

# Impaired olfactory ability associated with larger left hippocampus and rectus volumes at earliest stages of schizophrenia

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Impaired olfactory ability associated with larger left hippocampus and rectus volumes at earliest stages of schizophrenia: a sign of neuroinflammation?

Yuri Masaoka<sup>a,e</sup>, Dennis Velakoulis<sup>a</sup>, Warrick Brewer<sup>b</sup>, Vanessa Cropley<sup>a</sup>, Cali F. Bartholomeusz<sup>a,b,c</sup>, Alison Yung<sup>b,f</sup>, Barnaby Nelson<sup>b,c</sup>, Dominic Dwyer<sup>a,g</sup>, Cassandra Wannan<sup>a</sup>, Masahiko Izumizaki<sup>e</sup>, Patrick McGorry<sup>b,c</sup>, Stephen J Wood<sup>b,c,d</sup>, Christos Pantelis<sup>a,h\*</sup>

<sup>a</sup> Melbourne Neuropsychiatry Centre, Department of Psychiatry, University of Melbourne and Melbourne Health, Carlton South, Victoria, Australia

<sup>b</sup> Orygen, The National Centre of Excellence in Youth Mental Health, Parkville, Victoria, Australia

<sup>c</sup> Orygen, Centre for Youth Mental Health, The University of Melbourne, Parkville, Victoria, Australia

<sup>d</sup> School of Psychology, University of Birmingham, Birmingham, UK

<sup>e</sup> Department of Physiology, Showa University School of Medicine, Tokyo, Japan

<sup>f</sup> Institute of Brain, Behaviour and Mental Health, University of Manchester, UK

<sup>g</sup> Department of Psychiatry and Psychotherapy, Ludwig-Maximilian University, Munich, Germany

<sup>h</sup> Florey Institute for Neuroscience and Mental Health, Parkville, Victoria, Australia

\*Correspondence to Christos Pantelis

Melbourne Neuropsychiatry Centre, Department of Psychiatry, University of Melbourne and Melbourne Health

161 Barry St, Level 3, Alan Gilbert Building, Carlton Parkville, Victoria, Australia 3053

[cpant@unimelb.edu.au](mailto:cpant@unimelb.edu.au)

All authors' address

a. 161 Barry St, Level 3, Alan Gilbert Building, Carlton South, Victoria, Australia 3053

<sup>b</sup> 35 Poplar Rd, Victoria, Australia 3052

<sup>c</sup> 35 Poplar Rd, Victoria, Australia 3052

<sup>d</sup> Hills Building, Edgbaston Park Rd, Birmingham B15 2TT

<sup>e</sup> 1-5-8 Hatanodai, Shinagawaku, Tokyo 142-8555, Japan

<sup>f</sup> Oxford Rd, Manchester M13 9PL, UK

<sup>g</sup> Geschwister-Scholl-Platz 1, 80539 München, Germany

<sup>h</sup> 30 Royal Parade, Parkville, VIC 3052, Australia

All authors'E-mail address

Yuri Masaoka [faustus@med.showa-u.ac.jp](mailto:faustus@med.showa-u.ac.jp)

Dennis Velakoulis [dennisv@unimelb.edu.au](mailto:dennisv@unimelb.edu.au)

Warrick Brewer [warrick.brewer@mh.org.au](mailto:warrick.brewer@mh.org.au)

Vanessa Cropley [vcropley@unimelb.edu.au](mailto:vcropley@unimelb.edu.au)

Cali Bartholomeusz [cali.bartholomeusz@orygen.org.au](mailto:cali.bartholomeusz@orygen.org.au)

Alison Yung [alison.yung@manchester.ac.uk](mailto:alison.yung@manchester.ac.uk)

Barnaby Nelson [nelsonb@unimelb.edu.au](mailto:nelsonb@unimelb.edu.au)

Dominic Dwyer [dominic.dwyer@med.uni-muenchen.de](mailto:dominic.dwyer@med.uni-muenchen.de)

Cassandra Wannan [cwannan@student.unimelb.edu.au](mailto:cwannan@student.unimelb.edu.au)

Masahiko Izumizaki [masahiko@med.showa-u.ac.jp](mailto:masahiko@med.showa-u.ac.jp)

Patrick McGorry [pmcgorry@unimelb.edu.au](mailto:pmcgorry@unimelb.edu.au)

Stephen J Wood [stephen.wood@orgen.org.au](mailto:stephen.wood@orgen.org.au)

Christos Pantelis [cpant@unimelb.edu.au](mailto:cpant@unimelb.edu.au)

Abstract

Impaired olfactory identification has been reported as a first sign of schizophrenia during the earliest stages of illness, including before illness onset. The aim of this study was to examine the relationship between volumes of these regions (amygdala, hippocampus, gyrus rectus and orbitofrontal cortex) and olfactory ability in three groups of participants: healthy control participants (Ctrls), patients with first-episode schizophrenia (FE-Scz) and chronic schizophrenia patients (Scz). Exploratory analyses were performed in a sample of individuals at ultra-high risk (UHR) for psychosis in co-submission paper (Masaoka et al., 2020). The relationship to brain structural measures was not apparent prior to psychosis onset, but was only evident following illness onset, with a different pattern of relationships apparent across illness stages (FE-Scz vs Scz). Path analysis found that lower olfactory ability was related to larger volumes of the left hippocampus and gyrus rectus in the FE-Scz group. We speculate that larger hippocampus and rectus in early schizophrenia are indicative of swelling, potentially caused by an active neurochemical or immunological process, such as inflammation or neurotoxicity, which is associated with impaired olfactory ability. The volumetric decreases in the chronic stage of Scz may be due to degeneration resulting from an active immune process and its resolution.

