Age-related deficits in intracortical myelination in young adults with bipolar disorder type I

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Cannabis: A potential efficacious intervention for PTSD or simply snake oil?

Alfonso Abizaid, PhD; Zul Merali, PhD; Hymie Anisman, PhD

The ancient Egyptians used willow bark as a remedy for aches and pains, even though they were unaware that salicylic acid was responsible for its anti-pyrogenic and anti-inflammatory actions. Based on anecdotal reports and social media chatter, cannabis might yet displace salicylic acid as the most prolific cure-all. Like the bark of the willow, the marijuana plant and its derivatives have been used to diminish treatment-resistant epilepsy and to reduce chronic pain, even before it was understood that the active components of the cannabis plant, Δ9-tetrahydrocannabinol (THC) and cannabidiol (CBD), contributed to these outcomes. Cannabis is also touted to be effective in attenuating a wide range of conditions, including asthma, inflammatory bowel disease, glaucoma, multiple sclerosis, menstrual cramps, AIDS, nausea and cancer. Beyond these effects on physical conditions, cannabis has been reported to improve neurocognitive and psychiatric conditions, such as Alzheimer disease, anxiety disorders and bipolar disorder.2,3

Progress in understanding the potential positive and negative impact of cannabis within clinical situations has been limited, and only a handful of high-quality clinical trials were reported to have used cannabinoids in the treatment of numerous illnesses.2 As a result, our understanding of the potential adverse effects of chronic cannabis use remains meager. Nonetheless, there are indications that cannabis promotes cognitive disturbances, impairs neuronal plasticity and organization in the adolescent brain, promotes persistent functional brain changes, promotes abuse liability and, in highly vulnerable individuals, may exacerbate the course of schizophrenia.4,6 At the same time, it may be essential not to go overboard; caution has been recommended concerning “the real risks” of marijuana, and calls have been made for evidence-based analyses of the links between this agent and psychiatric conditions, such as Alzheimer disease, anxiety disorders and bipolar disorder.2,3

Increased attention has focused on the potential use of cannabis in the treatment of posttraumatic stress disorder (PTSD). However, as we all know, treatment of PTSD has been a hard nut to crack. Ideally, the neurobiological mechanisms of psychological disorders could be assessed using animal models.8 Yet, even under the best conditions, this can be difficult to achieve, especially in relation to PTSD. Among other things, paradigms used to model PTSD are frequently the same as those used to model depressive disorders and anxiety (e.g., freezing in response to conditioned fear cues; response to novel stimuli after exposure to uncontrollable footshock; forced swim performance following prolonged restraint).9,10 To be sure, PTSD shares several endophenotypes with depression and anxiety, making it difficult to distinguish underlying processes within animal models. Furthermore, PTSD develops in only a small portion of individuals who experience extreme stressors, whereas this is rarely considered in most animal models.10 Several published reviews of the literature have offered varied suggestions that could be instrumental in enhancing the validity of animal models. Ultimately, these models would necessarily require multiple behavioural tests to simulate the presumed symptoms of PTSD in humans, although simulating intrusive thoughts is obviously not possible. As well, it is necessary to consider sex differences, the history of traumatic encounters across the lifespan, analyses of PTSD-related genetic and epigenetic influences (e.g., methylation of FKBP5 and NR3C1 genes, which affect glucocorticoid receptors, modulate glucocorticoid sensitivity and are associated with corticotropin releasing hormone receptor 1)11,12 and possibly even actions related to trauma encountered in earlier generations that might have engendered epigenetic changes.13

To appreciate the processes that govern the development, maintenance and treatment of PTSD, it would be necessary to consider the actions of stressors on varied neurobiological processes. Ordinarily, acute stressful events influence autonomic nervous system functioning, promote gut microbial changes, stimulate the release of numerous metabolic hormones and several neurotransmitters (e.g., glucocorticoids, monoamine functioning, γ-aminobutyric acid, glutamate), and affect neurotrophin functioning as well as microglia activity and the resultant release of proinflammatory cytokines within the brain.9,14 With chronic stressor experiences, some of these same neurobiological processes may be...
sustained, eventually leading to excessive neuronal activation or taxing of critical resources (allostatic overload), favouring the development of psychological pathologies. The sheer number of stressor-provoked neurobiological changes makes it difficult to identify which of these (or their interactions) contribute to the emergence of PTSD, and indeed, the disorder is likely biochemically heterogeneous.

