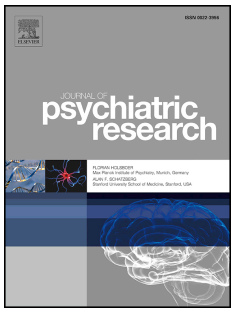

“First-episode psychosis: Structural covariance deficits in salience network correlate with symptoms severity”

The GET UP Group

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Short informative title:

"First-episode Psychosis: structural covariance deficits in salience network correlate with symptoms severity"

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First-episode Psychosis (FEP) → **Psychotic symptoms (PANSS)**

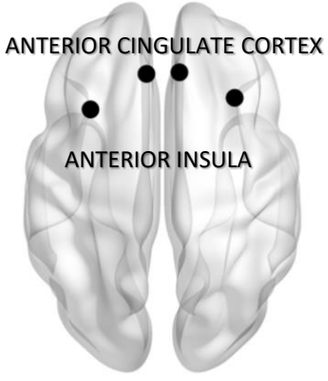
Progressive cortical reorganization

↓

Model of insular dysfunction

↓

Importance of Salience Network nodes



Does gray matter covariance (anatomical MRI) with Salience Network nodes distinguish FEP from healthy controls (HC)?

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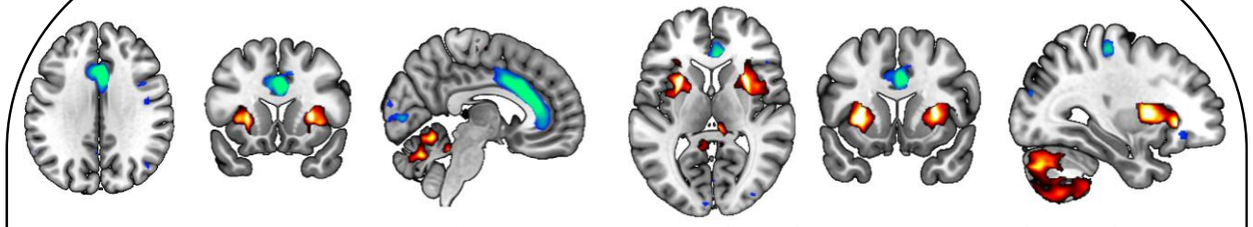
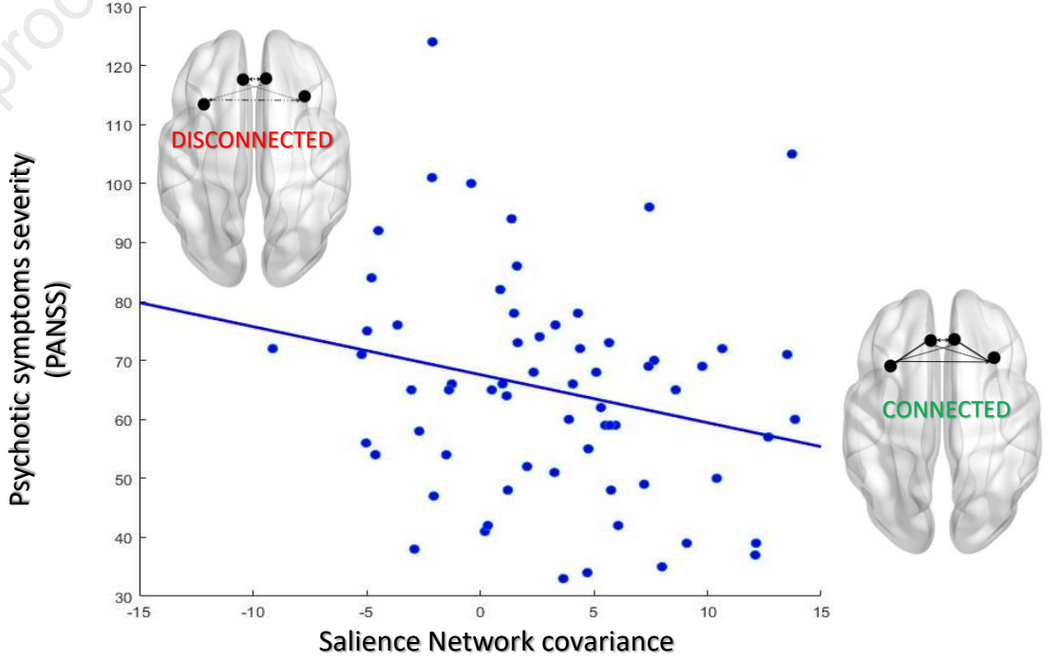


Gray matter structural covariance with Salience Network Nodes
(FEP=67, HC=67)



Yes! And Salience Network covariance is associated with psychotic severity (PANSS).
Potential MRI biomarker to help early detection/diagnosis

ABSTRACT

Background: Patterns of coordinated variations of gray matter (GM) morphology across individuals are promising indicators of disease. However, it remains unclear if they can help characterize first-episode psychosis (FEP) and symptoms' severity.

Methods: Sixty-seven FEP and 67 matched healthy controls (HC) were assessed with structural MRI to evaluate the existence of distributed GM structural covariance patterns associated to brain areas belonging to salience network. Voxel-based morphometry (VBM) and structural covariance differences, investigated with salience network seed-based Partial Least Square, were applied to explore differences between groups. GM density associations with Raven's intelligent quotient (IQ) and Positive and Negative Syndrome Scale (PANSS) scores were investigated.

Results: Univariate VBM results gave trend without significant GM differences across groups. GM and IQ correlated positively in both groups: in FEP, mostly in hippocampus, insula, and fronto-temporal structures, while in HC mostly in amygdala, thalamus and fronto-temporal regions. GM and PANSS scores correlated negatively in FEP, with widespread clusters located in limbic regions. Multivariate analysis showed strong and opposite structural GM covariance with salience network for FEP and HC. Moreover, structural covariance of the salience network in FEP correlated negatively with severity of clinical symptoms.

Conclusion: Our study provides evidence supporting the insular dysfunction model of psychosis. Reduced structural GM covariance of the salience network, with its association to symptom's severity, appears a promising morphometry feature for FEP detection.

KEYWORDS:

Magnetic Resonance Imaging; Neuroimaging; Psychosis; First-Episode; Salience Network; Structural covariance.

Abbreviations:

FEP: First-episode Psychosis

HC: healthy controls

GM: gray matter

SN: salience network

AI: anterior insula

ACC: anterior cingulate cortex

VBM: Voxel-based Morphometry

PLS: partial least square

1. Introduction

The diagnosis of first-episode psychosis (FEP) is challenging and has crucial implications for treatment and prognosis. Non-invasive neuroimaging techniques, such as magnetic resonance imaging (MRI), with the quantitative characterization of anatomical abnormalities, may contribute towards FEP diagnosis and monitoring. This could be of particular relevance at early disease stages, where abnormalities are less affected by chronicity and minimally influenced by exposure to medications.

Multiple neuroanatomical MRI studies support associations between gray matter (GM) atrophy and FEP. Cross-sectional studies comparing FEP relative to healthy controls (HC) show ventricular enlargement and distributed GM reductions mostly in temporal lobes (Delvecchio et al., 2017; Fannon et al., 2000; Segall et al., 2009) and hippocampus (Baglivo et al., 2018; Steen, Mull, Mcclure, Hamer, & Lieberman, 2004). Longitudinal studies, which are more specific to disease stage, further corroborated these results, suggesting a decrease in GM evolving dynamically after the first psychotic episode (Gallardo-Ruiz, Crespo-Facorro, Setién-Suero, & Tordesillas-Gutierrez, 2019; Wood et al., 2001). Indeed, metanalytic results (Vita, De Peri, Deste, & Sacchetti, 2012), highlight how the greater magnitude of GM changes in psychosis is strictly related to early stages of the pathology (FEP).

Starting from the idea that maximal GM reductions occurs at early stages of psychosis, the *"progressive cortical reorganization"* model (Lena Palaniyappan, 2017) proposes psychosis onset to be characterized by distributed cortical reorganization deficits. This reorganization would explain the multidimensional psychopathology of psychosis,

characterized by variety of symptoms affecting cognitive systems supported by several cortical areas. Indeed, from a clinical perspective, psychosis and all schizophrenic spectrum disorders are mainly characterized by three symptom domains: neurocognitive, positive and negative (Addington, Addington, & Maticka-Tyndale, 1991; Andreasen & Olsen, 1982). Overall, (i) both psychotic symptoms and cognitive performance are associated to GM changes/reductions (Brambilla et al., 2013; Dusi et al., 2017; Morgan et al., 2010; Whitford et al., 2005); and (ii) cortical reorganization deficits have been related to higher positive symptom burden (M. Li et al., 2019; Rosa et al., 2015). However, the GM areas or networks that are most vulnerable and show the earliest signs of cortical reorganization in psychosis remain debated.