**Keywords:** olfaction, schizophrenia, ultra-high risk, first episode, chronic, hippocampus, amygdala, rectus, orbitofrontal cortex.

## 1. Introduction

Declining olfactory ability has been suggested as a biomarker for neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease (Doty et al., 1988; Hawkes, 2003), attributed to pathology in the amygdala (AMG), hippocampus (HI), and orbitofrontal cortex (OFC). Evidence has also identified

impaired olfactory ability as a first sign of schizophrenia from the earliest stages of illness, including before illness onset (Brewer et al., 2001; 2003). Another recent study in pre-psychotic individuals suggests that impaired olfactory identification ability may be a predictor of poor outcome rather than diagnosis (Lin et al., 2015). Morphological brain changes related to olfaction, such as a shallow olfactory sulcus, have been reported in ultra-high risk (UHR) individuals and patients with psychotic disorders (Takahashi et al., 2014). Further, HI volumetric reduction is reported across stages of psychosis and schizophrenia (Velakoulis et al., 2006) with evidence of progressive HI volume loss (Ho et al., 2017). Gray matter reductions across multiple areas, including frontal and temporal regions relevant to smell ability, have also been found to occur during and following the first-episode of psychosis (Bartholomeusz et al., 2017).

Olfactory information bypasses the thalamus to ascend directly to the olfactory limbic structures including AMG, entorhinal cortex (ENT) and HI (Yashurum and Sobel, 2010). This neural cascade is hypothesized to directly link olfactory perception with emotional salience processing in the AMG and memory systems in the HI (Masaoka et al., 2012, 2014). Outputs from ENT and AMG subsequently converge on the OFC where higher-order processing takes place, including smell identification and emotional labeling (Rolls, 2001). AMG, HI and OFC play important roles for olfactory perception and identification; however, no research has examined the link between structural changes and olfactory abilities across stages of schizophrenia.

The aim of this study was to investigate the relationship between olfactory ability and volume differences in olfactory brain regions at differing stages of schizophrenia (first-episode schizophrenia, FE-Scz; chronic schizophrenia, Scz), compared with healthy control subjects (Ctls), taking account of WAIS-estimated IQ (Wechsler, 1999). Further, exploratory analyses were performed in a sample of individuals at ultra-high risk (UHR) for psychosis divided into subgroups, which are reported as 'Data in Brief' [Masaoka et al., 2020]. We hypothesized that impairments in olfactory identification ability would be related to brain volumes in olfactory-related regions in patients, in a stage-specific manner.

## 2. Methods

### 2.1 Participants

Subjects included in this study are a subset of the subjects from previously published studies (Brewer et al., 2001, 2003; Velakoulis et al., 2006; Bartholomeusz et al., 2017; Lavoie et al., 2014; Eritaia et al., 2000; Lin et al, 2015), who had both olfactory and imaging (structural MRI) data available. Ctls, FE-Scz and Scz subject numbers and demographic information are shown in Table 1. There was no personal or family history of anosmia across all participants. Subject numbers and demographic information including UHR subgroups (UHR subjects who did not become psychotic, UHR-NP; UHR who were diagnosed as having psychosis other than schizophrenia, UHR-other; UHR who progressed to psychotic disorder than schizophrenia, UHR-Scz) are indicated in Table 1 in Data in Brief [Masaoka et al., 2020]. Participant recruitment information and diagnosis criteria are reported in co-submission paper [Masaoka et al., 2020]. All subjects provided written informed consent, including parental consent for those less than 18 years of age, in accordance with guidelines provided by the local mental health service research and ethics committees and the Departments of Psychiatry and Psychology at the University of Melbourne.

## 2.2 Measurement of olfactory ability and intelligence quotient (IQ)

Olfactory identification ability was measured with the University of Pennsylvania Smell Identification Test (UPSIT), a standardized, self-administered multiple-choice scratch-and-sniff test consisting of four booklets, each containing 10 items (Doty, et al., 1984). This test has been normatively adjusted for Australian samples (Mackay-Sim and Doty, 2001). IQ was assessed by the Wechsler Adult Intelligence Scale –Revised (WAIS-R) (Wechsler 1981).

## 2.3 MRI acquisition, image processing and volume measurements

Image processing and guidelines for estimations of whole brain volume (WBV) and intracranial volume (ICV), and tracing of HI and AMG are described in Velakoulis et al. (2006). In brief, Ctls, FE-Scz and Scz participants underwent MRI using the same

instrument (1.5 T Signa; General Electric Medical Systems, Milwaukee, Wisconsin) at the Royal Melbourne Hospital. A 3-dimensional volumetric spoiled gradient recalled echo in the steady-state sequence generated 124 contiguous, 1.5-mm coronal sections. Imaging parameters were as follows: echo time, 3.3 ms; repetition time, 14.3 ms; flip angle, 30°; matrix size, 256 × 256; field of view, 24 × 24-cm matrix; and voxel dimensions, 0.938 × 0.938 × 1.5 mm. Head movement was minimized by using foam padding and Velcro straps across the forehead and chin. Each scanner was calibrated fortnightly using the same proprietary phantom to ensure stability and accuracy of measurements. UHR subgroups MRI acquisition and processing were reported in the co-submission paper. The boundaries of HI and AMG were manually traced for each subject via the software ANALYZE (Mayo Clinic, Rochester, NY; <http://www.mayo.edu/bir/>).

