in SH-SY5Y cells. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology. 2009;19:356-362.

38. Binder DK, Scharfman HE. Brain-derived neurotrophic factor. Growth factors. 2004;22:123-131.
39. Spalding KL, Bergmann O, Alkass K, Bernard S, Salehpour M, Huttner HB, Bostrom E, Westerlund I, Vial C, Buchholz BA, Possnert G, Mash DC, Druid H, Frisen J. Dynamics of hippocampal neurogenesis in adult humans. Cell. 2013;153:1219-1227.
40. Deng W, Aimone JB, Gage FH. New neurons and new memories: how does adult hippocampal neurogenesis affect learning and memory? Nature reviews Neuroscience. 2010;11:339-350.

Supplementary Material for Article 4.

Table S1. Medication Information for each FEP Client from Entry until Scan2. Groupings based on interscan interval data.

	Entry to Scan1			Interscan Interval (Scan1 to Scan2)					
Identifier	Time1	Medication Taken [AP:Months (Adh %)]	CPZ1 Total	Time2	Medication Taken [AP:Months (Adh %)]	CPZ2 Total	CPZ2 Avg	2nd AP %.	AD or Mood
Risperidone									
1 (13)	5.9	Risp:5.9 (100)	978	13.9	Risp:13.9 (94)	899	64.6	--	--
2 (24)	1.7	Risp:1.7 (100)	121	14.5	Risp: 14.5 (95)	1204	82.9	--	--
3 (27)	6.9	Risp:6.9 (90)	891	15.0	Risp:2.1 (50); Quet:3.0 (75); RLAI:6.0 (50); Unkn:3.9	1421	94.9	37%	--
4 (38)	2.9	Risp:2.9 (92)	298	12.2	Risp:12.2 (57)	469	38.4	--	--
5 (40)	2.1	Risp:2.1 (100)	160	12.9	Risp:0.9 (100); Risp/Olan:3.0 (100); RLAI:9.0 (92)	1714	133.1	23%	--
6 (41)	6.3	Risp:6.3 (100)	843	11.3	Risp:11.3 (100)	563	50.0	--	--
7 (44)	2.4	Olan:2.4 (42)	143	12.6	Olan:0.6 (50); NT:3.0; Risp:9.0 (100)	946	75.0	--	AD
8 (46)	3.4	Olan:1.0 (50); NP:2.4	75	14.2	NP:3.6; Olan:3.0 (50); RLAI:7.6 (100)	2223	156.3	39%	--
9 (56)	2.2	Risp:2.2 (100)	220	14.6	Risp:9.8 (64); NT:4.8	502	34.3	--	--
10 (58)	5.5	Risp:5.5 (88)	676	14.3	Risp:6.5 (96); RLAI:7.8 (100)	2419	169.7	--	--
11 (59)	2.3	Risp:2.3 (100)	150	14.7	Risp:14.7 (100)	1380	93.8	--	--
12 (62)	4.4	Risp/RLAI:4.5 (100)	654	17.0	RLAI:1.6 (100); RLAI/Zipr:3.0 (100); RLAI:9.0 (100); NT:3.4	1191	70.0	22%	AD
13 (69)	4.0	Olan:2.0 (50); NP:2.0	225	12.8	NP:5.0; NT:3.0; RLAI:5.8 (100)	541	42.5	--	--
14 (80)	3.9	Risp:3.9 (94)	856	14.7	Risp:2.1 (75); RLAI:12.0 (75); NT:0.6	868	59.1	--	--
15 (89)	3.1	Risp/RLAI:3.1 (100)	649	15.5	R-LAI:8.9 (75); NT:6.6	1984	127.7	--	--
16 (90)	7.9	Zipr:2.0 (100); Risp:5.9 (83)	387	14.6	Risp:14.6 (98)	360	24.7	--	--
Paliperidone									
1 (113)	6.3	NP:1.0; PLAI:5.3 (100)	943	12.3	PLAI:12.3 (100)	2188	178.5	--	--
2 (114)	1.7	NT:1.0; PLAI:0.7 (100)	169	13.3	PLAI:13.3 (100)	1622	121.7	--	--
3 (140)	2.3	NT:1.0; PLAI:1.3 (100)	274	12.2	PLAI:12.2 (100)	1455	119.0	--	--
4 (142)	2.9	Palp:2.9 (100)	572	12.2	Palp:12.2 (100)	2444	200.0	--	AD
5 (143)	6.1	Arip:6.0 (100); PLAI:0.1 (100)	1145	13.8	PLAI:13.8 (100)	3274	237.8	--	
Olanzapine									
1 (07)	8.0	Olan:8.0 (94)	2100	12.6	Olan:12.6 (100)	2569	204.1	--	--
2 (16)	1.7	Olan:1.7 (100)	534	14.7	Olan:14.7 (100)	3320	226.6	--	AD
3 (18)	1.2	Olan:1.2 (100)	327	11.8	Olan:11.8 (89)	1261	107.2	--	--
4 (25)	1.7	Risp:1.7 (100)	221	12.0	Risp:0.3 (100); Olan:10.0 (100); Olan/Quet:1.7 (100)	3956	329.9	--	--