In this framework, the salience network (SN) has been found to be particularly important (Huang et al., 2019; V. Menon, 2015; Vinod Menon, 2011; L. Palaniyappan, White, & Liddle, 2013; Lena Palaniyappan & Liddle, 2012). The SN is defined as an intrinsic functional brain network characterized by two main hubs (Yeo et al., 2011) anchored in anterior insula (AI) and anterior cingulate cortex (ACC). Different cognitive processes, such as communication, social behavior and self-awareness, are related to SN's activity, as well as integrative functions at sensory, emotional and cognitive level. Indeed, all the above cited mental operations, can underlie the ability of a stimulus to become critical, prominent and extremely outstanding (i.e. salient) respect to the surrounding environment. The salience of an object can be defined as a combination and integration of attentional and volitional/motivational processes competing towards the definition of the most relevant stimulus (V. Menon, 2015). In such circumstances the SN, it thought to be the neural core of the salience processing, guiding the individual through goal-directed behavior.

One of the main features of psychosis is the incorrect classification of salience between internal and external stimuli, which created the idea of neural interference proposed in the model of "*aberrant salience*" in schizophrenia (Kapur, 2003). This model, presents the psychotic brain to be in a persistent hyperdopaminergic state, giving rise to misleading assignment of object's salience, where, recurrently, internal cues of the individual are processed and attended rather than external one's. The neural reflection of this incorrect salience processing seems to be further supported by the critical role of the SN in psychosis, being functionally disconnected with other brain areas or large networks (Lena Palaniyappan & Liddle, 2012; Supekar, Cai, Krishnadas, Palaniyappan, & Menon, 2019). In light of this, the behavioral impairment of aberrant salience processing could disrupt functional and structural neural networking and potentially establish psychotic symptoms from the early phases of the disease. Indeed, this is further corroborated by the found GM volume reductions in each of the SN's hubs, while considered per se, in schizophrenic patients (White, Joseph, Francis, & Liddle, 2010). Nevertheless, the degree to which distributed GM changes related to the SN in a networking framework at early illness stages, such as in FEP, and its relationship with positive symptomatology, is still unknown.

The observed impairment of salience processing in psychosis has been associated to damage in the SN, which shows overall reduced functional brain connectivity (Lena Palaniyappan & Liddle, 2012; Supekar, Cai, Krishnadas, Palaniyappan, & Menon, 2019).). In addition, a critical role of the SN is further supported by brain morphometry studies that found focal GM volume reductions in schizophrenia patients, particularly at each of the SN's hubs (White, Joseph, Francis, & Liddle, 2010). However, several open questions remain. Are the observed focal morphological changes part of an abnormal large-scale brain

microstructure network? If so, are abnormalities in this structural network related to positive symptomatology in early psychosis, such as FEP?

This study sought to address those questions. We hypothesized that, relative to HC, FEP subjects would show an abnormal brain structural covariance network relative to the SN hubs. Moreover, we expect these changes to be widely correlated with severity of the present psychotic symptoms, since the tested population allows the early detection of the pathology and the reduction of other confounds (e.g. pharmacological treatments, chronicity, etc.).

Summarizing, the goal of this study is to investigate if neuroanatomical MRI could help differentiate FEP from HC with respect to: 1) GM density effects ; 2) correlations between GM density and clinical and cognitive variables; and 3) GM density covariation with SN as defined from selected high-confidence seeds (DuPre & Spreng, 2017).

2. Materials and Methods

2.1. Participants and assessment measures

Sixty-seven FEP patients (mean age: 29±9 years; 27 females) and sixty-seven age and gender-matched HC (mean age: 31±8 years; 38 females) participated in this study. However, a trend effect for age difference between the two group (FEP>HC, T-score=-1.70, p-value=0.9) was found. Patients were recruited by the multisite GET UP project (Genetic Endophenotype and Treatment: Understanding early Psychosis), for eligibility criteria (<3 months antipsychotic medications) see Ruggeri *et al.*, 2012; Ruggeri *et al.*, 2015. HC were recruited by the University of Verona in the context of other projects.

The clinical sample consists of 67 patients with an ICD-10 diagnosis of schizophrenia (F20, n=18); schizotypal disorder (F21, n=2); schizoaffective disorders (F25, n=8); delusional disorders (F22, n=10); brief psychotic disorder (F23, n=14); acute and transitory psychotic episode (F23.3, n=1); unspecified psychosis not due to a substance or known physiological condition (F29, n=1); manic episode, severe with psychotic symptoms (F30.2, n=5); major depressive disorder, single episode, severe with psychotic features (F32.3, n=6); unspecified bipolar disorder (F31.9, n=1); bipolar disorder, mixed episode (F31.6, n=1). Duration of Untreated Psychosis (DUP, Table 1) in the FEP group ranged between 0 and 2240 days, with 31.25 % of the sample with DUP < 1 months, 31.25 % DUP < 3 months, 18.75 % DUP < 6 months and 18.75 % DUP > 6 months.

The following cognitive and clinical measures were acquired: i) Progressive Raven's Matrices (Caffarra, Vezzadini, Zonato, Copelli, & Venneri, 2003), a non-verbal set of tests aimed at measuring Intelligence quotient (IQ, both FEP and HC); ii) Positive and Negative Syndrome Scale (PANSS, only FEP) (Kay, Fiszbein, & Opler, 1987) to measure severity of psychotic symptoms.

Additional demographic, clinical and ethical approval information are summarized in Table 1 and in *Supplementary Materials 1.1.*

2.2.Brain structural MRI data acquisition and pre-processing

MRI data were obtained using a Siemens 3.0 T Magnetom Allegra MRI scanner (Neuroradiology Section of AOUI Verona, Italy). Structural T1-weighted 3D MPRAGE images were acquired with the following acquisition parameters: 1×1×1 mm^3, TR=2060 ms, TE=3.93 ms, flip angle 15°. Image pre-processing steps are described in *Supplementary Materials 1.2*.

2.3. Gray matter density differences between FEP and HC

Subject-level local GM density was quantified using Voxel-based Morphometry (VBM), a univariate whole-brain volume analysis method. GM differences across groups were estimated using two-sample t-test (N_{FEP}=67, N_{HC}=67). Voxel-wise multiple linear regression was used to quantify associations between GM density and cognitive profile (N_{FEP}=66, N_{HC}=58) and clinical symptom severity (N_{FEP}=66). These latter analyses were performed on subsets of the original dataset were all the relevant scores were acquired by assuming samples as independent and with unequal variance across groups. Voxels with GM density absolute value above 0.2 were included in analysis, using total intracranial volume, age and gender as nuisance covariates. Statistical thresholds were set to $p < 0.05$ with family wise error (FWE) correction for multiple comparisons.

2.4. Gray matter density covariance with functional SN

A multivariate analysis was used to investigate patterns of voxel-wise distributed GM volume associations with GM volume in nodes of the functional SN. A seed-based partial least square (PLS) method was used due to its advantages for modelling association between brain regional GM volume and multivariate cognitive or clinical factors (Giessing, Fink, Rösler, & Thiel, 2007; Krishnan, Williams, McIntosh, & Abdi, 2011; Menzies et al., 2007). In this study, seed PLS was performed in the attempt to detect differences in the pathological FEP condition (i.e. being our clinical variable) compared to HC.

Briefly, seed PLS shows how GM volume at whole brain level correlates with GM volume of the chosen seeds defining different structural covariance patterns between groups. In our study structural integrity of all the SN nodes were extracted and then

correlated across participants with all other brain voxels which PLS then uses to recognize patterns of correlation (i.e. structural covariance network, R. N. Spreng & Turner, 2013). For this purpose, seed PLS was applied to determine structural GM density covariance between SN seed ROIs and the rest of brain structures, through PLSgui (https://www.rotman-baycrest.on.ca), on pre-processed T1-weighted images (N_{FEP}=67, N_{HC}=67). The following main functional SN nodes (10.5 mm^3 spheres centered in the MNI seed coordinates) were used as seeds for PLS analysis (Figure 1): anterior cingulate cortex (ACC) and anterior insula (AI), using hemispheric spatial coordinates from previous studies (Buckner et al., 2011; DuPre and Spreng, 2017). Then, the between-subject correlation matrix of GM volume between SN seeds and all other voxels in the brain was decomposed into latent variables (*LV*) describing distinctive patterns of structural correlation (e.g. structural covariance). Importantly, using decomposition and resampling techniques, PLS takes into account all voxels simultaneously, avoiding multiple comparison issues (for more details see *Supplementary Materials 1.3*).

3. Results

3.1. Gray matter density differences and associations with cognitive and clinical variables

The VBM analysis showed no significant differences in GM density between FEP and HC (two sample t-test, N_{FEP}=67, N_{HC}=67, FEW correction p<0.05). A few clusters showed decreased GM volume (p <.001, uncorrected), mainly located in fronto-temporal regions with a strong presence in superior-frontal and superior-temporal gyri (Figure S1, Table S2).

Intelligence scores were significantly different between the groups (p <.0001; FEP>HC

T-score=-21.43): FEP: range [75-102], mean $_{IQ}$=94±7 and HC: range [100-128], mean $_{IQ=}$122±8. For FEP, the severity of psychotic symptoms measured by PANSS results in: range [33-124], mean=65±19. The associations between these scores and GM density were investigated separately.