Detailed description of OFC morphology other than volumetric measures (i.e., sulcogyral pattern and cortical thickness) were reported in previous studies (Bartholomeusz et al., 2013; Lavoie et al., 2014). HI, AMG, OFC, and rectus were corrected for ICV using the formula previously reported (Velakoulis et al. 2006). Detailed description for estimating volumes are provided in the co-submission paper (Masaoka et al., 2020) and elsewhere (Velakoulis et al, 2006; Eritea et al, 2000; Bartholomeusz et al, 2017).

#### 2.4 Statistical analysis

All statistical analyses were performed using SPSS (IBM SPSS Statistics Version 23, Chicago). Gender was compared using a Chi-squared test. One-way analysis of variance (ANOVA) was used for testing group differences in age and height. Group comparisons of WAIS and UPSIT were performed with analysis of covariance (ANCOVA) with age as a covariate. Comparisons of WBV, ICV, and left and right HI, AMG, OFC, and rectus between groups were analyzed by ANCOVA with age and sex as covariates. Post-hoc testing was performed with the Bonferroni method. UHR subgroups were also analyzed in the same manner. Pearson's correlation coefficient was calculated to examine the relationship between UPSIT and WAIS.

Path analysis was conducted to examine relationships between IQ, olfactory ability and volumes of interest. Before undertaking path analysis, two analyses were performed



to refine our hypotheses. First, within-group partial correlations between UPSIT and each brain region volume were performed controlling for IQ and gender. Second, multiple regression with interaction analysis was performed to compare slopes between Ctls vs FE-Scz and FE-Scz vs. Scz to assess whether differential relationships existed between olfactory ability and volumes of interest. UPSIT score was the dependent variable in each regression model. For independent variables, we entered the volume of interest, group and all interactions (eg., HI  $\times$  FE-Scz, and HI  $\times$  Ctls). To test for differences in slopes for structural volumes against UPSIT scores between Ctls vs FE-Scz and FE-Scz vs Scz, we used ordered dummy variables to test whether the slope for the FE-Scz differed from the slope for the Ctls, and to test whether slope for the Scz group differed from the slope for the FE-Scz group. We determined the statistical significance of all model parameters from the unstandardized estimates calculated on the mean-centered continuous independent variables. A 'variance inflation factor' (VIF)  $\leq 4$  indicated significant multicollinearity. Path analysis was then used to examine how volumes of the regions of interest (HI, AMG, OFC and rectus) impacted UPSIT and WAIS scores in each group separately. The structural equation modeling program employed (AMOS; IBM SPSS Statistics, Version 23) specified statistical significance for path coefficients; a model fitness close to 1 was adopted for each model. Given the small sample size of UHR subgroups, exploratory path analysis of UHR was undertaken (UHR-other and UHR-Scz were combined to improve statistical power; results are reported in Data in Brief co-submission paper [Masaoka et al., 2020]).

### 3. Results

#### 3.1 Demographic and structural data

Table 1 shows the demographic characteristics of each group, the mean and standard deviation (SD) of WAIS, UPSIT, ICV and WBV, and volumes for the regions of interest. Statistical results indicated in the Data in Brief [Masaoka et al., 2020]. In brief, WAIS IQ scores differed significantly between groups with FE-Scz and Scz having significantly lower IQ scores than Ctls ( $p$  values  $< 0.001$ ), while the patient groups did not differ from each other ( $p = 0.44$ ). Illness duration was measured in chronic schizophrenia patients. For exploratory analysis, partial correlation covarying WAIS-R

and illness duration for the Scz group was examined; we found similar results - there was a partial correlation between UPSIT and right HI ( $r=0.62$ ,  $P=0.005$ ) and between UPSIT and left HI ( $r=0.74$ ,  $P<0.0001$ ). In Pearson correlation analysis, there was no correlation between illness duration and UPSIT and between illness duration and each brain region ( $P>0.05$ ).

Significantly lower UPSIT scores were evident in patients compared with Ctls (FE-Scz,  $p < 0.01$ ; Scz,  $p < 0.0001$ ), while FE-Scz and Scz groups did not differ from each other ( $p = 0.14$ ). There was no significant group effect in volumes of interest.

Additional demographic and structural data, and analyses including UHR subgroups (total six groups comparison), are reported in the co-submission article. UHR-subgroups, FE-Scz and Scz had significantly lower WAIS scores than Ctls (all  $P<0.05$ ). There was a significant difference in UPSIT, and post-hoc testing indicated that there were significant differences between Ctls and FE-Scz ( $P<0.01$ ), Ctls and Scz ( $P<0.001$ ), UHR-NP and Scz ( $P<0.001$ ), and between UHR-other and Scz ( $P<0.001$ ). There were no differences between the UHR-Scz, FE-Scz and Scz groups ( $P>1$ ). All statistical results including volume comparisons were comparable to our previous findings (Brewer et al., 2001, 2003; Velakoulis et al., 2006; Bartholomeusz et al., 2017; Lavoie et al., 2014; Eritaia et al., 2000; Lin et al, 2015).