5 (33)	4.9	Olan:4.9 (100)	393	11.1	Olan:11.1 (100)	479	43.2	--	--
6 (37)	5.1	Risp:1.0 (100); Olan:4.1 (100)	940	12.3	Olan:12.3 (100)	3859	313.2	--	--
7 (42)	2.1	Olan:2.1 (100)	328	12.4	Olan:12.4 (91)	1699	137.1	--	AD
8 (65)	1.9	Olan:1.9 (100)	144	14.5	Olan:10.1 (100); NP:3.4	444	30.7	--	Mood
9 (66)	2.4	Olan:2.4 (58)	190	14.9	Olan:0.6 (25); NT:3.0; Olan:6.0 (100); Unkn: 5.3	898	60.4	--	--
10 (68)	3.4	Olan:3.4 (100)	585	15.2	Olan:14.6 (100); NP:0.6	1215	80.2	--	Mood
11 (79)	3.0	Olan:3.0 (100)	1147	13.0	Olan: 13.0 (100)	2399	184.9	--	AD
12 (103)	6.7	Olan:6.7 (100)	2435	11.8	Olan:11.3 (100); PLAI:0.5 (75)	2764	234.3	--	AD
Aripiprazole									
1 (95)	2.1	Arip:2.1 (100)	302	12.5	Arip:12.5 (100)	852	68.4	--	--
2 (104)	7.5	Arip/Olan:7.5 (100)	3241	11.0	Arip/Olan:3.5 (100); Arip:6.5 (100)	4494	407.1	35%	--
3 (105)	5.1	Arip:5.1 (100)	811	14.6	Arip:12.9 (63); NP:1.7	1084	74.1	--	--
4 (108)	4.4	Risp/Arip:3.0 (92); Arip:1.4 (75)	505	12.2	Arip:12.2 (87)	1411	116.1	--	--
5 (112)	3.6	Olan:1.0 (100); Arip:2.6 (100)	1003	12.1	Arip:12.1 (100)	1047	86.3	--	--
6 (116)	3.3	Olan:1.0 (100); Arip:2.3 (100)	709	12.2	Arip:5.7 (100); NT:3.0; Arip:6.5 (79)	1249	102.5	--	--
7 (117)	1.3	Arip:1.3 (100)	85	15.0	Arip:7.7 (72); NT:3.0; Arip:4.3 (50)	504	33.7	--	AD
8 (123)	1.9	Arip:1.9 (100)	188	13.2	Arip:13.2 (100)	1629	123.6	--	AD
9 (124)	4.0	NP:1.0; Arip:3.0 (100)	356	13.0	Arip:5.0 (100); Unkn:8.0	428	32.9	--	--
10 (127)	3.4	Risp/Arip:3.0 (100); Arip:0.4 (100)	820	12.1	Arip:12.1 (100)	3787	314.1	--	--
11 (129)	5.4	Arip:5.4 (67)	697	10.4	Arip:10.4 (96)	2054	198.5	--	--
12 (133)	5.8	NT:1.0; Arip:4.8 (100)	636	13.6	Arip:12.2 (75); Palp:1.4 (100)	7830	577.1	--	--
13 (137)	2.9	Arip:2.9 (100)	350	12.6	Arip:12.6 (100)	504	39.9	--	AD
14 (139)	3.6	Arip:3.6 (100)	316	14.0	Arip:14.0 (100)	1246	66.5	--	--
15 (141)	6.2	Arip:6.2 (100)	1025	12.2	Arip:12.2 (100)	1625	133.0	--	--
16 (149)	5.1	Arip:5.1 (100)	677	12.3	Arip:12.3 (100)	1275	104.0	--	--
Mix									
1 (15)	6.8	Olan:4.6 (100); NT:2.2	1225	15.1	NT:2.2; Risp:3.0 (100); RLAI:6.0 (100); Cloz:3.9 (100)	1801	119.2	43%	AD
2 (34)	2.6	Quet:2.6 (100)	822	12.0	Quet:6.4 (57); RLAI:5.6 (100)	2264	189.4	88%	--
3 (39)	7.5	NT:1.0; Olan:5.0 (100); Risp:1.5 (50)	2073	13.5	Risp:4.5 (45); Quet/Risp:9.0 (100)	5234	387.6	67%	AD
4 (50)	2.3	NP:1.0; Risp:1.3 (88)	87	12.0	Risp:3.7 (100); Quet:8.3 (92)	8230	711.3	46%	AD
5 (70)	6.0	Olan:6.0 (100)	745	12.9	Olan:3.0 (75); Risp/Olan:3.0 (100); Risp:6.9 (96)	2894	225.3	61%	--