Gray matter density associations with global cognition in FEP and HC

A multiple regression analysis between GM density and Raven's intelligence scores, done separately in each group (66 FEP and 58 HC), showed associations between GM and global cognition. The positive correlation resulted in: (i) supra-threshold clusters in FEP mainly localized in hippocampal, superior frontal and temporal regions, amygdala and para-hippocampal gyrus (Figure S2, panel A; FWE correction at p <.05, Table S3); and (ii) supra-threshold clusters in HC mainly localized in superior medial gyrus and inferior temporal gyrus (Figure S2, panel B; FWE correction at p <.05, Table S4). The distribution of clusters was relatively similar across groups, with subtle differences in terms of cluster peak positions.

Gray matter density associations with symptom severity in FEP

Significant negative correlations were found between GM density and clinical scores of psychosis severity (Figure S3, Table S5) considering global PANSS scores (Figure S3, Panel A), mainly located in thalamus, para-hippocampal gyrus and temporal regions (FWE correction set at p <.05). The correlation analysis was also done separating Positive Syndrome Scale (items from P1 to P7) and Negative Syndrome Scale (items from N1 to N7) scores. GM

correlated with Positive scores in a similar way as it did with global PANSS (cluster peaks: hippocampus, thalamus and insula; Figure S3, panel B, Table S6). No correlation between Negative scores and GM was found (FWE correction for $p < .05$).

3.2. Gray matter density covariance with the functional Salience Network

The PLS framework was used to conduct a multivariate analysis to evaluate if FEP and HC had different structural GM covariance patterns with respect to GM volume in nodes of functional SN. Only one *LV* resulted significant in describing a GM covariance pattern. Figure 2 shows the salience map of the LV (bilateral ACC and AI nodes) in the full group (Fig. 2A, FEP and HC, T =-5.8, p<.01), only FEP patients (Fig. 2B, p=.04) and only HC (Fig. 2C, p=.01). The salience of the significant *LV* is characterized by opposite signs between groups (Fig. 2A), indicating group differences in structural integrity of the SN (Mcintosh & Gonzalez-Lima, 1998).

The group covariance network identifies brain regions where the two groups had significantly different GM volume with respect to the SN seeds. The *LV* (p <.05) reflected structural covariance differences to SN seeds in patients and controls, with FEP exhibiting significantly reduced spatial pattern in right hemisphere of both seeds (rACC and rAI) than other seed regions. Correlation between the *BrainScore* (e.g. how much each subject contributes to SN covariance pattern, see Supplementary Materials) and SN seeds (Fig. 2D) consistently showed, via bootstrap testing and multiple runs, reversed signs and non-overlapping confidence intervals in the two groups, highlighting how structural integrity in each seed significantly differs across groups, besides right ACC in FEP. Indeed, for FEP the most salient region for *LV* is left ACC (r=.42) whereas for HC is right AI (r=.50). FEP's spatial

pattern of positive covariance extended through cingulate cortex to bilateral culmen, inferior parietal lobule and right dorsolateral prefrontal cortex. Negative covariance in FEP was observed in left insula, middle frontal gyrus, inferior temporal lobe and middle temporal area (Table S7). On the other hand, in HC, the spatial pattern of positive covariance extended through bilateral insular cortex to cerebellar structures and negative covariance was observed in bilateral thalamus, left middle temporal gyrus and right cingulate cortex (Table S8).

Structural covariance strength within the functional SN in FEP correlated with psychosis severity as measured by PANSS. We found that the *BrainScore* of the significant LV, representing the SN, was negatively and significantly correlated with PANSS scores, representing occurrence of symptomatology (r=-.25, p=.03, Figure 3 and Table S9, see Supplementary Materials 2.1 section for correlation with PANSS sub-scales). This shows how a higher *BrainScore* (e.g. structural integrity of SN's covariance pattern) is related to lower PANSS symptoms burden, regardless the SN nodes (Figure S4).

4. Discussion

Motivated by searching early neuroanatomical MRI markers of psychosis, the overall goal of this study was to investigate structural GM differences between FEP patients and age- and gender-matched HC. The main findings are as follows: *4.1*) we found no significant GM differences between FEP and HC groups using a univariate VBM analysis; *4.2)* Raven's IQ showed similar patterns of correlations with GM density in both groups, thereby suggesting no disease specificity; *4.3*) in the FEP group, GM density correlated negatively with clinical scores of symptoms severity (PANSS), particularly in limbic system and fronto-temporal

regions; *4.4*) our multivariate analysis showed significant GM covariance between limbic system and SN, with opposite patterns in FEP and HC groups. Specifically, in FEP, SN covariance integrity correlated negatively with symptoms severity.

4.1. FEP and HC do not differ in gray matter density using univariate morphometry analyses

Several single- and meta-studies done mostly with adult subjects have reported significant GM differences between early psychosis patients and matched HC (Baglivo et al., 2018; Fusar-Poli et al., 2011). Other studies, mostly on adolescent populations, have reported no GM differences between early onset FEP and healthy matched controls, both in longitudinal (Castro-Fornieles et al., 2018) and cross-sectional designs (Pagsberg et al., 2007) even if GM abnormalities are considered to be strictly related to the symptomatic development of the pathology rather than its progression (Borgwardt et al., 2007; Satterthwaite et al., 2016). Our study, which includes only adult subjects, found trends of decreased GM in FEP relative to HC, mostly in fronto-temporal regions and in superior orbitofrontal gyrus. However, the GM differences observed did not survive statistical corrections for multiple comparisons. Several reasons may explain why our GM differences were not significant, including smaller sample size than other studies (Fusar-Poli et al., 2011) and brain morphometry methods differences (Baglivo et al., 2018). In fact, previous studies have shown that morphometry pipelines differences can lead to different results even if the same FEP sample is considered (Glahn et al., 2008; Grimm et al., 2015; Hartzell et al., 2016; Voets et al., 2008).

An additional reason for having detected only subtle yet not significant GM differences between FEP and HC may relate to our FEP population, which was scanned

immediately after hospitalization. Since GM loss/reorganization in FEP takes place around 30 months after onset, and not in the first 10 months of disease (Theberge et al., 2007), FEP may have not developed sufficient GM atrophy. Moreover, it is also plausible that since the relatively short DUP ranges of our sample (see Table 1), we failed detecting the acknowledged GM reorganization caused by long untreated psychotic state (Lappin et al., 2006; Malla, Bodnar, Joober, & Lepage, 2011).

4.2. Gray matter density associations with global cognition seem unspecific to FEP

In agreement with previous FEP studies, we found that intelligence, as a global cognitive measure estimated by Raven's IQ, was lower in FEP than in controls (Woodberry et al., 2008) and correlated with GM density in multiple brain structures including hippocampus, thalamus, insula and fronto-temporal regions (Ferro et al., 2015; X. Li et al., 2015; Minatogawa-Chang et al., 2009). The FEP and HC groups showed similar correlations between IQ and GM density, both in space and in strength. This suggests that in our groups and with our experimental design, the GM density associations with IQ may be general (Frangou, Chitins, & Williams, 2004) and not specific to the FEP pathology.

4.3. Gray matter density reduction is associated with severity of psychotic symptoms in FEP

Even though we found no significant GM density differences between the FEP and HC groups, we found that in the FEP group, distributed GM density reductions correlated with

psychosis symptoms severity. The strongest effects were found in para-hippocampus, hippocampus, thalamus and insula regions, previously known to be related to early onset psychosis (Okada et al., 2016; Uddin, 2015). These findings are consistent with the recent proposed model of "*progressive cortical reorganization"* in schizophrenia (Lena Palaniyappan, 2017). This model proposes that in psychotic patients and in association with symptoms severity, especially the positive ones, GM undergoes an inefficient reorganization leading to GM reductions in specific regions such as insula and ACC.

4.4. Gray matter covariance with the salience network is reduced in FEP and correlates with psychosis symptoms severity

In recent years there has been a growing interest in measuring distributions of structural covariance of GM across subjects as an indicator of brain networks disruption (V. Menon, 2015; Vinod Menon, 2011; L. Palaniyappan et al., 2013; Lena Palaniyappan & Liddle, 2012; Supekar et al., 2019). Indeed, the importance of such neural networks for the pathophysiology of psychosis has been established by one of the major theoretical frameworks of schizophrenia: the "*disconnection hypothesis*" (Friston, Brown, Siemerkus, & Stephan, 2016; Pettersson-Yeo, Allen, Benetti, McGuire, & Mechelli, 2011). By explicitly making reference to the definition of the diagnostic entity of schizophrenia per se (i.e. split/disassociated mind), the "*disconnection hypothesis"* states disruptions in anatomical and functional connections, seen as neuromodulators, to be at the basis of the psychotic experience. With the advent of connectomics, an increasing body of literature has looked at how particular functional brain networks are aberrant in psychosis and additional specific models has been proposed, such as the "*triple-network model*" (Vinod Menon, 2011) and

the "*insular dysfunction model*" (Lena Palaniyappan & Liddle, 2012). These models highlight how not only the psychotic disorder arises from a series of aberrant functional networking organization, but also these disruptions are particularly targeting the SN. These have been confirmed and studied at multiple levels, such as functional and dynamic networking (L. Palaniyappan et al., 2013; Supekar et al., 2019), at neurobiological and neurotransmitter scale (Roiser, Howes, Chaddock, Joyce, & McGuire, 2013) and in terms of GM atrophy (L. Palaniyappan, Mallikarjun, Joseph, White, & Liddle, 2011).