### 3.2 Partial correlations and multiple regression with interaction analyses

These analyses, undertaken prior to path analysis are reported in Tables 2, 3A-3B and Fig. 2 in the co-submission article. In brief, in FE-Scz there was a negative correlation between left HI and UPSIT ( $r=-0.48$ ,  $P=0.04$ ), between left AMG and UPSIT ( $r=-0.53$ ,  $P=0.02$ ), and between left rectus and UPSIT ( $r=-0.6$ ,  $P=0.01$ ).

In multiple regressions with interaction analyses, there was a significant interaction between FE-Scz and Scz for the relationship between UPSIT and left HI ( $P=0.02$ ), and between UPSIT and right HI ( $p=0.005$ ). There was a significant interaction between FE-Scz and Ctls for the association between olfactory ability and left rectus ( $P=0.05$ ). Significant interactions on these are also reflected in the path analyses.

### 3.3 Path analyses

Path analyses were used to investigate how volumes of each brain region interacted in predicting WAIS and UPSIT scores for each group. Fig. 2 shows the path diagram and standardized path coefficients for Ctls, FE-Scz and Scz. We first tested the full model, including volumes of all regions of interest, UPSIT and WAIS scores. The final path model was constructed by successively eliminating non-significant paths. For Ctls, smaller right HI volume negatively predicted IQ ( $\beta = -0.34$ ,  $P < 0.01$ ) but had no relationship with UPSIT. In the FE-Scz model, lower UPSIT was predicted by larger left rectus ( $\beta = -0.56$ ,  $P < 0.001$ ) and larger left HI ( $\beta = -0.33$ ,  $P < 0.001$ ). There was an indirect path from the left AMG to UPSIT through the left rectus ( $\beta = -0.27$ ). In the chronic Scz model, left HI volume was positively associated with UPSIT score ( $\beta = 0.42$ ,  $P < 0.001$ ), whereas the left AMG volume was negatively associated with UPSIT ( $\beta = -0.32$ ,  $P < 0.05$ ). The right AMG had an indirect effect on UPSIT, via WAIS IQ, as AMG had a direct effect on WAIS. Examination of the impact of illness duration in the path analyses for the chronic Scz group did not change our findings; paths between illness duration and UPSIT, and between illness duration and each brain region were eliminated as non-significant paths (all path,  $P > 0.05$ ).

Path analysis for UHR (combined subgroups) are reported in Fig. 3 in Data in Brief [Masaoka et al., 2020]. The analysis showed that smaller right HI and AMG volume negatively predicted IQ (HI,  $\beta = -0.34$ ,  $P < 0.05$ ; AMG,  $\beta = -0.27$ ,  $P < 0.05$ ). The left OFC and right OFC positively associated with ipsilateral rectus (left,  $\beta = 0.39$ ,  $P < 0.05$ ; right,  $\beta = 0.41$ ,  $P < 0.05$ ). There were no regions associated with UPSIT, with the path diagram showing similarities to that of Ctls.

#### 4. Discussion

In this cross-sectional study of patients at differing stages of schizophrenia, we investigated the relationship of olfactory identification ability and IQ to structural volumes of relevant olfactory-related brain areas, including AMG, HI, gyrus rectus and

OFC.

The main finding of this study was that HI, AMG and rectus volumes were significantly associated with UPSIT scores independently of IQ, and these associations differed significantly between FE-Scz and Scz illness groups. Path analyses showed a schematic view of the relationships between UPSIT, IQ and brain volume for each group (Fig 2). The findings suggest that olfactory deficits are associated with larger volumes of left HI and rectus in first-episode schizophrenia (FE-Scz). In contrast, at the chronic stage of schizophrenia (Scz), volume reduction of the left HI was associated with poor olfactory ability, indicating the opposite relationship of HI to UPSIT compared with FE-Scz. In addition, at the Scz stage the left AMG was negatively associated with UPSIT, indicating that a larger AMG volume was linked to poor olfactory ability, the same phenomenon as that observed for the left HI in FE-Scz. We speculate that the change in relationship between early and late stage illness might be explained by neuroinflammation at earlier illness stages associated with increased water content in tissue (see Cropley et al 2013; Laskaris et al, 2016; Di Biase et al., 2016), associated with larger apparent size of hippocampus and amygdala, which diminishes at the later stages of illness. These observations accord with our volumetric and T2-relaxometry findings previously reported (Velakoulis et al, 2006; Wood et al, 2010), and with evidence of immune activation related to the complement system (Laskaris et al, 2019). With regard to the AMG, this does not show the same variation in size according to illness stage (Velakoulis et al, 2006), suggesting that the association is not explained by this mechanism.