6 (72)	3.6	Olan:3.6 (100)	1065	13.3	Olan:2.4 (100); Quet:6.0 (100); Quet/Risp:4.9 (100)	4926	369.3	67%	--
7 (73)	6.7	Palp:6.7 (90)	1290	13.6	Palp:5.3 (75); Olan:8.3 (75)	1894	139.9	64%	AD
8 (78)	3.5	Olan:3.5 (78)	704	11.7	Olan:2.5 (75); Olan/Zipr:3.0 (50); NP:6.2	473	40.4	55%	AD
9 (110)	2.1	Arip:1.0 (100); PLAI:1.1 (88)	355	13.8	PLAI:0.9 (100); NT:6.0; Arip/RLAI:3.0 (100); Arip/PLAI:3.9 (100)	2869	208.4	88%	--
10 (111)	5.9	Arip:5.9 (100)	1263	13.1	Arip:6.1 (35); PLAI:6.0 (100); NT:1.0	1692	129.0	98%	--
11 (118)	4.1	NT:1.0; Palp/PLAI:3.1 (100)	1362	12.8	PLAI:7.9 (100); PLAI/Arip:4.9 (100)	7925	620.1	38%	--
12 (122)	4.4	Palp:2.0 (100); PLAI/Olan:2.4 (100)	2732	11.8	PLAI/Olan:1.6 (100); Cloz:3.0 (100); Cloz/PLAI:7.2 (100)	6944	587.1	71%	--
13 (130)	6.7	Olan:6.0 (100); PLAI:0.7 (75)	2032	12.0	PLAI:2.3 (75); NT:3.0; PLAI/Quet:6.7 (56)	8495	710.3	74%	--
14 (134)	1.8	NP:1.8	0	11.9	NP:0.2; Asen:4.0 (50); Asen/Arip:7.7 (66)	1056	89.0	66%	AD
Refusal (Not Taking/Refusing)									
1 (17)	2.0	Risp:2.0 (100)	141	11.6	NT:7.0; NP:4.5	0	0.0	--	--
2 (20)	3.6	Olan:3.6 (94)	933	16.1	Olan:2.4 (75); NP:12.0; Olan:1.7 (100)	851	53.0	--	Both
3 (30)	4.5	NP:4.5	0	12.9	NP:12.9	0	0.0	--	--
4 (32)	5.1	Olan:5.1 (100)	1259	12.2	Olan:3.9 (100); NP:8.3	241	19.8	--	Mood
5 (63)	6.0	Risp:3.0 (100); NT:3.0	425	13.2	Risp:3.0 (75); NT:3.0; Palp:7.2 (34)	1269	95.4	42%	--
6 (64)	2.4	Palp:2.0 (88); Olan:0.4 (50)	372	14.3	Olan:14.3 (42)	746	52.3	--	--
7 (93)	2.2	Arip:2.0 (100); NT:0.2	133	11.7	NT:11.7	0	0.0	--	--
8 (107)	3.5	Arip:2.0 (88); NT:1.5	77	12.4	NT:2.5; NP:3.0; Quet:3.0 (100); NP:3.9	1330	107.1	--	--
9 (115)	1.7	NT:1.0; PLAI:0.7 (75)	132	12.5	P-LAI:4.3 (50); NT:6.0; Arip:2.2 (25)	478	38.2	--	--
10 (120)	5.1	NP:5.1	0	11.8	NP:11.8	0	0.0	--	--
11 (121)	2.6	Arip:2.6 (67)	133	14.4	NT:0.4; NP:6.0; Unkn:8.0	0	0.0	--	--
12 (125)	5.5	Arip:5.5 (75)	336	13.6	Arip:6.5 (39); NP:7.1	166	12.2	--	--
13 (132)	4.2	Arip:4.2 (88)	707	14.5	Arip:1.8 (100); NP:3.0; Arip:3.0 (50); NP:6.0; Arip:0.7 (25)	446	30.8	--	--
Asenapine									
1 (138)	3.8	Arip:1.0 (100); Asen:2.0 (25); NP:0.8	316	12.6	NP:5.2; Asen:7.4 (50)	372	29.5	--	--
Haloperidol									
1 (08)	2.9	Olan:1.0 (100); Halo:1.9 (100)	865	19.9	Halo:19.9 (80)	1375	69.2	--	--
Ziprasidone									
1 (74)	1.8	Olan:1.8 (100)	724	14.6	Olan:0.2 (100); Zipr:14.4 (100)	3394	232.2	--	--
2 (99)	2.7	Arip/Olan:2.0 (100); Olan/Zipr:0.7 (100)	410	13.8	Olan/Zipr:0.3 (100); Zipr:13.5 (92)	856	62.0	--	AD
3 (100)	5.7	Olan:1.0 (100); Arip:2.0 (100); Quet:2.7 (100)	1464	14.1	Quet:0.3 (100); Zipr:12.0 (88); Palp:1.8 (75)	1053	74.6	--	Mood