Focusing on structural GM changes in schizophrenia, previous evidence demonstrated how psychosis is strongly characterized by altered irregular covariance between different brain regions, with a particular involvement of cortices involved in salience processing (Heinze et al., 2015; Kuang et al., 2017; Lena Palaniyappan, Park, Balain, Dangi, & Liddle, 2015; Rosengard et al., 2020; Zugman et al., 2015). To-date, regardless of the extensive research about neuroanatomical covariance in different psychosis populations (Lena Palaniyappan et al., 2019), very few studies focusing on precise GM networks (R Nathan Spreng et al., 2019). Our study is the first to focus specifically on the structural covariance of the SN in FEP. We found distinct, specific and dissociating structural covariance pattern trends in FEP and HC populations involving AI and ACC comparable to results previously reported in schizophrenia (R Nathan Spreng et al., 2019). In the following paragraphs, we discuss our findings and the roles of these two structural hubs in early psychosis stages.

Regarding insula, we found morphometric and structural covariance differences between conditions (FEP vs HC). The AI is thought to be a key region in the brain, not only because of its functional role in SN, but also as a relevant center of multisensory integrations (Kurth, Zilles, Fox, Laird, & Eickhoff, 2010; Mutschler et al., 2009). Previous

studies showed insular cortex notably involved in psychosis-related structural and functional abnormalities (Corradi-Dell'Acqua et al., 2012; Lee et al., 2016; Mutschler et al., 2009; Nickl-Jockschat et al., 2011; Radua et al., 2012). More recent studies (Clos, Rottschy, Laird, Fox, & Eickhoff, 2014) looked at combined connectivity of AI, only in left hemisphere, and found this region to be involved in multiple cognitive domains at functional level which are all impacted by establishment of the psychotic disorder. These functional findings further corroborate the inversion of the SN's covariance pattern in FEP found in this study, which may be driven by a large and robust decrease of the insular involvement in GM covariance networking possibly resulting in an abnormal activation of the region. In this framework, our study on FEP population clarifies the establishment of the insular dysfunction in psychosis, even at GM structural covariance level, previously stated volumetrically in Palaniyappan et al., 2012b. Indeed, the psychosis neural model proposed by Palaniyappan & Liddle, 2012a talks about an insular dysfunction not only present in real time processing of salience stimuli, so as functional disengagement of region, but also as an abnormal structural characteristic. This model specifically proposes a strong association between a dysfunction in salience networking and positive psychotic symptoms. Here, firstly, we further confirmed this statement by showing brain structural covariance changes in psychotic patients; secondly, we clarified the presence of salience disruption driven by GM insular volume at the first stages of the pathology. Furthermore, our analysis negatively associated GM covariance in AI with symptoms severity, measured by PANSS, supporting an association that was previously detected only at the functional level (Pang et al., 2017). This may show an implication of insular structure in positive psychotic symptoms formations, since we have shown correlation effect with GM brain structures is mainly driven by Positive sub-scale of PANSS. Recent studies, in fact, showed: (i) decreased functional connectivity in insula leads

to over-activity in auditory networking at the basis of one of most common positive symptoms of psychosis such as auditory hallucinations (Mallikarjun et al., 2018), (ii) clinical symptomatology variability is explained by intrinsic insula connectivity changes characteristic of each individual (Tian, Zalesky, Bousman, Everall, & Pantelis, 2019) and (iii) functional connectivity is reduced in term of differentiation between anterior and posterior insula and significantly interacting with diagnosis (Tian et al., 2019). Therefore, we may infer that the strong negative covariance pattern found in FEP in AI, combined with negative correlation with PANSS, may explain a decrease in its functional connections and the resulting behavioral outcome of psychotic symptoms.

Regarding cingulate cortex, previous studies in psychosis patients have shown this brain region undergoing progressive structural changes impacting on large scale connectivity in the individuals at high-risk of psychosis (Allen et al., 2010; Borgwardt, McGuire, Fusar-Poli, Radue, & Riecher-Rössler, 2008; Cui et al., 2015) and with progress of the disorder (Pettersson-Yeo et al., 2011). Anyhow, whether these morphometric changes may worsen, interact or be ameliorated by means of long treatments with antipsychotic medications is still a matter of debate (Leung et al., 2011; Pressler, Nopoulos, Ho, & Andreasen, 2005; Radua et al., 2012). For this reason, by design, our study excluded FEP patients that have taken any type of anti-psychotic medication for more than 3 months, in the attempt of investigating of early brain morphometry effects by reducing as much as possible contribution of medication effects. We found that in our FEP group structural covariance involving left ACC was different from that found in HC group, showing not only a reversed but also a more confined structural GM covariance pattern. These observations suggest a new interpretation about antipsychotic medication effects on SN. A recent reward functional MRI study (Schmidt et al., 2016) reports evidence supporting the model of insular

dysfunction, with reduced functional connectivity in SN during reward prediction even in untreated FEP. The authors propose decrease in functional connectivity could be related to the formation of psychotic symptoms, and in this sense antipsychotic drugs may reverse this brain connectivity pattern. In our study, we expand those findings by confirming them at GM structural level. We show, in FEP patients with minimal pharmacological treatment history (less than 3 months), abnormal structural covariance patterns between ACC and SN. Therefore, these distributed network effects seem to be related to the disease before effects of long-term medications are visible reconfirming the hypothesis of GM reorganization occurring during the first stages of the disease (Lena Palaniyappan, 2017).

4.5. Potential limitations and future directions

Our study has potential limitations. First, due to relatively small sample size, we did not stratify our sample by diagnostic group (e.g. affective and non-affective psychosis) and we were not able to control for potential confounds due to the diagnostic heterogeneity of the sample. Future longitudinal studies with larger sample size would be useful to better understand the effect of sub-types of psychosis on SN structural covariance as well as their temporal psychopathological evolution. Second, our evaluation of global cognition was limited to IQ from Raven Matrices. Future studies should include evaluations of additional high cognitive functions to more specifically characterize them in relation to structural covariance of the SN, as well their temporal profiles in longitudinal assessments. Lastly, regarding potential pharmacological treatment confounds: FEP patients with more than 3 months of antipsychotic treatment were excluded and only a small percentage of them underwent typical antipsychotics, which are thought to largely impact GM volumes (Leung et al., 2011; Pressler, Nopoulos, Ho, & Andreasen, 2005; Radua et al., 2012). However, since

we cannot exclude that even this small period of pharmacological treatment could influence cortical reorganization, further work is needed.

4.6.Conclusions

Our brain morphometry study in FEP patients with minimal treatment provides evidence in support of the insular dysfunction model of psychosis. The multivariate analysis of GM structural covariance was more sensitive than the univariate VBM to differentiate FEP patients from matched HC. The GM structural findings showed that the main hubs of the SN have distinct and opposite brain structure covariance patterns depending on the presence or absence of the pathology. Moreover, we identified a negative association between the SN node's structural covariance pattern and positive psychosis symptoms. The findings of this study suggest that insular and ACC GM covariance reduction with the rest of the brain may be a signature of altered brain morphometry in early psychosis. The use of such neuroanatomical MRI measures may be of interest in longitudinal or treatment response studies of psychosis.

References

Addington, J., Addington, D., & Maticka-Tyndale, E. (1991). Cognitive functioning and positive and negative symptoms in schizophrenia. *Schizophrenia Research*, *5*(2), 123–134. https://doi.org/10.1016/0920-9964(91)90039-T

Allen, P., Stephan, K. E., Mechelli, A., Day, F., Ward, N., Dalton, J., … McGuire, P. (2010). Cingulate activity and fronto-temporal connectivity in people with prodromal signs of psychosis. *NeuroImage*, *49*(1), 947–955. https://doi.org/10.1016/j.neuroimage.2009.08.038

Andreasen, N. C., & Olsen, S. (1982). Definition and Validation. *Arch Gen Psychiatry*, *39*(7), 789–794.