We previously (Cropley et al, 2013; Laskaris et al, 2019) suggested that brain expansion reflects swelling potentially caused by an active neurochemical or immunological process, such as inflammation or neurotoxicity, associated with processes of the acute psychosis, and that subsequent volumetric decreases are due to partial remission from the active pathophysiological process. The source of brain swelling in schizophrenia may be due to neurochemical changes involving dopaminergic or glutamatergic function (Stone et al, 2007), or processes involving inflammation (Miller et al., 2011), or HPA-axis changes (Phillips et al., 2006). Interestingly, a previous study reported that increased left HI volume was associated with transition to psychosis in a UHR sample (Phillips et al., 2002). Another study has reported that elevated T2 relaxation time is observed in the left hippocampus in UHR

individuals who later transition to a full threshold psychotic disorder, and that higher T2 signal was associated with greater level of positive symptoms (Wood et al., 2010). Further, Garver et al. (2000) demonstrated that brain volume increases, and ventricular volume reductions were associated with an exacerbation of psychosis. These findings suggest that stages when psychosis is particularly active may be associated with pathology resulting in tissue swelling in specific brain regions such as hippocampus. In support of this hypothesis, we show that HI expansion at the earlier stages of illness is associated with olfactory impairment, especially in the left HI (Velakoulis et al., 2006; Phillips et al., 2006). In contrast, in patients at a chronic stage of schizophrenia (Scz), smaller HI was associated with lower olfactory abilities. This relationship was in the opposite direction to that observed in FE-Scz. Longitudinal studies are needed to examine this further, and to examine the possible role of inflammation or other immune-related factors.

The HI plays an important role in memory retrieval (Herz, 2016; Masaoka et al., 2012) and has been associated with reduction in memory in patients with schizophrenia (Wannan et al., 2017; Wannan et al., 2018). Hippocampal function is also crucial for accurate olfactory identification; for example, memory retrieval of the perceived scent coupled with the recognition of the name/label that the given scent is associated with, requires HI involvement. Pathological changes of the HI affect olfactory identification, and olfactory impairment has been reported as a first sign of Parkinson's and Alzheimer's diseases (Doty et al., 1988, Hawkes, 2003). In this study, poor olfactory ability was observed from the UHR-Scz stage as previously reported (Brewer et al., 2001, 2003), and this sign was associated with HI and rectus volume (rectus discussed below). A dynamic relationship over the course of the illness may be relevant to dynamic changes in functions relevant to these structures, including olfactory ability and memory function. Clinically, olfactory testing might be an early indication for abnormality of the olfactory brain, which plays an important role in emotion and memory.

In addition to finding an association between larger left HI and olfactory impairment, we observed that the left rectus was also involved in olfactory ability in the FE-Scz group. The rectus has been reported to play a role in the reward system (Keating and Rossel, 2013), and emotional behavior (Delom-Martin et al., 2013), however, few studies have investigated its role in olfaction. In humans, interestingly, an increase of

gray matter volume in the gyrus rectus has been observed in professional perfumers, and the volume of the left rectus and medial OFC is positively correlated with experience as perfumers (Delon-Martin et al, 2013). In a clinical study, Takahashi et al (2014) have reported that depth of the olfactory sulcus is significantly shallower in schizophrenia patients, and suggested that olfactory sulcus depth may be a vulnerability marker of schizophrenia reflecting an early neurodevelopmental abnormality. Based on the studies above, we suggest that the rectus volume may be linked to olfactory abilities, and we conclude that an expanded rectus volume, in addition to HI volume changes, may be involved in the impairment of olfactory abilities in the first episode stages of schizophrenia.

We did not identify a relationship between OFC volume and olfactory ability. This was contrary to our expectation, as the OFC has been suggested to play a role in identification and recognition of odors (Rolls, 2004), and serves as a secondary olfactory area. Further, ascending fibers from the HI and AMG converge in the OFC for naming and recognition of odor perception (Rolls, 2004; Gottfried et al., 2002; Royet et al., 2003). It may be that OFC volume does not accurately reflect olfactory function, which may be due to the breadth of involvement of the OFC in numerous higher and lower-order processes, thus other modalities of imaging such as functional magnetic resonance imaging may be required to elucidate the role of the OFC in olfactory ability in healthy and schizophrenia populations.

## **5. Limitations and further study**

Our study was cross-sectional and the findings require validation in longitudinal studies to examine whether larger hippocampus and rectus volumes might be due to neuroinflammation. Additionally, this study did not measure neurochemical or neuroinflammatory markers to investigate the potential cause of brain swelling over the course of schizophrenia development (Cropley et al., 2013; Laskaris et al, 2016). The use of longitudinal assessments of such brain imaging markers and olfactory ability would inform the current findings and examine the dynamic changes over the course of the illness. In our study, there were more male schizophrenia patients than female patients. Group comparison was performed with ANCOVA covarying for gender,

therefore, the results may be specific both for males and females. For partial correlations, we added gender as a covariate in addition to IQ; there were significantly negative correlations between left HI and UPSIT, and between left rectus and UPSIT in FE-Scz. We used these analyses in the main text and Data in Brief. Thus, we did not specifically examine the effects of gender here; rather our main findings showing large HI and rectus associated with lower UPSIT scores were after controlling for this variable. However, future studies with larger samples of females would be important in order to investigate the relevance of gender for our findings.

Also, the samples in this study may not be representative of all FE-Scz patients, as they were selected from a larger cohort where MRI scans were also available. Despite this, the interesting findings in our analyses indicated that the structural differences of left HI and rectus between FE-Scz and Scz differed according to illness stage.