Quetiapine									
1 (11)	6.8	NP:1.0; NT:2.0; Quet:3.8 (75)	1051	12.4	Quet:12.4 (94)	4544	365.9	--	--
2 (43)	3.4	Quet:3.4 (100)	2135	12.4	Quet:8.6 (92); Quet/Olan:3.8 (100)	6394	514.8	31%	--
3 (51)	1.9	Quet:1.9 (100)	1368	13.5	Quet:10.1 (93); Olan:3.4 (50)	5443	403.1	34%	AD
4 (75)	7.6	NT:1.0; Zipr:6.6 (100)	953	13.5	Zipr:1.4 (100); Quet:12.1 (100)	6292	467.1	--	--
Removed									
1 (28)	1.9	Risp:1.9 (100)	156	11.5	Risp:11.5 (85)	622	54.2	--	--
2 (49)	4.7	Olan:4.7 (100)	2600	12.0	Olan:4.3 (65); NT:3.0; Olan/Risp:4.7 (75)	2280	190.2	52%	--
3 (94)	6.9	Risp/Cloz:3.0 (100); Cloz:3.9 (100)	2265	14.6	Cloz:2.1 (100); Unkn:9.0; Arip:3.5 (100)	963	66.0	--	--
4 (98)	2.8	Olan:2.8 (100)	719	12.9	Olan:0.2 (100); Quet:3.0 (100); NP:9.7	1229	94.9	--	Mood
5 (135)	5.6	PLAI:5.6 (100)	1004	14.6	PLAI:14.6 (100)	2611	178.6	--	--

Abbreviations: Risp – Risperidone; Olan – Olanzapine; Cloz – Clozapine; Quet – Quetiapine; Halo – Haloperidol; Zipr – Ziprasidone; Palp – Paliperidone; Arip – Aripiprazole; Asen – Asenapine; RLAI – Risperidone Long-Acting Injectable; PLAI – Paliperidone Long-Acting Injectable; CPZ – Chlorpromazine; Adh – Medication Adherence; NP – Not Prescribed; NT – Not Taking (0% adherence); Unkn – Unknown; AD – anti-depressant; Mood – mood stabilizer.

Time1 – months from entry until Scan1; **Time2** – months from Scan1 until Scan2; **CPZ1 Total** – total antipsychotic dosage in CPZ equivalents (mg/day) from Entry until Scan1, accounting for Adh; **CPZ2 Total** – same as CPZ1 Total except from Scan1 until Scan2; **2ndAP** – percent of time taking a secondary antipsychotic in comparison to the primary antipsychotic; **CPZ2 Avg.** – CPZ equivalents (mg/day) per month during intercan interval, accounting for Adh.