The brain processes that govern the development and persistence of PTSD have yet to be identified fully, and most of what is known comes from preclinical animal studies (despite the problems inherent with animal models). Sites involved in threat detection, fear learning (or alternatively disturbed fear extinction), contextual processing, emotion regulation and executive function appear salient in this regard. Accordingly, PTSD features in humans were attributed to neuronal dysfunction within the medial prefrontal cortex, anterior cingulate cortex and hippocampus and with diminished connectivity between the ventromedial prefrontal cortex and amygdala. Furthermore, low endocannabinoid (eCB) tone contributes to the amygdala hyperactivation as well as the anxiety and hyperarousal symptoms characteristic of PTSD. The hyperarousal anxiety may, in turn, be fundamental in promoting many of the most debilitating aspects of PTSD, including sleep disturbances, memory and cognitive impairments, altered pain sensitivity, as well as depression, anxiety, emotional numbing and suicidality. Hyperarousal anxiety may also serve as a driver for symptoms that comprise re-experiencing, avoidance and emotional numbing. In addition, other features of PTSD, such as dissociation, are more prominent in females than in males. These distinguishing characteristics of PTSD may offer clues as to the most efficacious treatments of the disorder.

Given that eCB processes are affected by stressors and can affect anxiety and fear, it was hypothesized that eCB functioning is tied to the development of PTSD, possibly through a corticotropin-releasing hormone-mediated reduction of anandamide in several brain regions. Paralleling this view, it was maintained that pharmacological manipulations of endogenous cannabinoids could be used in the treatment of PTSD. As in the case of many other purported benefits of cannabis, much of the supportive evidence in humans has come from anecdotal or case reports as well as observational studies that provide little evidence of a causal connection. For instance, individuals presenting with PTSD characteristics frequently use cannabis in an effort to self-medicate, reporting that the drug diminished anxiety and arousal and enhanced sleep.

Importantly, PTSD was associated with increased expression of cannabinoid receptor type 1 (CB1) and reduced peripheral levels of the eCB anandamide as well as a compensatory increase of CB1 availability, which was linked to excessive threat processing and with features of anxious arousal. In essence, a deficiency of eCB signalling reflects a stress endophenotype underlying PTSD, raising the possibility that endocannabinoid manipulations could be potentially useful in a therapeutic capacity.

There have been indications that cannabis or some of its components, primarily THC and CBD, diminish particular symptoms of PTSD. In this regard, in a small study (n = 10), 5 mg of THC twice a day as an add-on treatment enhanced sleep quality and reduced the frequency of nightmares, PTSD hyperarousal (based on the Clinician-Administered PTSD Scale) and global symptom severity. The synthetic analogue of THC, nabilone, similarly enhanced sleep, reduced nightmares and diminished other PTSD symptoms among patients. It seems, however, that the positive effects of THC in relation to PTSD are limited, leaving many features of this condition unaffected. Unfortunately, the available data showing a cannabinoïd–PTSD link in human clinical trials have been relatively sparse and yielded mixed results, ranging from ameliorated symptoms to cautions concerning its efficacy. Furthermore, because PTSD is so often comorbid with depressive illnesses and anxiety disorders, it is uncertain whether the eCB links reflect a direct causal connection to PTSD or actions related to anxiety or depression.

In addition to potentially reducing PTSD symptoms, cannabis also mitigates the propensity for inflammation and may be useful in psychological conditions that involve elevated inflammatory processes within the brain. This would include a subset of depressed individuals in whom inflammation may be a component of the illness and may contribute to threat processing linked to PTSD in trauma survivors. In fact, anti-inflammatory agents can diminish PTSD features in an animal model, and in humans PTSD was accompanied by elevated circulating proinflammatory cytokines. Thus, cannabinoids could potentially act against PTSD by activation of cannabinoid receptor type 2 (CB2) receptors, which promote anti-inflammatory actions involving microglia.

Behavioural and neurobiological changes in rodents vary as a function of the nature of the stressor experienced and its chronicity and vary with the passage of time, as observed in the development of neuronal sensitization. The evolution of PTSD may likewise involve dynamic processes, including a phase soon after trauma, wherein features of the illness incubate and emerge with time-dependent variations in the sensitization of neuronal functioning. Whether further changes in the processes subserving PTSD develop over the ensuing months is uncertain; nor is it clear whether different treatments at various phases of the illness would be most effective. It has been maintained that treatment with cortisol (or related compounds) or those that affect norepinephrine at specific post-trauma periods may prevent the development of PTSD, possibly by affecting the consolidation or reconsolidation of fear-related memories. The actions of cannabinoids on PTSD symptomatology may likewise come about owing to any number of processes, including disruption of fear memory consolidation, decreasing salience of ordinarily significant stimuli, or facilitating the extinction of fear memories. As cannabinoid variations may function as a fundamental component of adaptation to a stressor, such changes may also evolve with time following trauma experiences, as observed in relation to trauma memories. It should also be considered that the cluster of PTSD symptoms as well as the magnitude and type of trauma experienced in humans vary over time.
following the trauma and in turn that the efficacy of cannabinoid-related treatments may also vary.38