Baglivo, V., Cao, B., Mwangi, B., Bellani, M., Perlini, C., Lasalvia, A., … Brambilla, P. (2018). Hippocampal Subfield Volumes in Patients With First-Episode Psychosis. *Schizophrenia Bulletin*, *44*(3), 552–559. https://doi.org/10.1093/schbul/sbx108

Borgwardt, S. J., McGuire, P., Fusar-Poli, P., Radue, E. W., & Riecher-Rössler, A. (2008). Anterior cingulate pathology in the prodromal stage of schizophrenia. *NeuroImage*, *39*(2), 553–554. https://doi.org/10.1016/j.neuroimage.2007.08.047

Borgwardt, S. J., McGuire, P. K., Aston, J., Berger, G., Dazzan, P., Gschwandtner, U., … Riecher-Rössler, A. (2007). Structural brain abnormalities in individuals with an at-risk mental state who later develop psychosis. *British Journal of Psychiatry*, *191*(SUPPL. 51). https://doi.org/10.1192/bjp.191.51.s69

Brambilla, P., Perlini, C., Rajagopalan, P., Saharan, P., Rambaldelli, G., Bellani, M., … Thompson, P. M. (2013). Schizophrenia severity, social functioning and hippocampal neuroanatomy: Three-dimensional mapping study. *British Journal of Psychiatry*, *202*(1), 50–55. https://doi.org/10.1192/bjp.bp.111.105700

Caffarra, P., Vezzadini, G., Zonato, F., Copelli, S., & Venneri, A. (2003). A normative study of a shorter version of Raven's progressive matrices 1938. *Neurological Sciences*, *24*(5), 336–339. https://doi.org/10.1007/s10072-003-0185-0

Castro-Fornieles, J., Bargalló, N., Calvo, A., Arango, C., Baeza, I., Gonzalez-Pinto, A., … de la Serna, E. (2018). Gray matter changes and cognitive predictors of 2-year follow-up abnormalities in early-onset first-episode psychosis. *European Child and Adolescent Psychiatry*, *27*(1), 113–126. https://doi.org/10.1007/s00787-017-1013-z

Clos, M., Rottschy, C., Laird, A. R., Fox, P. T., & Eickhoff, S. B. (2014). Comparison of structural covariance with functional connectivity approaches exemplified by an investigation of the left anterior insula. *NeuroImage*, *99*, 269–280. https://doi.org/10.1016/j.neuroimage.2014.05.030

Corradi-Dell'Acqua, C., Tomelleri, L., Bellani, M., Rambaldelli, G., Cerini, R., Pozzi-Mucelli, R., … Brambilla, P. (2012). Thalamic-insular dysconnectivity in schizophrenia: Evidence from structural equation modeling. *Human Brain Mapping*, *33*(3), 740–752. https://doi.org/10.1002/hbm.21246

Cui, L.-B., Liu, J., Wang, L.-X., Li, C., Xi, Y.-B., Guo, F., … Lu, H. (2015). Anterior cingulate cortex-related connectivity in first-episode schizophrenia: a spectral dynamic causal modeling study with functional magnetic resonance imaging.

Frontiers in Human Neuroscience, *9*(November), 1–10. https://doi.org/10.3389/fnhum.2015.00589

Delvecchio, G., Lorandi, A., Perlini, C., Barillari, M., Ruggeri, M., Altamura, A. C., … Brambilla, P. (2017). Brain anatomy of symptom stratification in schizophrenia: a voxel-based morphometry study. *Nordic Journal of Psychiatry*, *71*(5), 348–354. https://doi.org/10.1080/08039488.2017.1300323

DuPre, E., & Spreng, R. N. (2017). Structural covariance networks across the lifespan, from 6-94 years of age. *Network Neuroscience*, 1–38. https://doi.org/10.1162/NETN_a_00016

Dusi, N., Bellani, M., Perlini, C., Squarcina, L., Marinelli, V., Finos, L., … Brambilla, P. (2017). Progressive disability and prefrontal shrinkage in schizophrenia patients with poor outcome: A 3-year longitudinal study. *Schizophrenia Research*, *179*, 104–111. https://doi.org/10.1016/j.schres.2016.09.013

Fannon, D., Chitnis, X., Doku, V., Tennakoon, L., O'Ceallaigh, S., Soni, W., … Sharma, T. (2000). Features of structural brain abnormality detected in first-episode psychosis. *American Journal of Psychiatry*, *157*(11), 1829–1834. https://doi.org/10.1176/appi.ajp.157.11.1829

Ferro, A., Roiz-Santiáñez, R., Ortíz-García de la Foz, V., Tordesillas-Gutiérrez, D., Ayesa-Arriola, R., de La Fuente-González, N., … Crespo-Facorro, B. (2015). A cross-sectional and longitudinal structural magnetic resonance imaging study of the post-central gyrus in first-episode schizophrenia patients. *Psychiatry Research - Neuroimaging*, *231*(1), 42–49. https://doi.org/10.1016/j.pscychresns.2014.10.023

Frangou, S., Chitins, X., & Williams, S. C. R. (2004). Mapping IQ and gray matter density in healthy young people. *NeuroImage*, *23*(3), 800–805. https://doi.org/10.1016/j.neuroimage.2004.05.027

Friston, K., Brown, H. R., Siemerkus, J., & Stephan, K. E. (2016). The dysconnection hypothesis (2016). *Schizophrenia Research*, *176*(2–3), 83–94. https://doi.org/10.1016/j.schres.2016.07.014

Fusar-Poli, P., Borgwardt, S., Crescini, A., Deste, G., Kempton, M. J., Lawrie, S., … Sacchetti, E. (2011). Neuroanatomy of vulnerability to psychosis: A voxel-based meta-analysis. *Neuroscience and Biobehavioral Reviews*, *35*(5), 1175–1185. https://doi.org/10.1016/j.neubiorev.2010.12.005

Gallardo-Ruiz, R., Crespo-Facorro, B., Setién-Suero, E., & Tordesillas-Gutierrez, D. (2019). Long-Term Grey Matter Changes in First Episode Psychosis: A Systematic Review. *Psychiatry Investigation*, *16*(5), 336–345. https://doi.org/10.30773/pi.2019.02.10.1

Giessing, C., Fink, G. R., Rösler, F., & Thiel, C. M. (2007). fMRI data predict individual differences of behavioral effects of nicotine: A partial least square analysis. *Journal of Cognitive Neuroscience*, *19*(4), 658–670. https://doi.org/10.1162/jocn.2007.19.4.658

Glahn, D. C., Laird, A. R., Ellison-Wright, I., Thelen, S. M., Robinson, J. L., Lancaster, J. L., … Fox, P. T. (2008). Meta-Analysis of Gray Matter Anomalies in Schizophrenia: Application of Anatomic Likelihood Estimation and Network Analysis. *Biological Psychiatry*, *64*(9), 774–781. https://doi.org/10.1016/j.biopsych.2008.03.031

Grimm, O., Pohlack, S., Cacciaglia, R., Winkelmann, T., Plichta, M. M., Demirakca, T., & Flor, H. (2015). Amygdalar and hippocampal volume: A comparison between manual segmentation, Freesurfer and VBM. *Journal of Neuroscience Methods*, *253*, 254–261. https://doi.org/10.1016/j.jneumeth.2015.05.024

Hartzell, J. F., Davis, B., Melcher, D., Miceli, G., Jovicich, J., Nath, T., … Hasson, U. (2016). Brains of verbal memory specialists show anatomical differences in language, memory and visual systems. *NeuroImage*, *131*, 181–192. https://doi.org/10.1016/j.neuroimage.2015.07.027

Heinze, K., Reniers, R. L. E. P., Nelson, B., Yung, A. R., Lin, A., Harrison, B. J., … Wood, S. J. (2015). Discrete alterations of brain network structural covariance in individuals at ultra-high risk for psychosis. *Biological Psychiatry*, *77*(11), 989–996. https://doi.org/10.1016/j.biopsych.2014.10.023

Huang, H., Xu, L., Qian, Z., Zhang, T., Tang, X., Botao, Z., … Liu, X. (2019). Aberrant resting-state functional connectivity of salience network in first-episode schizophrenia. *Brain Imaging and Behavior*. https://doi.org/10.1007/s11682-019-00040-8

Kapur, S. (2003). Psychosis as a state of aberrant salience: A framework linking biology, phenomenology, and pharmacology in schizophrenia. *American Journal of Psychiatry*, *160*(1), 13–23. https://doi.org/10.1176/appi.ajp.160.1.13

Kay, S. R., Fiszbein, A., & Opler, L. A. (1987). The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophrenia Bulletin*, *13*(2), 261–276. https://doi.org/10.1093/schbul/13.2.261

Krishnan, A., Williams, L. J., McIntosh, A. R., & Abdi, H. (2011). Partial Least Squares (PLS) methods for neuroimaging: A tutorial and review. *NeuroImage*, *56*(2), 455–475. https://doi.org/10.1016/j.neuroimage.2010.07.034

Kuang, C., Buchy, L., Barbato, M., Makowski, C., MacMaster, F. P., Bray, S., … Addington, J. (2017). A pilot study of cognitive insight and structural covariance in first-episode psychosis. *Schizophrenia Research*, *179*, 91–96. https://doi.org/10.1016/j.schres.2016.09.036

Kurth, F., Zilles, K., Fox, P. T., Laird, A. R., & Eickhoff, S. B. (2010). A link between the systems: functional differentiation and integration within the human insula revealed by meta-analysis. *Brain Structure & Function*, *214*(5–6), 519–534. https://doi.org/10.1007/s00429-010-0255-z

Lappin, J. M., Morgan, K., Morgan, C., Hutchison, G., Chitnis, X., Suckling, J., … Dazzan, P. (2006). Gray matter abnormalities associated with duration of untreated psychosis. *Schizophrenia Research*, *83*(2–3), 145–153. https://doi.org/10.1016/j.schres.2005.11.018

Lee, S. H., Niznikiewicz, M., Asami, T., Otsuka, T., Salisbury, D. F., Shenton, M. E., & McCarley, R. W. (2016). Initial and progressive gray matter abnormalities in insular gyrus and temporal pole in first-episode schizophrenia contrasted with first-episode affective psychosis. *Schizophrenia Bulletin*, *42*(3), 790–801. https://doi.org/10.1093/schbul/sbv177