Medication Taken – presented as "**antipsychotic prescribed: number of months (medication adherence in percent)**". Not Prescribed **(NP)** is when a client was not prescribed any antipsychotic in agreement with treating team and not considered a refusal of medication. Not taking **(NT)** is when a client outright refuses (0% adherence). Unknown **(Unkn)** is where medication information (type and months) was not available (due to missed appointments or no longer followed). Information in red indicates refusal of treatment (adherence less than 50%). For a better clarity of antipsychotic polypharmacy or switching, clients who took a different antipsychotic for 3 or more consecutive months during the interscan interval are highlighted in blue.

Cases requiring further explanation (highlighted in yellow).

Risperidone	
3 (27)	No clinical follow-up at month 18 or after but returned for Scan2; client took RLAI for 6 months (50% adh) prior to follow-up ending.
Olanzapine	
9 (66)	No clinical follow-up at month 12 or after but returned for Scan2; client took olanzapine for 6 months (100% adh) prior to follow-up ending.
Aripiprazole	
9 (124)	No clinical follow-up at month 9 or after but returned for Scan2; client took aripiprazole for 5 months (100% adh) prior to follow-up ending.
None (Not Taking/Refusing)	
7 (121)	No clinical follow-up at month 9 or after but returned for Scan2; client stopped AP treatment 6 months prior to ending clinical follow-up.
Removed	
1 (28)	Was in "Risp" subgroup. Error in processing MRI data; extreme changes in hippocampal values.
2 (49)	Was in "Mix" subgourp. Error in processing MRI data; extreme changes in hippocampal values.
3 (94)	Key clinical data was missing as client missed appointments at month 6 and month9; could not determine subgroup.
4 (98)	Was in "None" subgroup. Error in processing MRI data; extreme changes in hippocampal values.
5 (135)	Was in "Palp" subgroup. Error in processing MRI data; extreme changes in hippocampal values.

Table S2. Hippocampal Volumes at Scan1 and Scan2 for Patient Subgroups and Controls. Includes Mix subgroup data with patients taking Aripiprazole removed *

Time_Region	Group	N	M	SD	Minimum	Maximum
SCAN1_CA1	Refusal	13	661.5985	109.73997	499.42	904.67
	Rsip/Palp	21	671.6481	82.70358	506.11	849.55
	Olan	12	674.6358	76.70515	547.93	794.70
	Arip	16	676.9181	75.94636	538.18	792.67
	Mix	14	633.3721	52.30057	532.64	743.07
	*Mix: no Arip**	*10*	*642.1000*	*58.01986*	*532.64*	*743.07*
	Control	46	651.3715	67.93241	493.78	806.29
	Total	122	659.5247	76.01286	493.78	904.67
SCAN2_CA1	Refusal	13	667.2123	112.79203	518.17	924.56
	Rsip/Palp	21	671.3376	81.57335	520.72	862.96
	Olan	12	664.6375	67.34162	570.80	743.21
	Arip	16	690.9331	55.89575	577.60	782.58
	Mix	14	624.5636	59.30397	527.95	771.21
	*Mix: no Arip**	*10*	*628.0310*	*68.08134*	*527.95*	*771.21*
	Control	46	654.8791	70.35509	514.26	837.29
	Total	122	661.2357	75.43289	514.26	924.56
SCAN1_CA2.3	Refusal	13	2029.5423	299.26547	1579.12	2734.08
	Rsip/Palp	21	2066.0048	291.67668	1609.39	2839.01
	Olan	12	2041.4408	260.72241	1637.20	2446.94
	Arip	16	2111.0350	233.75951	1675.29	2397.01
	Mix	14	1925.3879	212.14891	1321.73	2148.53
	*Mix: no Arip**	*10*	*1927.1490*	*240.08517*	*1321.73*	*2148.53*
	Control	46	1988.1478	187.19593	1649.42	2606.55
	Total	122	2020.1166	238.12878	1321.73	2839.01
SCAN2_CA2.3	Refusal	13	2017.5015	295.76527	1611.69	2711.93
	Rsip/Palp	21	2064.4871	260.30984	1590.71	2791.83
	Olan	12	2043.8225	238.93647	1665.35	2377.24
	Arip	16	2134.7094	232.82304	1691.70	2444.32
	Mix	14	1904.1086	217.61625	1364.39	2278.71
	*Mix: no Arip**	*10*	*1889.1950*	*224.48979*	*1364.39*	*2171.03*
	Control	46	1988.2617	195.78937	1623.59	2608.95
	Total	122	2019.5125	234.96111	1364.39	2791.83
SCAN1_CA4.DG	Refusal	13	1134.4300	163.89184	887.14	1499.57
	Rsip/Palp	21	1156.7967	151.35187	905.99	1556.50
	Olan	12	1137.7083	140.54789	927.91	1360.57
	Arip	16	1165.6019	122.32291	932.85	1316.78
	Mix	14	1059.8843	115.98948	757.66	1269.71
	*Mix: no Arip**	*10*	*1049.9180*	*118.11354*	*757.66*	*1165.42*