Much still needs to be assessed concerning the efficacy and safety of cannabis in treating PTSD and other conditions. Among other things, questions remain concerning effective doses for different conditions, how long the drug needs to be taken before positive effects can be expected, potential sex differences in the effectiveness of cannabinoid action, and to what extent adverse outcomes can be expected in some people. Moreover, given the differential effects of THC and CBD in relation to affective behaviours and cognitive functioning,40 it is necessary to determine the ratio of the different cannabis components (e.g., THC in relation to CBD) that are most effective at promoting therapeutic effects while minimizing adverse effects. It is also possible that cannabis or some of its components might serve as a useful tool in creating openness or a bridge to assist psychotherapy, much as 3,4-methylenedioxymethamphetamine might have such actions,41 although perhaps through different mechanisms.42

Considerable research in animals has pointed to benefits of cannabinoids in the treatment of PTSD. Legal restrictions that existed regarding access and use of cannabis had, unfortunately, limited the evaluation of the medical use of cannabis in humans,39 including treatment of psychological disorders. The limited research in humans has nevertheless suggested that cannabis can ameliorate particular features of PTSD. However, these studies had a small number of participants, did not distinguish between the conditions that promoted the disorder (e.g., an acute traumatic experience, chronic stressor encounters, multiple trauma experiences), and did not consider the time at which the disorder was treated relative to when the trauma was experienced. In effect, the studies were typically of low quality, and large-scale studies remain to be conducted evaluating the efficacy of cannabis use in the treatment of PTSD. Nonetheless, the preclinical studies, together with the few clinical studies reported, support further detailed investigation into the use of cannabinoids in the treatment of PTSD. As in the case of so many other medical conditions for which new treatments have emerged at a rapid pace, it is important to distinguish between actual remedies versus those that are simply hopes. It is unfortunate that research pertaining to cannabis safety and efficacy for various illnesses has not kept pace with social reforms concerning its use. As indicated in a recent headline, “Legal weed is everywhere — unless you’re a scientist.” The recent legalization of cannabis within Canada may provide the opportunity to conduct the necessary research to determine whether cannabis-based treatments are more than snake oil.

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Competing interests: The Institute of Mental Health, headed by Z. Merali, received a Chair in Military Mental Health from the Department of National Defense. No other competing interests declared.

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Age-related deficits in intracortical myelination in young adults with bipolar disorder type I

Manpreet Sehmbi, PhD*; Christopher D. Rowley, PhD*; Luciano Minuzzi, MD, PhD; Flavio Kapczinski, MD, PhD; Jacek M. Kwiecien, DVM, MSc, PhD; Nicholas A. Bock, PhD; Benicio N. Frey, MD, MSc, PhD

Introduction

White-matter abnormalities are among the most consistently replicated neurobiological findings in people with bipolar disorder. Neuroimaging studies focusing on subcortical tracts have linked white-matter abnormalities with a greater number of hospitalizations, increased treatment resistance, suicide attempts and history of psychosis. White matter is composed primarily of myelinated axons: a myelin sheath formed by oligodendrocytes acts as an electrical insulator, allowing for faster axonal signal propagation via saltatory conduction and increased signal integrity. Myelin also provides trophic support essential for neuronal survival. As well, oligodendrocyte precursor cells can receive presynaptic input from neurons and respond to neurotransmitters, allowing them to regulate neural circuits. Myelin is essential for establishing and maintaining neuronal circuitry, and myelinated fibres are widely distributed throughout the brain, including in the cerebral cortex. In healthy humans, the development of intracortical myelin (ICM) follows an inverted-U trajectory and occurs in a heterochronous pattern: association areas (frontal, temporal and parietal lobes) commence myelination after primary process areas (motor and sensory cortices). Intracortical myelin consists of myelinated axons in the cortical grey matter; deeper layers of the cortex contain greater amounts of myelin. Importantly, animal studies have begun to link ICM with changes in behaviour and show that neuronal activity stimulates proliferation of oligodendrocyte precursor cells in the cortex and promotes oligodendrogenesis. This thickening of the myelin sheath enhances motor learning in mice. Furthermore, mice subjected to social isolation early in life displayed long-standing thinning of the myelin sheath of cortical axons and worse cognitive performance in adulthood. Extensive research has shown both grey-matter and white-matter abnormalities in bipolar disorder, but most knowledge