## **6. Conclusion**

Lower olfactory ability was related to volume increases in the left hippocampus and rectus in first-episode schizophrenia. In contrast, reductions of left and right hippocampi were associated with lower olfactory ability in established schizophrenia, suggesting a shift in the relationship across different illness stages. We speculate that larger hippocampus and rectus in early schizophrenia are indicative of swelling, potentially caused by an active neurochemical or immunological process, such as inflammation or neurotoxicity, which is associated with impaired olfactory ability. The volumetric decreases observed in those at later stages of schizophrenia may be due to partial remission of such an active pathophysiological process.

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## Author Statement

**Yuri Masaoka:** Conceptualization, Methodology, Formal analysis, Writing-Original draft preparation **Dennis Velakoulis:** Investigation, Resources, Data Curation **Warrick Brewer:** Investigation, Resources, Methodology, Reviewing and Editing **Vanessa Cropley:** Investigation, Resources, Reviewing and Editing **Cali Bartholomeusz:** Investigation, Resources, Reviewing and Editing **Alison Yung:** Investigation, Resources, Reviewing and Editing **Barnaby Nelson:** Investigation, Resources, Reviewing and Editing **Dominic Dwyer:** Investigation, Resources **Cassandra Wannan:** Investigation, Resources **Masahiko Izumizaki:** Reviewing and Editing **Patrick McGorry:** Reviewing and Editing **Stephen J Wood:** Reviewing and Editing **Christos Pantelis:** Conceptualization, Reviewing and Editing, Supervision, Project administration

## Conflict of interest

Christos Pantelis has participated on Advisory Boards for Janssen-Cilag, Astra-Zeneca, Lundbeck, and Servier. He has received honoraria for talks presented at educational meetings organised by Astra-Zeneca, Janssen-Cilag, Eli-Lilly, Pfizer, Lundbeck and Shire



## References

- Bartholomeusz, CF, Cropley, V.L., Wannan, C., Di Biase, M., McGorry, P.D., Pantelis, C. 2017 Structural neuroimaging across early-stage psychosis: Aberrations in neurobiological trajectories and implications for the staging model. *Aust N Z J Psychiatry*. 51(5), 455-476.
- Brewer, W.J., Pantelis, C., Anderson, V., Velakoulis, D., Singh, B., Copolov, D.L., McGorry, P.D. 2001. Stability of olfactory identification deficits in neuroleptic-naïve patients with first-episode psychosis. *Am J Psychiatry*, 158, 107-115.
- Brewer, W.J., Wood, S.J., McGorry, P.D., Fraincey, S.M., Phillips, L.J., Yung, A.R., Anderson, V. 2003. Impairment of olfactory identification ability in individuals at ultra-high risk for psychosis who later develop schizophrenia. *Am. J. Psych.* 160, 1790-1794.
- Cropley, V., Wood, S.J, Pantelis, C. 2013. Brain structural, neurochemical and neuroinflammatory markers of psychosis onset and relapse: Is there evidence for a psychosis relapse signature? *Int. Clin. Psychopharmacol.* May10.
- Delon-Martin, C., Plailly, J, Fonlupt, P., Veyrac, A., Royet, J.P. 2013. Perfumers' expertise induces structural reorganization in olfactory brain regions. *Neuroimage*. 68, 55-62.
- Di Biase, M. A., Everall, I., Chana, G., Christopoulos, A., Skafidas, E., Cropley, V. L., Pantelis, C. 2016. Microglial activation and progressive brain changes in schizophrenia. *Br J Pharmacol*, 173(4), 666-680. doi:10.1111/bph.13364
- Doty, R.L., Shaman, P., Dann, M. 1984. Development of the University of Pennsylvania Smell Identification Test: a standardized microencapsulated test of olfactory function. *Physiol. Behav.* 1984, 32:489–502

Doty, R.L., Deems, D.A., Stellar, S. 1988. Olfactory dysfunction in parkinsonism: general deficit unrelated to neurologic signs, disease stage, or disease duration. *Neurology*. 38, 1237-44.

Eritaia, J., Wood, S. J., Stuart, G. W., Bridle, N., Dudgeon, P., Maruff, P., Velakoulis, D., & Pantelis, C. 2000. An optimized method for estimating intracranial volume from magnetic resonance images. *Magnetic Resonance in Medicine*, 44(6), 973-977.

Garver, D.L., Nair, T.R., Christensen, J.D., Holcomb, J.A., Kingsbury, S.J. 2000. Brain and ventricle instability during psychotic episodes of the schizophrenias. *Schizophr. Res.* 44, 11–23.

Gottfried, J.A., Deichmann, R., Winston, J.S., Dolan, R.J. 2002. Functional heterogeneity in human olfactory cortex: an event-related functional magnetic resonance imaging study. *J. Neurosci.* 22, 10819-10828.

Hawkes, C. 2003. Olfaction in neurodegenerative disorder. *Mov. Disord.* 18, 364-372.

Herz, R.S. 2016. The role of odor-evoked memory in psychological and physiological health. *Brain Sci.* 19,6 (3).

Ho, N.F., Holt, D.J., Cheung, M., Iglesias, J.E., Goh, A., Wang, M., Lim, J.K., de Souza, J., Poh, J.S., See, Y.M., Adcock, A.R., Wood, S.J., Chee, M.W., Lee, J., Zhou, J. 2017. Progressive Decline in Hippocampal CA1 Volume in Individuals at Ultra-High-Risk for Psychosis Who Do Not Remit: Findings from the Longitudinal Youth at Risk Study. *Neuropsychopharmacology*. 42(6), 1361-1370.