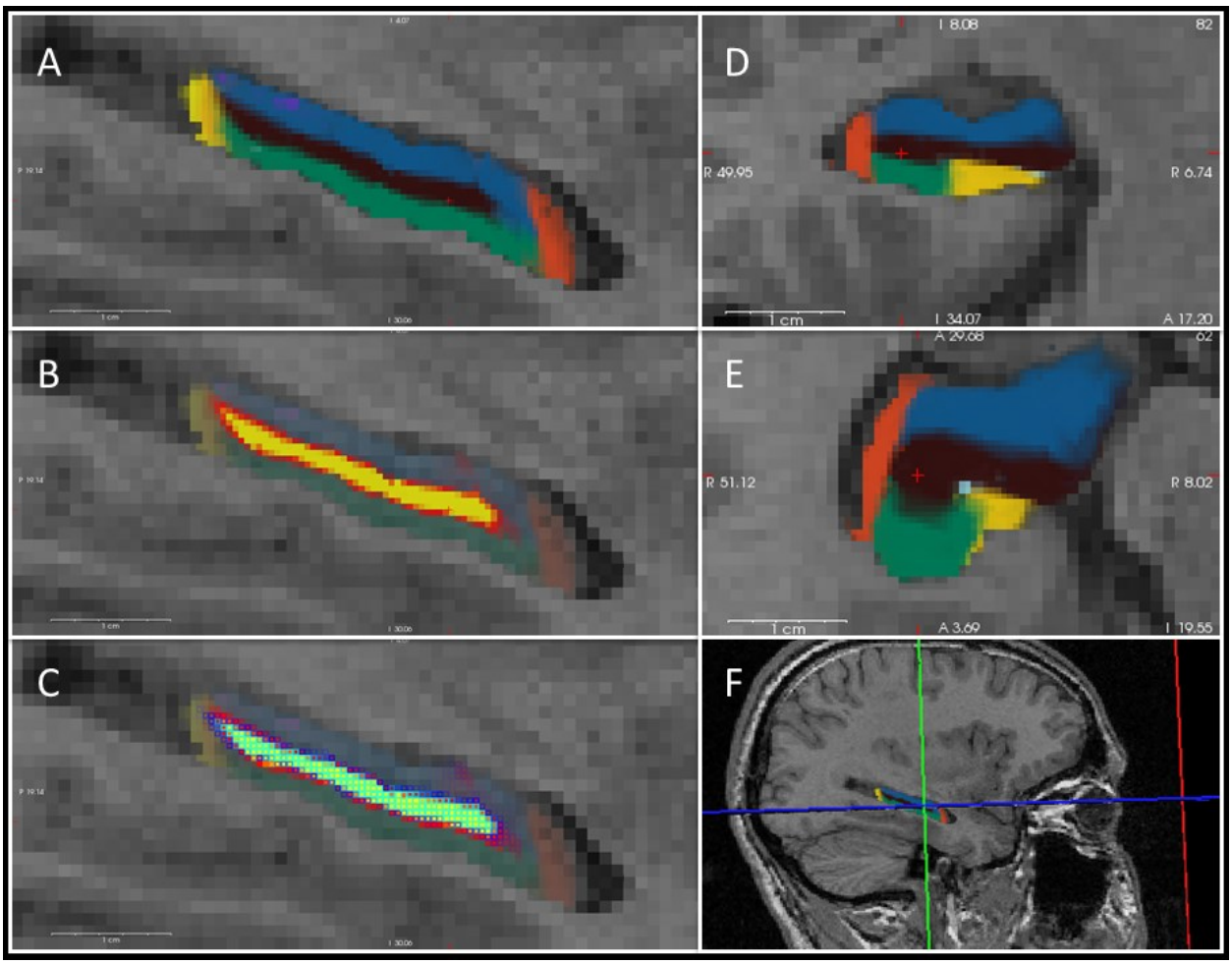
	Control	46	1122.8389	108.26852	920.48	1443.27
	Total	122	1129.7657	129.59264	757.66	1556.50
SCAN2_CA4.DG	Refusal	13	1124.6308	165.84630	889.92	1500.13
	Rsip/Palp	21	1149.3690	145.78969	896.52	1539.69
	Olan	12	1132.1175	138.50328	903.92	1329.64
	Arip	16	1190.8319	114.88465	977.16	1338.55
	Mix	14	1068.9743	123.44470	794.55	1341.95
	*Mix: no Arip**	*10*	*1048.4470*	*113.40162*	*794.55*	*1177.55*
	Control	46	1116.1728	112.84392	893.80	1473.97
	Total	122	1128.7316	130.59042	794.55	1539.69
SCAN1_Presubiculm	Refusal	13	1004.9985	157.49929	748.26	1277.42
	Rsip/Palp	21	1042.8662	105.63766	836.83	1248.21
	Olan	12	949.6850	107.49854	786.34	1109.24
	Arip	16	1056.7819	132.82252	872.17	1351.75
	Mix	14	958.6421	79.63030	856.25	1095.77
	*Mix: no Arip**	*10*	*970.1480*	*87.60483*	*863.18*	*1095.77*
	Control	46	1024.0224	125.83738	754.01	1307.05
	Total	122	1014.7207	123.73474	748.26	1351.75
SCAN2_Presubiculum	Refusal	13	1000.1362	155.52415	776.13	1293.44
	Rsip/Palp	21	1032.5067	114.76576	837.13	1284.25
	Olan	12	946.4492	123.11085	756.74	1132.34
	Arip	16	1067.7844	118.53565	892.34	1350.70
	Mix	14	956.6186	66.63569	876.36	1059.28
	*Mix: no Arip**	*10*	*963.6560*	*71.70093*	*876.36*	*1059.28*
	Control	46	1020.4763	126.66398	781.79	1328.10
	Total	122	1011.9748	124.28667	756.74	1350.70
SCAN1_Subiculum	Refusal	13	1328.4662	198.33308	955.06	1637.11
	Rsip/Palp	21	1344.9567	145.18896	1135.91	1628.47
	Olan	12	1301.3133	134.69826	1083.25	1531.44
	Arip	16	1405.7219	145.42225	1142.31	1681.92
	Mix	14	1236.3136	95.24101	1023.35	1405.66
	*Mix: no Arip**	*10*	*1225.6970*	*101.19175*	*1023.35*	*1405.66*
	Control	46	1328.7267	147.87342	1071.72	1623.51
	Total	122	1328.2892	150.51648	955.06	1681.92
SCAN2_Subiculum	Refusal	13	1325.3608	202.61604	974.75	1695.24
	Rsip/Palp	21	1341.2338	153.92219	1111.90	1670.77
	Olan	12	1296.4125	141.03627	1056.37	1486.60
	Arip	16	1429.0844	114.57799	1238.75	1708.60
	Mix	14	1226.9429	102.00945	1044.43	1375.89
	*Mix: no Arip**	*10*	*1205.7680*	*106.22531*	*1044.43*	*1375.89*
	Control	46	1325.4448	145.45289	1075.33	1633.14