Background: Previous studies have implicated white-matter-related changes in the pathophysiology of bipolar disorder. However, most of what is known is derived from in vivo subcortical white-matter imaging or postmortem studies. In this study, we investigated whole-brain intracortical myelin (ICM) content in people with bipolar disorder type I and controls. Methods: Between Sept. 1, 2014, and Jan. 31, 2017, we used a 3 T General Electric scanner to collect T1-weighted images in 45 people with bipolar disorder type I and 60 controls aged 17 to 45 years using an optimized sequence that was sensitive to ICM content. We analyzed images using a surface-based approach. We used general linear models with quadratic age terms to examine the signal trajectory of ICM across the age range. Results: In healthy controls, the T1-weighted signal followed an inverted-U trajectory over age; in people with bipolar disorder type I, the association between ICM and age followed a flat trajectory (p < 0.05, Bonferroni corrected). Exploratory analyses showed that ICM signal intensity was associated with duration of illness, age of onset, and anticonvulsant and antipsychotic use in people with bipolar disorder type I (p < 0.05, uncorrected). Limitations: Because of the cross-sectional nature of the study, we were unable to comment on whether the effects were due to dysmyelination or demyelination in bipolar disorder. Conclusion: This foundational study is, to our knowledge, the first to show global age-related deficits in ICM maturation throughout the cortex in bipolar disorder. Considering the impact of myelination on the maintenance of neural synchrony and the integrity of neural connections, this work may help us better understand the cognitive and behavioural deficits seen in bipolar disorder.
about white-matter anomalies stems from subcortical anatomic or diffusion tensor imaging studies of white-matter tracts. Postmortem human studies report fewer overall oligodendrocytes, a decreased ratio of oligodendrocytes per neuron and lower expression of myelin-related genes in the prefrontal cortex of people with bipolar disorder,14-18 all suggesting possible ICM deficits in bipolar disorder.

Fortunately, it is now possible to map ICM across the cortical surface in humans, using high-resolution MRI. For instance, the MRI tissue parameter longitudinal relaxation time (T₁) and its inverse, longitudinal relaxation rate (R₁ = 1/T₁), were sensitive to the presence of myelin in the cortex in a nonhuman primate MRI and histology study,19 and in a post-mortem human MRI and histology study.20 Furthermore, T₁-weighted images and R₁ maps have been used to map myelin densities in humans over the entire cortex,21-24 colocalize specific auditory25 and visual26 areas for functional MRI, and map ICM changes associated with age.7

In the present study, we studied ICM in young adults with bipolar disorder using an optimized MRI technique that has been validated for ICM measurement in humans and non-human primates.27,28 We hypothesized that people with bipolar disorder would show deficits in ICM relative to matched controls. In exploratory analyses, we investigated the association between ICM and clinical variables such as age of onset; number of manic, depressive, mixed or hypomanic episodes; duration of illness; lifetime psychosis; and medication use.

Methods

Study participants

This study was approved by the Hamilton Integrated Research Ethics Board, and signed informed consent was obtained from each participant. We imaged 49 people with a diagnosis of bipolar disorder type I (28 female, 21 male) and 67 matched healthy controls (37 female, 30 male). All participants were right-handed and aged 17 to 45 years. All female participants were premenopausal. All participants completed the Structured Clinical Interview for DSM-IV to confirm diagnosis. Controls did not meet criteria for any current or lifetime axis I psychiatric conditions. Participants with bipolar disorder type I did not meet criteria for any current axis I psychiatric comorbidities. Other exclusion criteria were unstable medical or inflammatory conditions, alcohol or substance abuse within the last year (excluding caffeine or nicotine), past or current history of neurologic disorders (including head trauma and migraines) or any MRI contraindications.

Imaging acquisition

We acquired the images on a 3 T General Electric scanner (software version 22.0) using a 32-channel receive-only radiofrequency coil for the head (MR Instruments) and a transmit radiofrequency body coil (General Electric) to produce a T₁-weighted image with optimized intracortical contrast for ICM analysis.28,29 All images were acquired with 1 mm isotropic resolution, and the total time for the protocol was about 35 minutes. We recently reported age-related ICM mapping in healthy controls.9 The imaging protocol is summarized below, with further details and parameter specifications available in our previous work and in the Supplementary Methods (Appendix 1, available at jpn.ca/170220).

First, we acquired a typical 3D T₁-weighted anatomic reference image of the whole head using a 3D inversion-recovery gradient echo sequence (GE 3D BRAVO). We used this image for registration in processing.

Next, we acquired another 3D T₁-weighted whole-head image with strong intracortical contrast using an inversion-recovery gradient echo sequence (GE 3D BRAVO) to form the basis of our ICM maps.