Leung, M., Cheung, C., Yu, K., Yip, B., Sham, P., Li, Q., … McAlonan, G. (2011). Gray matter in first-episode schizophrenia before and after antipsychotic drug treatment. Anatomical likelihood estimation meta-analyses with sample size weighting. *Schizophrenia Bulletin*, *37*(1), 199–211. https://doi.org/10.1093/schbul/sbp099

Li, M., Li, X., Das, T. K., Deng, W., Li, Y., Zhao, L., … Li, T. (2019). Prognostic utility of multivariate morphometry in schizophrenia. *Frontiers in Psychiatry*, *10*(APR), 1–9. https://doi.org/10.3389/fpsyt.2019.00245

Li, X., Black, M., Xia, S., Zhan, C., Bertisch, H. C., Branch, C. A., & DeLisi, L. E. (2015). Subcortical structure alterations impact language processing in individuals with schizophrenia and those at high genetic risk. *Schizophrenia Research*, *169*(1–3), 76–82. https://doi.org/10.1016/j.schres.2015.08.001

Malla, A. K., Bodnar, M., Joober, R., & Lepage, M. (2011). Duration of untreated psychosis is associated with orbital-frontal grey matter volume reductions in first episode psychosis. *Schizophrenia Research*, *125*(1), 13–20. https://doi.org/10.1016/j.schres.2010.09.021

Mallikarjun, P. K., Lalousis, P. A., Dunne, T. F., Heinze, K., Reniers, R. L., Broome, M. R., … Upthegrove, R. (2018). Aberrant salience network functional connectivity in auditory verbal hallucinations: A first episode psychosis sample. *Translational Psychiatry*, *8*(1). https://doi.org/10.1038/s41398-018-0118-6

Mcintosh, A. R., & Gonzalez-Lima, F. (1998). Large-scale functional connectivity in associative learning: Interrelations of the rat auditory, visual, and limbic systems. *Journal of Neurophysiology*, *80*(6), 3148–3162. https://doi.org/10.1152/jn.1998.80.6.3148

Menon, V. (2015). Salience Network. In *Brain Mapping: An Encyclopedic Reference* (Vol. 2). https://doi.org/10.1016/B978-0-12-397025-1.00052-X

Menon, Vinod. (2011). Large-scale brain networks and psychopathology: A unifying triple network model. *Trends in Cognitive Sciences*, *15*(10), 483–506. https://doi.org/10.1016/j.tics.2011.08.003

Menzies, L., Achard, S., Chamberlain, S. R., Fineberg, N., Chen, C. H., Del Campo, N., … Bullmore, E. (2007). Neurocognitive endophenotypes of obsessive-compulsive disorder. *Brain*, *130*(12), 3223–3236. https://doi.org/10.1093/brain/awm205

Minatogawa-Chang, T. M., Schaufelberger, M. S., Ayres, A. M., Duran, F. L. S., Gutt, E. K., Murray, R. M., … Busatto, G. F. (2009). Cognitive performance is related to cortical grey matter volumes in early stages of schizophrenia: A population-based study of first-episode psychosis. *Schizophrenia Research*, *113*(2–3), 200–209. https://doi.org/10.1016/j.schres.2009.06.020

Morgan, K. D., Dazzan, P., Morgan, C., Lappin, J., Hutchinson, G., Suckling, J., … David, A. S. (2010). Insight, grey matter and cognitive function in first-onset psychosis. *British Journal of Psychiatry*, *197*(2), 141–148. https://doi.org/10.1192/bjp.bp.109.070888

Mutschler, I., Wieckhorst, B., Kowalevski, S., Derix, J., Wentlandt, J., Schulze-Bonhage, A., & Ball, T. (2009). Functional

organization of the human anterior insular cortex. *Neuroscience Letters*, *457*(2), 66–70. https://doi.org/10.1016/j.neulet.2009.03.101

Nickl-Jockschat, T., Schneider, F., Pagel, A. D., Laird, A. R., Fox, P. T., & Eickhoff, S. B. (2011). Progressive pathology is functionally linked to the domains of language and emotion: Meta-analysis of brain structure changes in schizophrenia patients. *European Archives of Psychiatry and Clinical Neuroscience*, *261*(SUPPL. 2), 166–171. https://doi.org/10.1007/s00406-011-0249-8

Okada, N., Fukunaga, M., Yamashita, F., Koshiyama, D., Yamamori, H., Ohi, K., … Hashimoto, R. (2016). Abnormal asymmetries in subcortical brain volume in schizophrenia. *Molecular Psychiatry*, *21*(10), 1460–1466. https://doi.org/10.1038/mp.2015.209

P, S., D, G., M, M., S, S., A, C., S, T., … G, R. (2012). A multi-element psychosocial intervention for early psychosis (GET UP PIANO TRIAL) conducted in a catchment area of 10 million inhabitants: study protocol for a pragmatic cluster randomized controlled trial. *Trials*, *13*, 1–16. https://doi.org/10.1186/1745-6215-13-73

Pagsberg, A. K., Baaré, W. F. C., Raabjerg Christensen, A. M., Fagerlund, B., Hansen, M. B., LaBianca, J., … Hemmingsen, R. P. (2007). Structural brain abnormalities in early onset first-episode psychosis. *Journal of Neural Transmission*, *114*(4), 489–498. https://doi.org/10.1007/s00702-006-0573-8

Palaniyappan, L., Mallikarjun, P., Joseph, V., White, T. P., & Liddle, P. F. (2011). Reality distortion is related to the structure of the salience network in schizophrenia. *Psychological Medicine*, *41*(8), 1701–1708. https://doi.org/10.1017/S0033291710002205

Palaniyappan, L., White, T. P., & Liddle, P. F. (2013). The Concept of Salience Network Dysfunction in Schizophrenia: From Neuroimaging Observations to Therapeutic Opportunities. *Current Topics in Medicinal Chemistry*, *12*(21), 2324–2338. https://doi.org/10.2174/1568026611212210005

Palaniyappan, Lena. (2017). Progressive cortical reorganisation: A framework for investigating structural changes in schizophrenia. *Neuroscience and Biobehavioral Reviews*, *79*(May), 1–13. https://doi.org/10.1016/j.neubiorev.2017.04.028

Palaniyappan, Lena, Hodgson, O., Balain, V., Iwabuchi, S., Gowland, P., & Liddle, P. (2019). Structural covariance and cortical reorganisation in schizophrenia: A MRI-based morphometric study. *Psychological Medicine*, *49*(3), 412–420. https://doi.org/10.1017/S0033291718001010

Palaniyappan, Lena, & Liddle, P. F. (2012). Does the salience network play a cardinal role in psychosis? An emerging hypothesis of insular dysfunction. *Journal of Psychiatry and Neuroscience*, *37*(1), 17–27. https://doi.org/10.1503/jpn.100176

Palaniyappan, Lena, Park, B., Balain, V., Dangi, R., & Liddle, P. (2015). Abnormalities in structural covariance of cortical gyrification in schizophrenia. *Brain Structure and Function*, *220*(4), 2059–2071. https://doi.org/10.1007/s00429-014-

0772-2

Pang, L., Kennedy, D., Wei, Q., Lv, L., Gao, J., Li, H., … Song, X. (2017). Decreased functional connectivity of insular cortex in drug Naïve first episode schizophrenia: In relation to symptom severity. *PLoS ONE*, *12*(1), 1–12. https://doi.org/10.1371/journal.pone.0167242

Pettersson-Yeo, W., Allen, P., Benetti, S., McGuire, P., & Mechelli, A. (2011). Dysconnectivity in schizophrenia: Where are we now? *Neuroscience and Biobehavioral Reviews*, *35*(5), 1110–1124. https://doi.org/10.1016/j.neubiorev.2010.11.004

Pressler, M., Nopoulos, P., Ho, B. C., & Andreasen, N. C. (2005). Insular cortex abnormalities in schizophrenia: Relationship to symptoms and typical neuroleptic exposure. *Biological Psychiatry*, *57*(4), 394–398. https://doi.org/10.1016/j.biopsych.2004.11.010

Radua, J., Borgwardt, S., Crescini, A., Mataix-Cols, D., Meyer-Lindenberg, A., McGuire, P. K., & Fusar-Poli, P. (2012). Multimodal meta-analysis of structural and functional brain changes in first episode psychosis and the effects of antipsychotic medication. *Neuroscience and Biobehavioral Reviews*, *36*(10), 2325–2333. https://doi.org/10.1016/j.neubiorev.2012.07.012

Roiser, J. P., Howes, O. D., Chaddock, C. A., Joyce, E. M., & McGuire, P. (2013). Neural and behavioral correlates of aberrant salience in individuals at risk for psychosis. *Schizophrenia Bulletin*, *39*(6), 1328–1336. https://doi.org/10.1093/schbul/sbs147

Rosa, P. G. P., Zanetti, M. V., Duran, F. L. S., Santos, L. C., Menezes, P. R., Scazufca, M., … Schaufelberger, M. S. (2015). What determines continuing grey matter changes in first-episode schizophrenia and affective psychosis? *Psychological Medicine*, *45*(4), 817–828. https://doi.org/10.1017/S0033291714001895