	Total	122	1327.5866	151.90287	974.75	1708.60
SCAN1_Tail	Refusal	13	770.3815	120.1084	523.42	968.07
	Rsip/Palp	21	813.0204	130.6135	622.78	1203.61
	Olan	12	724.2625	68.5677	601.46	819.96
	Arip	16	768.1925	86.7745	619.07	919.24
	Mix	14	727.7657	79.8286	603.26	846.64
	*Mix: no Arip**	*10*	*723.2740*	*85.7943*	*603.26*	*846.64*
	Control	46	759.5989	95.9599	581.25	1004.73
	Total	122	763.9417	102.2752	523.42	1203.61
SCAN2_Tail	Refusal	13	765.7338	127.0665	502.36	973.26
	Rsip/Palp	21	818.7681	116.2664	640.92	1137.90
	Olan	12	733.4300	68.9026	625.47	834.81
	Arip	16	769.2531	85.7534	593.56	937.24
	Mix	14	716.0957	85.1934	579.57	876.46
	*Mix: no Arip**	*10*	*709.0420*	*91.5939*	*579.57*	*876.46*
	Control	46	756.7450	96.9596	575.81	998.36
	Total	122	763.0614	101.7682	502.36	1137.90
SCAN1_WholeHippo	Refusal	13	6159.0331	860.89474	4669.79	8052.85
	Rsip/Palp	21	6282.2705	710.67884	5134.86	8046.00
	Olan	12	6104.7758	630.22358	5162.97	6910.26
	Arip	16	6416.0563	591.09665	5327.52	7365.74
	Mix	14	5813.5986	470.23041	4538.65	6542.68
	*Mix: no Arip**	*10*	*5815.0110*	*538.69952*	*4538.65*	*6542.68*
	Control	46	6115.1043	570.50309	5134.98	7658.39
	Total	122	6152.4139	638.25006	4538.65	8052.85
SCAN2_WholeHippo	Refusal	13	6134.8354	867.71213	4809.27	8125.29
	Rsip/Palp	21	6258.9333	689.87871	5044.56	8091.46
	Olan	12	6083.4367	633.73489	5131.17	6984.31
	Arip	16	6513.3406	519.90741	5586.21	7474.84
	Mix	14	5781.2057	477.79217	4654.97	6518.19
	*Mix: no Arip**	*10*	*5735.0950*	*516.92966*	*4654.97*	*6518.19*
	Control	46	6105.2341	561.48770	5027.29	7672.69
	Total	122	6149.0393	633.72652	4654.97	8125.29