Finally, we collected a 3D proton-density-weighted image of the whole head to normalize intensity inhomogeneities in the T₁-weighted image with high intracortical contrast that would otherwise obscure ICM patterns. This image was made using a 3D gradient-echo sequence (GE 3D SPGR). We registered the proton-density-weighted image to the T₁-weighted image with high intracortical contrast using a 6-parameter rigid transform (FSL) and filtered it using a 3D median filter with a kernel size of 5 × 5 × 5 mm. We then divided the T₁-weighted image by the filtered proton-density-weighted image to create the ratio image, which was a strongly T₁-weighted image that removed the radiofrequency receive field (B₁−) inhomogeneity, radiofrequency transmit field (B₁+) inhomogeneity and T₁*-weighting arising from the gradient echo readout in the inversion-recovery image. The ratio image still contained some B₁+ inhomogeneity from the magnetization preparation portion of the inversion-recovery pulse sequence (see Bock and colleagues29 for the mathematical derivation of the ratio image from magnetic resonance signal equations).

ICM processing

We performed image processing to map ICM content in the cortex of each participant and register participants’ maps to a common space for group analysis. To map ICM in a participant, we found the pial surface and a surface at the grey-matter/white-matter boundary of the cortex. We calculated a new surface at the middle depth of the cortex, and we mapped the signal intensity of the ratio image onto this surface to depict ICM. This process is further described in Appendix 1, Supplementary Methods.

Statistical analysis

Demographics

We completed all statistical analyses using R version 3.3.2 (https://www.r-project.org). We used the Shapiro–Wilks test and the Bartlett test to determine the normality and homogeneity of variance for continuous variables, respectively. We tested between-group differences in age, sex, body mass index (BMI), years of education and smoking status using 2-tailed independent t tests, Mann–Whitney tests, or χ² tests, as appropriate (Table 1).
Age and ICM content
We used the MarsAtlas\(^\text{30}\) to parcellate the cortex into 82 regions of interest (ROIs) for analysis. We generated ICM maps using Surfstat (http://www.math.mcgill.ca/keith/surfstat/) in Matlab (version R2015a). We investigated signal intensity in the ratio image as a function of age for the ROIs using a general linear model: age + age\(^2\) + BMI + diagnosis + diagnosis \(\times\) age\(^2\), where diagnosis was the participant diagnosis. By investigating the interaction term diagnosis \(\times\) age\(^2\), we could determine whether a diagnosis of bipolar disorder affected the inverted-U shape of the ICM trajectory seen in healthy participants. We included BMI as a covariate because it influences white-matter integrity.\(^\text{31}\) We have recently shown that there were no sex differences in ICM trajectory over age in healthy individuals.\(^\text{9}\) We were unable to analyze 6 ROIs per hemisphere (12 in total) because of topological errors in identifying the pial or grey-matter/white-matter boundary surfaces in the cortex. These corresponded to the isthmus of the cingulate, the insula, the rostral medial visual cortex, the medial and rostral inferior temporal cortex and the rostral superior temporal cortex. We used the remaining 70 regions to compare age trajectories in \(T_1\)-weighted signals between controls and people with bipolar disorder type I. We averaged the signal across each ROI and analyzed the findings in R. We extracted \(p\) values for the linear model and the interaction term from the general linear models. We calculated partial \(\eta^2\) to determine the effect size of the interaction term, and to visualize the amount of variance in the data that could be attributed to the interaction term. We used coefficients for age\(^2\) to determine the shape of the ICM signal trajectory over age in each population. We used bootstrapping analysis to verify whether findings related to the interaction term in the general linear models were driven by outliers.

History of psychosis
To determine whether history of psychosis affected ICM, we ran general linear models in each ROI in the bipolar disorder type I group only: age + age\(^2\) + BMI + psychosis + psychosis \(\times\) age\(^2\), where psychosis was a binary variable (1 if the person with bipolar disorder type I had experienced lifetime delusions or hallucinations, 0 if they had not). Our goal was to investigate the final interaction term to determine whether a history of psychosis had any effect on ICM over age after controlling for BMI.

Clinical correlates
As per Foland-Ross and colleagues,\(^\text{32}\) we used partial correlation analysis to determine the potential effects of age of onset, duration of illness and number of episodes (depressive, manic, hypomanic, mixed) on ICM throughout the cortex in participants with bipolar disorder type I, both individually and as a total sum. We included age, sex and BMI as covariates in all analyses, except when the dependent variable was expected to be highly collinear with age, such as duration of illness. In that case, we included only sex and BMI as covariates.