Rosengard, R. J., Makowski, C., Chakravarty, M., Malla, A. K., Joober, R., Shah, J. L., & Lepage, M. (2020). Pre-onset sub-threshold psychotic symptoms and cortical organization in the first episode of psychosis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *100*, 109879. https://doi.org/10.1016/j.pnpbp.2020.109879

Ruggeri, M., Bonetto, C., Lasalvia, A., Fioritti, A., De Girolamo, G., Santonastaso, P., … Meneghelli, A. (2015). Feasibility and effectiveness of a multi-element psychosocial intervention for first-episode psychosis: Results from the cluster-randomized controlled GET UP PIANO trial in a catchment area of 10 million inhabitants. *Schizophrenia Bulletin*, *41*(5), 1192–1203. https://doi.org/10.1093/schbul/sbv058

Satterthwaite, T. D., Wolf, D. H., Calkins, M. E., Vandekar, S. N., Erus, G., Ruparel, K., … Gur, R. E. (2016). Structural brain abnormalities in youth with psychosis spectrum symptoms. *JAMA Psychiatry*, *73*(5), 515–524. https://doi.org/10.1001/jamapsychiatry.2015.3463

Schmidt, A., Palaniyappan, L., Smieskova, R., Simon, A., Riecher-Rössler, A., Lang, U. E., … Borgwardt, S. J. (2016). Dysfunctional insular connectivity during reward prediction in patients with first-episode psychosis. *Journal of*

Psychiatry and Neuroscience, *41*(6), 367–376. https://doi.org/10.1503/jpn.150234

Segall, J. M., Turner, J. A., Van Erp, T. G. M., White, T., Bockholt, H. J., Gollub, R. L., … Calhoun, V. D. (2009). Voxel-based morphometric multisite collaborative study on schizophrenia. *Schizophrenia Bulletin*, *35*(1), 82–95. https://doi.org/10.1093/schbul/sbn150

Spreng, R. N., & Turner, G. R. (2013). Structural Covariance of the Default Network in Healthy and Pathological Aging. *Journal of Neuroscience*, *33*(38), 15226–15234. https://doi.org/10.1523/JNEUROSCI.2261-13.2013

Spreng, R Nathan, DuPre, E., Ji, J. L., Yang, G., Diehl, C., Murray, J. D., … Anticevic, A. (2019). Structural Covariance Reveals Alterations in Control and Salience Network Integrity in Chronic Schizophrenia. *Cerebral Cortex*, (May), 1–16. https://doi.org/10.1093/cercor/bhz064

Steen, A. N. T., Mull, C., Mcclure, R., Hamer, R. M., & Lieberman, J. A. (2004). REVIEW ARTICLE AUTHOR ' S PROOF Brain volume in first-episode schizophrenia Systematic review and meta-analysis of magnetic resonance imaging studies. *Imaging*, *i*. https://doi.org/10.1192/bjp.188.6.510

Supekar, K., Cai, W., Krishnadas, R., Palaniyappan, L., & Menon, V. (2019). Dysregulated Brain Dynamics in a Triple-Network Saliency Model of Schizophrenia and Its Relation to Psychosis. *Biological Psychiatry*, *85*(1), 60–69. https://doi.org/10.1016/j.biopsych.2018.07.020

Theberge, J., E.Williamson, K., Aoyama, N., Drost, D. J., Theberge, R., Manchanda, A. K. M., … C.Williamson, and P. (2007). Longitudinal grey-matter and glutamatergic losses in first-episode schizophrenia. *British Journal of Psychiatry*, *191*, 325–334.

Tian, Y., Zalesky, A., Bousman, C., Everall, I., & Pantelis, C. (2019). Insula Functional Connectivity in Schizophrenia: Subregions, Gradients, and Symptoms. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging*, *4*(4), 399–408. https://doi.org/10.1016/j.bpsc.2018.12.003

Uddin, L. Q. (2015). Salience processing and insular cortical function and dysfunction. *Nature Reviews Neuroscience*, *16*(1), 55–61. https://doi.org/10.1038/nrn3857

Vita, A., De Peri, L., Deste, G., & Sacchetti, E. (2012). Progressive loss of cortical gray matter in schizophrenia: A meta-analysis and meta-regression of longitudinal MRI studies. *Translational Psychiatry*, *2*(11), e190-13. https://doi.org/10.1038/tp.2012.116

Voets, N. L., Hough, M. G., Douaud, G., Matthews, P. M., James, A., Winmill, L., … Smith, S. (2008). Evidence for abnormalities of cortical development in adolescent-onset schizophrenia. *NeuroImage*, *43*(4), 665–675. https://doi.org/10.1016/j.neuroimage.2008.08.013

White, T. P., Joseph, V., Francis, S. T., & Liddle, P. F. (2010). Aberrant salience network (bilateral insula and anterior cingulate cortex) connectivity during information processing in schizophrenia. *Schizophrenia Research*, *123*(2–3), 105–115. https://doi.org/10.1016/j.schres.2010.07.020

Whitford, T. J., Farrow, T. F. D., Gomes, L., Brennan, J., Harris, A. W. F., & Williams, L. M. (2005). Grey matter deficits and symptom profile in first episode schizophrenia. *Psychiatry Research - Neuroimaging*, *139*(3), 229–238. https://doi.org/10.1016/j.pscychresns.2005.05.010

Wood, S. J., Velakoulis, D., Smith, D. J., Bond, D., Stuart, G. W., McGorry, P. D., … Pantelis, C. (2001). A longitudinal study of hippocampal volume in first episode psychosis and chronic schizophrenia. *Schizophrenia Research*, *52*(1–2), 37–46. https://doi.org/10.1016/S0920-9964(01)00175-X

Yeo, B. T., Krienen, F. M., Sepulcre, J., Sabuncu, M. R., Lashkari, D., Hollinshead, M., ... & Fischl, B. (2011). The organization of the human cerebral cortex estimated by intrinsic functional connectivity. Journal of neurophysiology.

Zugman, A., Assunção, I., Vieira, G., Gadelha, A., White, T. P., Oliveira, P. P. M., … Sato, J. R. (2015). Structural covariance in schizophrenia and first-episode psychosis: An approach based on graph analysis. *Journal of Psychiatric Research*, *71*, 89–96. https://doi.org/10.1016/j.jpsychires.2015.09.018

Tables

Table 1: **Demographic and clinical information of First-episode Psychosis (FEP) and Healthy Control (HC) participants.** More details about the pharmacological molecules used in psychosis treatments can be found in the supplementary materials (Table S1).

Variable	HC (N=67)	FEP (N=67)	Group Statistic	P value
Age, years, mean (SD)	31(8)	29(9)	FEP>HC *T-score*=-1.70	*p*=0.09
Sex, No. (%)				
Men	40(60)	40(60)	FEP>HC χ2=0	*p=1*
Women	27(40)	27(40)	FEP>HC *T-score*=-1.68	*p*=0.09
Handedness, mean (SD)				
Right	16(6)	17(5)	FEP>HC *T-score*=0.48	*p*=0.63
Left	-1(6)	-1(4)	FEP>HC *T-score*=0.01	*p*=0.99
Years of education, mean (SD)	15(3)	12(3)	FEP>HC *T-score*=-6.77	*p*<.0001
Premorbid IQ on the Raven Matrices, mean (SD)	122(8)	94(7)	FEP>HC *T-score*=-21.43	*p*<.0001
Total Intracranial Volume, cm^3, mean (SD)	1508(132)	1593(135)	FEP>HC *T-score*=0.63	*p*=0.52
Duration of untreated psychosis (DUP), days, mean (SD)	N.A.	134(291)	N.A.	
Antidepressant treatment, No. (%)	N.A.	8(12)	N.A.	
Antipsychotic treatment, No. (%)	N.A.	44(66)	N.A.	
GAF, total score, mean (SD)	N.A.	48(14)	N.A.	
PANSS, Positive sub-scale, mean (SD)	N.A.	14(6)	N.A.	
PANSS, Negative sub-scale, mean (SD)	N.A.	16(7)	N.A.	
PANSS, General sub-scale, mean (SD)	N.A.	34(10)	N.A.	
PANSS, Total score, mean (SD)	N.A.	65(19)	N.A.	