Table S3. Verbal Memory Performance among the FEP Subgroups and Controls separated between the Logical Memory subtest (WMS-III) and Shopping List (CogState). Data presented as z-scores determined from the M and SD of the FEP sample. Effect size (ES) is Cohen's d.

			Change			Baseline		Follow-up	
		N	M	SD	ES	M	SD	M	SD
Refusal	Logical Memory	4	.140	.365	0.06	-.181	1.486	-.041	1.220
	Shopping List	4	-.875	1.130	0.78	1.054	.692	.180	.599
	Total	**8**	**-.367**	**.948**	**0.13**	**.437**	**1.260**	**.069**	**.898**
Risp/Palp	Logical Memory	12	-.051	1.130	0.02	.057	.738	.007	.912
	Shopping List	3	.224	1.081	0.19	-.823	.603	-.599	1.044
	Total	**15**	**.004**	**1.087**	**0.00**	**-.119**	**.783**	**-.114**	**.934**
Olan	Shopping List	6	-.286	.532	0.12	.709	1.094	.423	.971
	Total	**6**	**-.286**	**.532**	**0.12**	**.709**	**1.094**	**.423**	**.971**
Arip	Logical Memory	8	.204	1.047	0.06	-.114	1.437	.090	1.321
	Total	**8**	**.204**	**1.047**	**0.06**	**-.114**	**1.437**	**.090**	**1.321**
Mix	Logical Memory	6	-.095	1.455	0.03	-.450	.912	-.545	1.513
	Shopping List	4	.443	.409	0.37	-1.522	.688	-1.078	.705
	Total	**10**	**.184**	**1.146**	**0.04**	**-.878**	**.962**	**-.758**	**1.231**
Mix: no Arip	*Logical Memory*	*6*	*-.095*	*1.455*	*0.03*	*-.450*	*.912*	*-.545*	*1.514*
	Total	*6*	*-.095*	*1.455*	*0.03*	*-.450*	*.912*	*-.545*	*1.514*

Figure S1 Hippocampal Subfields as Segmented using FreeSurfer v5.3



Panel A, D, and E present the hippocampal subfields in the sagittal, coronal, and transverse orientations, respectively; subfields are colored as: CA1 = Red, CA2/3 = Blue, CA4/DG = Brown, Subiculum = Green, Presubiculum = Dark Yellow, and Hippocampal Tail = Bright Yellow. **Panel B** highlights the CA4/DG at Scan1 in bright yellow and red. **Panel C** superimposes the CA4/DG at Scan2 (in blue lines) over the CA4/DG at Scan1 (bright yellow/red). **Panel F** displays the location of the hippocampus within the brain. Note: the scales represent 1cm.