Medications
We categorized medications for each participant as lithium, antidepressants, antipsychotics, anticonvulsants and anxiolytics, and we coded the dose for each class as 0 (absent), 1 (low) or 2 (high).\(^\text{33}\) We converted anticonvulsants and antidepres- sant doses to high/low groupings according to Sackeim.\(^\text{34}\) We grouped levels 1 and 2 as low-dose, and levels 3 and 4 as high-dose. We converted antipsychotics to chlorpromazine dose equivalents. Participants who took a dose equivalent below the mean effective daily dose were defined as low-dose, and participants who took a dose equivalent at or above the mean effective daily dose were defined as high-dose.\(^\text{35}\) We converted anxiolytic doses to lorazepam dose equivalents, and classified them as low-dose or high-dose using the Physician’s Desk Reference recommended daily dose median.\(^\text{36}\) We summed all individual low-/high-dose classifications to determine the total medication load. We used partial correlation analysis to determine the effects of individual medication classes and the composite medication score on ICM in bipolar disorder type I using age, sex and BMI as covariates.

Results
Five participants from the control group and 2 from the bipolar disorder type I group were excluded from the analysis because of missing BMI values. As well, 2 each from the control and bipolar disorder type I groups were removed because of poor-quality ICM maps. We conducted the final age trajectory analysis with 60 participants in the control group and 45 in the bipolar disorder type I group.

Demographics
Controls and participants with bipolar disorder type I were well matched according to age, sex, BMI, years of education and smoking status (Table 1). The clinical characteristics of the study population are described in Table 2.

Age trajectories of the ICM signal
Consistent with previous literature,\(^\text{7}\) the ICM signal followed an inverted-U trajectory over age in controls, but it was sig- nificantly flattened over age in participants with bipolar

| Table 1: Sociodemographic characteristics of the study population (\(n = 105\))\(^\text{*}\) |
|-----------------|-----------------|-----------------|-----------------|
| Characteristic | Control (\(n = 60\)) | Bipolar disorder type I (\(n = 45\)) | Test statistic | \(p\) value |
| Age, yr | 30.7 ± 8.3 | 31.9 ± 8.1 | \(t = 0.73\) | 0.47 |
| Female, \(n\) (%) | 32 (53.3) | 25 (55.6) | \(\chi^2 = 0.82\) | 0.84 |
| Body mass index, kg/m\(^2\) | 25.6 ± 5.2 | 26.6 ± 4.4 | \(t = 1.00\) | 0.32 |
| Education, yr | 15.9 ± 2.8 | 15.9 ± 3.3† | \(t = 0.09\) | 0.92 |
| Smoking status, \(n\) (%) | 16 (26.7) | 13 (28.9) | \(\chi^2 = 5.37\) | 0.07 |
| Past | 2 (3.3) | 7 (15.6) |

\(^\text{*}\)Data are presented as mean ± standard deviation unless otherwise specified. |
\(^\text{†}\)Missing data for 1 participant.
Table 2: Clinical characteristics of the study population*†

<table>
<thead>
<tr>
<th>Characteristic</th>
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<td>Age at onset, yr</td>
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<td>Duration of illness, yr</td>
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<td>Current mood state, n</td>
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<td>Depressed</td>
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<td>Manic</td>
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<td>Hypomanic</td>
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<tr>
<td>Mixed</td>
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<td>MEDRS score</td>
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<td>Control</td>
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<tr>
<td>Euthymic</td>
<td>4.9 ± 5.6</td>
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<td>YMRS score</td>
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<tr>
<td>Euthymic</td>
<td>2.3 ± 3.9</td>
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<td>Lifetime psychosis, n (%)</td>
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<td>Medication use, n‡</td>
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<td>Mixed</td>
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</tr>
<tr>
<td>Total</td>
<td>40.0 ± 46.9</td>
</tr>
<tr>
<td>Lifetime comorbid psychiatric diagnoses, n</td>
<td></td>
</tr>
<tr>
<td>Panic disorder</td>
<td>14</td>
</tr>
<tr>
<td>Agoraphobia</td>
<td>10</td>
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<tr>
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<td>14</td>
</tr>
<tr>
<td>Obsessive–compulsive disorder</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Posttraumatic stress disorder</td>
<td>8</td>
</tr>
<tr>
<td>Generalized anxiety disorder</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Anorexia nervosa</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Binge-eating disorder</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Alcohol dependence</td>
<td>5</td>
</tr>
<tr>
<td>Alcohol abuse</td>
<td>14</td>
</tr>
<tr>
<td>Substance dependence</td>
<td>7</td>
</tr>
<tr>
<td>Substance abuse</td>
<td>13</td>
</tr>
</tbody>
</table>

MADRS = Montgomery–Åsberg Depression Rating Scale; YMRS = Young Mania Rating Scale.