Keating, C., Rossell, S. 2013. Gyrus rectus volume and the related pleasantness of the sucrose solution in eating disorder. *Am. J. Psych.* 170, 11:1366-1367.

Lavoie, S., Bartholomeusz, C. F., Nelson, B., Lin, A., McGorry, P. D., Velakoulis, D., Whittle, S. L., Yung, A. R., Pantelis, C., & Wood, S. J. 2014. Sulcogyral pattern and

sulcal count of the orbitofrontal cortex in individuals at ultra high risk for psychosis. *Schizophr Res*, 154(1-3), 93-99.

Laskaris, L.E., Di Biase, M.A., Everall, I., Chana, G., Christopoulos, A., Skafidas, E., Cropley, V.L., Pantelis, C. 2016. Microglial activation and progressive brain changes in schizophrenia. *Br J Pharmacol*. 173(4), 666-80.

Laskaris, L., Zalesky, A., Weickert, C. S., Di Biase, M. A., Chana, G., Baune, B. T., Bousman, C., Nelson, B., McGorry, P., Everall, I., Pantelis, C., Cropley, V. 2019. Investigation of peripheral complement factors across stages of psychosis. *Schizophr Res*, 204, 30-37.

Lin A, Brewer WJ, Yung AR, Nelson B, Pantelis C, Wood SJ. 2015. Olfactory identification deficits at identification as ultra-high risk for psychosis are associated with poor functional outcome. *Schizophr Res*. 161(2-3),156-62.

Mackay-Sim, A., Doty, R.L. 2001. The university of Pennsylvania Smell Identification test: normative adjustment for Australian subjects. *Australian J Oto-laryngol*. 4,174-177.

Masaoka, Y., Sugiyama, H., Katayama, A., Kashiwagi, M., Homma, I., 2012. Remembering the past with slow breathing associated with activity in the parahippocampus and amygdala. *Neurosci. Lett.* 521(2), 98-103.

Masaoka, Y, Harding, I.H, Koiwa, N, Yoshida, M, Harrison, B.J, Lorenzetti, V, Ida, M, Izumizaki, M, Pantelis, C, Homma, I. 2014. The neural cascade of olfactory processing: A combined fMRI-EEG study. *Respir. Physiol.& Neurobiol*. 204, 71-77.

Masaoka, Y., Velakoulis, D., Brewer, W., Cropley, V., Bartholomeusz, C., Yung, A., Nelson, B., Dwyer, D., Wannan, C., Izumizaki, M., McGorry, P., Wood, S.J., Pantelis, C. 2020. A data article on olfactory ability associated with olfactory brain regions at earliest stages of schizophrenia. *Data in Brief* in press.

Miller, B.J., Buckley, P., Seabolt, W., Mellor, A., Kirkpatrick, B. 2011. Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. *Biol. Psychiatry*. 70, 663–671.

Phillips, L.J., McGorry, P.D., Garner, B., Thompson, K.N., Pantelis, C., Wood, S.J., Berger, G. 2006. Stress, the hippocampus and the hypothalamic-pituitary-adrenal axis: implications for the development of psychotic disorders. *Aust. N.Z. J. Psychiatry*. 40, 725–741.

Phillips, L.J., Velakoulis, D., Pantelis, C., Wood, S., Yuen, H.P., Yung, A.R., et al. 2002. Non-reduction in hippocampal volume is associated with higher risk of psychosis. *Schizophr. Res.* 58, 145–158.

Rolls, E.T. 2001. The rules of formation of the olfactory representations found in the orbitofrontal cortex olfactory areas in primates. *Chem. Senses*. 26, 595-604.

Rolls, E.T., 2004. The functions of the orbitofrontal cortex. *Brain and Cognition* 55, 11-29.

Royet, J.P., Plailly, J., Delon-Martin, C., Kareken, D.A., Segebarth, C. 2003. fMRI of emotional responses to odors: influence of hedonic valence and judgment, handedness, and gender. *Neuroimage*. 20, 713-728.

Stone, J.M., Morrison, P.D., Pilowsky, L.S. 2007. Glutamate and dopamine dysregulation in schizophrenia—a synthesis and selective review. *J. Psychopharmacol.* 21, 440–452.

Takahashi, T., Wood, S.J., Yung, A.R., Nelson, B., Lin, A., Yucel, M., Phillips, L.J., Nakamura, Y., Suzuki, M., Brewer, W.J., Proffitt, T.M., McGorry, P.D., Velakoulis, D., Pantelis, C. 2014. Altered depth of the olfactory sulcus in ultra high-risk individuals and patients with psychotic disorders. *Schz. Res.* 153, 18-24.

Velakoulis, D., Wood, S.J., Wong, M.T.H., McGorry, P.D., Yung, A., Phillip, L., Brewer,

W., Proffitt, T., Desmond, P., Pantelis, C. 2006. Hippocampus and amygdala volumes according to psychosis stage and diagnosis. *Arch. Gen. Psych.* 63, 139-149.

Wannan C.M.J., Bartholomeusz C.F., Cropley, V.L., Van Rheenen, T.E. Panayiotou, A., Brewer, W.J., Proffitt, T.M., Henry, L., Harris, M.G., Velakoulis. D., McGorry. P., Pantelis, C., Wood, S.J. 2017. Deterioration of visuospatial associative memory following a first psychotic episode: a long-term follow-up study. *Psychol Med.* 48(1),132-141.