PANSS, Positive and Negative Syndrome Scale

N.A., not applicable

Table 2: **Performed analyses and their effect sizes in First-episode Psychosis (FEP) and Healthy Control (HC) participants.**

Type of analysis	HC	FEP	Group Statistic	P value	Effect size
Whole brain VBM analysis	N=67	N=67	FEP>HC *T score*=-4.0	*p* <.001 uncorr	N.A.
Multiple regression VBM IQ		N=66	*T score*=9.3	*p* <.0001**	*Cohen's d*= 2.07
	N=58		*T score*=9.1	*p* <.0001**	*Cohen's d*= 2.41
Multiple regression VBM PANSS		N=66	*T score*=32.5	*p*=.001**	*Cohen's d*= 6.86
Multiple regression VBM PANSS-P		N=66	*T score*=40.3	*p*=.001**	*Cohen's d* = 9.85
Structural covariance Salience Network seed PLS	N=67	N=67	FEP>HC *T* score=-5.8	*p*<.01*	*Cohen's d*= 0.98
Structural covariance Salience Network correlation with PANSS		N=66		*p* = .03*	*Cohen's d*= *=0.51*

VBM, Voxel-based Morphometry

N.A., not applicable

IQ, Intelligence Quotient measured with Raven Matrices

PANSS, Positive and Negative Syndrome Scale

PANSS-P, Positive Syndrome sub-Scale

PLS, Partial Least Square

Figures Legend

Figure 1: Location of salience network seeds used in Partial Least Square seed-based analysis. Anterior Cingulate cortex is represented in red, MNI coordinates (mm): [x=±5, y=15, z=32]; Anterior Insula is represented in blue, MNI coordinates (mm): [x=±31, y=11, z=8]. Seeds were selected based on previous studies (DuPre & Spreng, 2017), more details in the Materials and Methods section.

Figure 2. Structural covariance pattern of the salience network. A. Volumetric orthogonal views of BootStrap Ratio in the significant Latent Variable (*LV*) extracted from salience network seed-based PLS analysis performed jointly in both groups (N=134). Spatial pattern of seed-based PLS performed in FEP patients (**B,** N=67, *p*= .04) and in HC (**C,** N=67, *p*= .01). **D.** *BrainScore*–seed correlation patterns for the significant *LV* showing significant differences between FEP and HC in all nodes besides right ACC. The bars represent the weight that the salience network seeds have (bilateral Anterior Cingulate cortex, ACC, and bilateral Anterior Insula, AI) describing the GM covariance pattern in the LV as a function of presence (FEP) or absence (HC) of pathology. Error bars are plotted as 95% bootstrapped confidence intervals (C.I.). Absence of overlap in C.I. shows reliable difference for any pair of correlations, whereas overlap with 0 represents a correlation not significantly different from 0.

Figure 3. Correlation between psychotic symptoms severity and structural covariance pattern of the salience network

BrainScore correlation patterns with symptoms severity, measured by PANSS, for the significant latent variable in FEP group. The negative correlation shows (*r*=-.25, *p*=.03) how

the structural integrity of the SN is compromised in the presence of higher symptoms burden.

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Coordinating center: Maurizio Miceli.

Participating MHCs: TAU Arm: MOM SMA 5; MOM SMA 8; MOM SMA 11; MOM SMA 12. Experimental Arm: MOM SMA 3; MOM SMA 7; MOM SMA 9; MOM SMA 10.

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Experts supervising treatments in the experimental arm.

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Coordinator: Carlo Faravelli (Firenze).

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Leading administrative institution: University of Firenze.

Research unit: Communications skills.

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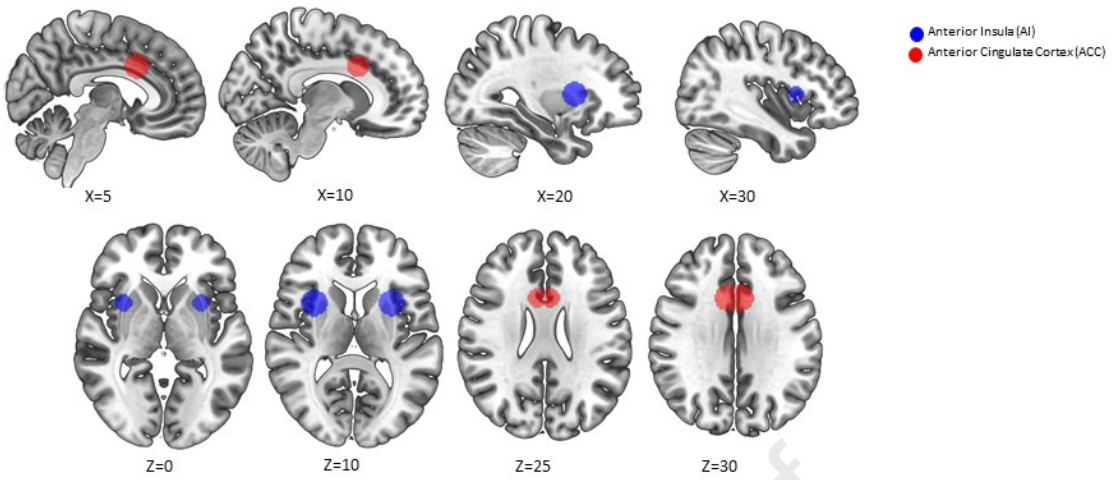

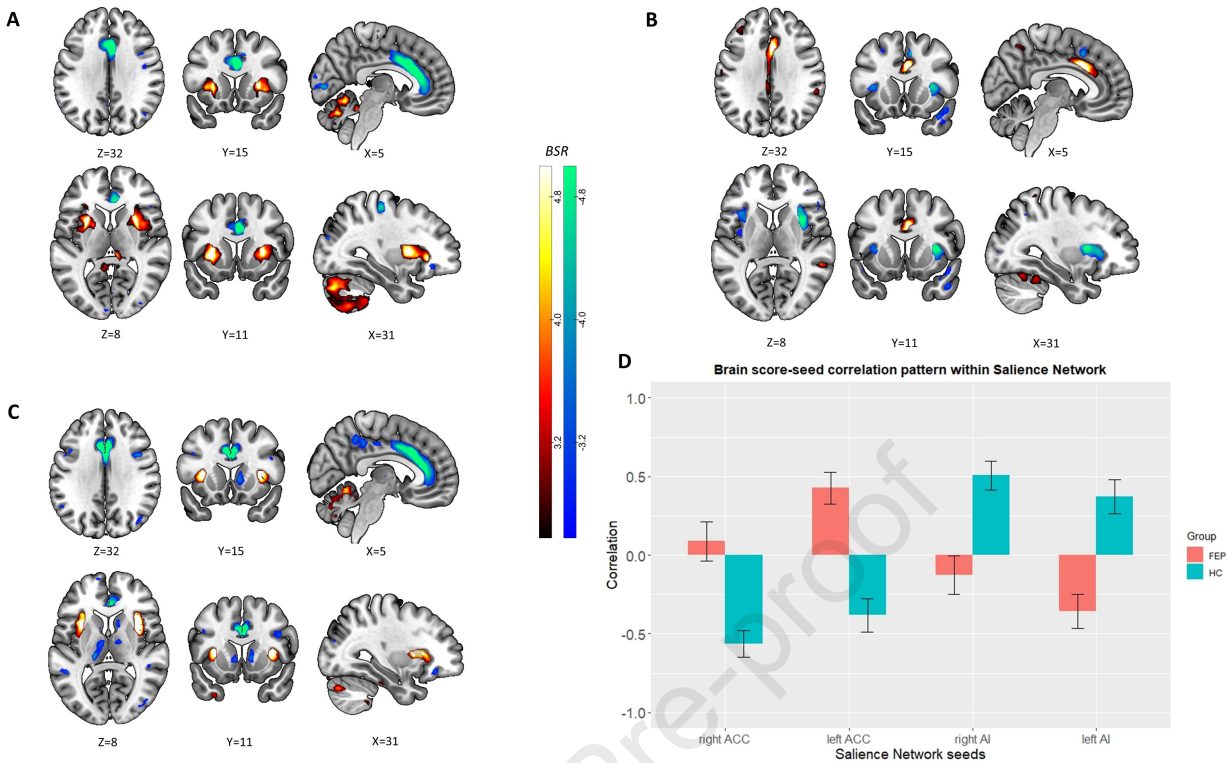

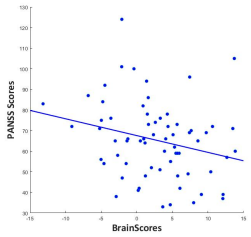

Highlights

- Multivariate structural covariance analysis from structural MRI differentiates first-episode psychosis patients from matched healthy controls, standard univariate morphometry analysis does not.
- In the structural gray matter nodes of the salience network, the structural covariance in psychosis and healthy controls show opposite patterns.
- Coordinated variation of cortical gray matter of the salience network is related to early untreated psychosis and its clinical symptoms severity.

Contributors

Francesca Saviola managed the literature searches, the analysis and wrote the first draft of the manuscript. Francesca Saviola, Marcella Bellani, Cinzia Perlini, Domenico Zacà, Paolo Brambilla and Jorge Jovicich designed the study rationale. Francesca Saviola, Domenico Zacà and Jorge Jovicich undertook the statistical analysis. Letizia Squarcina and Eleonora Maggioni helped with data storage and selection. Antonio Lasalvia, Nicola Dusi, Chiara Bonetto, Doriana Cristofalo, Franco Alessandrini, Giada Zoccatelli, Elisa Ciceri, Luca Mesiano, Enrico Semrov, Riccardo Lo Parrino, Karin Furlato and Michela Pratelli took part to the data collection. Mirella Ruggeri, as the coordinator of the GET UP Project, designed the project protocol together with Paolo Brambilla. All authors contributed to and have approved the final manuscript.

Conflict of interest

Authors have no conflict of interest to declare.