*Findings are for the bipolar type I group unless otherwise specified. Control group, n = 60; bipolar type I group, n = 45. Findings that applied to fewer than 5 participants have been suppressed owing to data privacy guidelines.
†Data are presented as mean ± standard deviation unless otherwise specified.
‡Most participants were on polypharmacy; therefore medication count totals are greater than the sample size (n = 45).

disorder type I, suggesting age-related deficits in myelin maturation in young adults with bipolar disorder (Fig. 1).

General linear models met the conditions required for analysis (linearity, normality, homoscedasticity and independence) and were Bonferroni-corrected, such that only models with a p value < 0.0007 (α = 0.05/70 tests) were considered significant. The interaction of diagnosis × age2 showed that the ICM signal trajectory over age was significantly different in participants with bipolar disorder relative to controls in several regions of the frontal, parietal and temporal cortices, most predominantly in the left hemisphere (Fig. 2). This suggests that ICM age-related deficits in young adults with bipolar disorder are widespread and not localized.

We calculated partial η² for diagnosis × age2 and mapped it onto the cortex (Fig. 3). In most ROIs, we observed an effect between 0.01 and 0.1, with the largest effects in the motor and premotor regions, mirroring the regions of greatest difference between bipolar disorder and controls seen in Figure 2.

To analyze the trajectories, we fitted each diagnostic group with predictors (BMI + age + age2) and mapped coefficients for age2 on the cortex (Fig. 4). Consistent with the diagnosis × age2 analyses, the coefficient was largely negative in controls, corresponding to an inverted-U trajectory for ICM; coefficient values in participants with bipolar disorder were much closer to 0.

We used bootstrapping analysis to explore any effect of outliers on the significance of diagnosis × age2 within each ROI (Appendix 1, Table S1). In the analysis, we drew samples of 105 participants with replacement over 10000 iterations to calculate confidence intervals on the interaction term. These samples did not necessarily contain potential outliers, making bootstrapping insensitive to outliers. The results showed that in most regions of the cerebral cortex, the significance of diagnosis × age2 was conserved with bootstrapping analysis, further supporting the main results shown in Figure 2. In the bilateral superior visual cortex, left caudal middle temporal cortex, left dorsal inferior parietal cortex, right rostral dorsal prefrontal cortex and right ventromedial orbitalfrontal cortex, the significance of diagnosis × age2 was lost following bootstrapping. In contrast, diagnosis × age2 became significant in the right rostral middle temporal cortex following bootstrapping and remained significant in all other cortical regions.

Intracortical myelin and clinical correlates

Age of onset of bipolar disorder was positively correlated with the ICM signal in regions of the bilateral parietal, premotor and prefrontal cortices, and in the left somatosensory cortex (R = 0.24–0.36, p = 0.002–0.046; Appendix 1, Table S2) suggesting that earlier age of onset was associated with less ICM.

Duration of illness was negatively correlated with the ICM signal in the bilateral visual, temporal, parietal, cingulate and premotor cortices, the bilateral cuneus, the left somatosensory and motor cortices, and the right prefrontal cortex (R = −0.24 to 0.37, p = 0.005–0.049; Appendix 1, Table S2), suggesting that a longer duration of illness was associated with less ICM.

The number of depressive episodes was not significantly associated with the ICM signal in any cortical region. The number of manic episodes was negatively correlated with the
ICM signal in the rostral middle temporal cortex ($R = -0.24$, $p = 0.047$). The number of hypomanic episodes was negatively correlated with the ICM signal in the bilateral posterior cingulate cortex (left $R = -0.27$, $p = 0.03$; right $R = -0.31$, $p = 0.009$; Appendix 1, Table S3) and the right anterior cingulate cortex ($R = -0.27$, $p = 0.02$; Appendix 1, Table S3). The number of mixed episodes was positively correlated with the ICM signal in regions of the bilateral orbitofrontal, temporal and prefrontal cortices, and in the right visual, parietal and somatosensory cortices ($R = 0.24–0.42$, $p = 0.0003–0.04$; Appendix 1, Table S3). In these exploratory analyses, only the correlation between the number of mixed episodes and ICM in the left ventromedial prefrontal cortex survived Bonferroni correction ($R = 0.42$, $p = 0.04$; Appendix 1, Table S3).

The total number of episodes was not significantly associated with ICM signal in any cortical region (Appendix 1, Table S3). Similarly, the interaction of psychosis × age$^2$ was not significant in any model, suggesting no differences in ICM signal between participants with bipolar disorder who did or did not have a history of psychosis.