Wannan C.M.J., Cropley, V.L., Chakravarty, M.M., Van Rheenen, T.E., Mancuso, S., Bousman, C., Everall, I., McGorry, P.D., Pantelis, C., Bartholomeusz, C.F. 2018. Hippocampal subfields and visuospatial associative memory across stages of schizophrenia-spectrum disorder. *Psychol Med.* 4:1-11.

Wechsler, D. 1981. Wechsler Adult intelligence Scale –Revised Manual. Cleveland, Psychological Corp, 1981.

Wood. S.J., Kennedy, D., Phillips, L.J., Seal, M.L., Yucel, M., Nelson, B., et al. 2010. Hippocampal pathology in individuals at ultra-high risk for psychosis: a multi-modal magnetic resonance study. *NeuroImage.* 52, 62–68.

Yeshurun, Y., Sobel, N., 2010. An odor is not worth a thousand words: from multidimensional odors to unidimensional odor objects. *Annu. Rev. Psychol.* 61,219-41.

## Figure legends

Figure 1

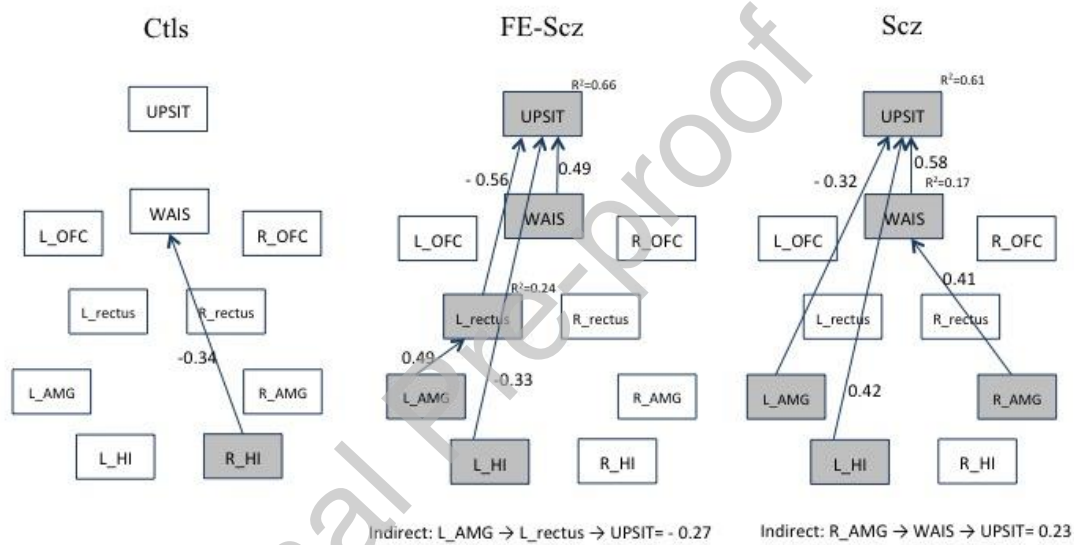


Fig.1

Path diagram and standardized path coefficients for the control, FE-Scz and Scz. R<sup>2</sup> values were indicated above each box. Path coefficients ( $\beta$ ) were considered significant at  $P < 0.05$  and model fitness close to 1 was presented.

Table 1 Demographic data, intelligence scale and olfactory abilities data of control subjects and patients.

	Controls	FE-Scz	Scz
Tota (M/F), No.	34 (24/10)	21 (17/4)	24 (21/3)
Age, y	22 ± 4.1	21.6 ± 3.9	33.1 ± 9.2 †
Height, cm	177.7 ± 9.1	174.2 ± 8	174.5 ± 7.9
Handness(R/L)	(32/2)	(18/3)	(21/3)
WAIS	111 ± 8.4	91.1 ± 11.6***	91.6 ± 16.2***
UPSIT	34.1 ± 2.9	29.3 ± 4**	26.8 ± 7****
ICV	1472869 ± 147913	1348323 ± 1542754***	1444300 ± 1089937*
WBV	1417991 ± 142098	1307000 ± 1593290**	1360703 ± 1104662*
L-HI	3036 ± 253	2856 ± 350	2873 ± 321
R-HI	3208 ± 288	3072 ± 299	2966 ± 291
L-AMG	1542 ± 216	1509 ± 197	1452 ± 252
R-AMG	1547 ± 250	1480 ± 238	1528 ± 361
L-OFC	6561 ± 817	6654 ± 661	5933 ± 702
R-OFC	6980 ± 748	7025 ± 935	6619 ± 693
L-rectus	2261 ± 308	2403 ± 389	2232 ± 338
R-rectus	1568 ± 262	1631 ± 314	1592 ± 231

Abbreviations: WAIS, Wechsler Adult Intelligence Scale; UPSIT, University of Pennsylvania Smell Identification Test; ICV, Intracranial volume; WBV, whole brain volume; HI, hippocampus; AMG, amygdala; OFC, orbitofrontal cortex; rectus, rectus gyrus. L, left; R, right.

Statistically significant compared with Ctls. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001, \*\*\*\*P<0.0001

Statistically significant compared with Ctls and FEP-Scz. †P<0.001