**Medication**

Lithium, antidepressant and anxiolytic use were not significantly associated with the ICM signal in any cortical region. Anticonvulsant use was negatively correlated with the ICM signal in regions of the bilateral prefrontal and right orbitofrontal cortices ($R = -0.23$ to $-0.25$, $p = 0.03–0.04$; Appendix 1, Table S4). Antipsychotic use was negatively correlated with the ICM signal in regions of the bilateral parietal, cingulate and motor cortices, the right cuneus and the left visual cortex ($R = -0.25$ to $-0.34$, $p = 0.003–0.04$; Appendix 1, Table S4). However, none of these medication analyses survived Bonferroni correction.

Total medication load was negatively associated with the ICM signal in regions of the bilateral visual and motor cortices, the right cuneus, the parietal and somatosensory cortices, and the left posterior cingulate cortex ($R = -0.25$ to $-0.38$, $p = 0.001–0.04$; Appendix 1, Table S4). The total medication count was negatively associated with the ICM signal in regions of the bilateral motor, premotor and prefrontal cortices, the left

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**Fig. 1**: Intracortical myelin signal over age in people with bipolar disorder (BD) ($n = 45$) and healthy controls ($n = 60$). Half-depth $T_1$-weighted intracortical myelin signal trajectory with age plotted in healthy controls and participants with bipolar disorder type I. The 4 chosen regions of interest were areas typically associated with bipolar disorder. In all regions, healthy controls showed an inverted-U quadratic trajectory of intracortical myelin signal over age. This association was lost in people with bipolar disorder, where the association of intracortical myelin signal over age was severely blunted. ACC = anterior cingulate cortex; Cu = cuneus; OFC = orbital frontal cortex; Pfrdls = rostral dorsolateral superior prefrontal cortex.
visual and orbitofrontal cortices, and the right temporal, parietal and somatosensory cortices (R = −0.24 to −0.33, p = 0.005–0.049; Appendix 1, Table S4). None of these analyses survived Bonferroni correction.

Discussion

Our main finding was that the age-related trajectory of the ICM signal in bipolar disorder did not follow the same inverted-U pattern seen in controls over young adulthood; this finding suggests that bipolar disorder is associated with age-related deficits in ICM development and/or maturation. These age-related deficits are widespread and primarily affect the frontal, parietal and temporal cortices. We observed the strongest effects in the motor and premotor regions, followed by the prefrontal and parietal regions.

Using a 1.5 T scanner, Jørgensen and colleagues recently reported increased T1-weighted grey-matter/white-matter contrast in bipolar disorder in the motor cortex only. The motor cortex is one of the most heavily myelinated cortical regions, and differences in this area are more readily identified using a variety of techniques, including increased T1-weighted grey-matter/white-matter contrast. Using a novel technique developed and optimized for enhanced sensitivity to the ICM signal, we found that age-related deficits in ICM occurred in several areas of the cerebral cortex in bipolar disorder. Previous techniques likely had insufficient sensitivity to detect differences in ICM in more lightly myelinated cortical regions. Our imaging methodology seems to endorse the increased sensitivity required for global analysis of ICM, considering the high variability in ICM content throughout the cortex.

Our results were consistent with a large study (n = 6503) that showed widespread patterns of reduced cortical thickness in frontal, temporal and parietal regions in people with bipolar disorder. They were also consistent with a recent systematic review that showed decreased cortical thickness in the left anterior cingulate/paracingulate and the left superior temporal gyrus, as well as several prefrontal regions in bipolar disorder. In the ENIGMA study, the strongest effects were observed in the left cortex — specifically in the left pars opercularis, left fusiform gyrus and left rostral middle frontal cortex. Our findings were consistent with a more prominent left lateralization of age-related ICM deficits. It is conceivable that abnormal cortical thickness in bipolar disorder may be due in part to changes in ICM, because ICM content can affect overall thickness measures. Although the literature emphasizes a propensity toward right-hemisphere effects in bipolar disorder, a number of studies have also shown left-hemisphere

![Fig. 2: The p values for the interaction term from the linear model of diagnosis × age2. All shaded regions (except those outlined in black) show significantly different trajectories of intracortical myelin signal over age between participants with bipolar disorder (n = 45) and healthy controls (n = 60). The p value for diagnosis × age2 was < 0.05, and the p value for the model was < 0.0007 following Bonferroni correction for 70 regions. We observed significant interactions between diagnosis and age2 (bipolar disorder v. control) throughout the medial and lateral cortices, with a potential left lateralization in the posterior lateral cortex. The effect was largely bilateral in frontal cortices. L = left hemisphere; R = right hemisphere.](image-url)

![Fig. 3: Partial η2 as an estimate of effect size for the interaction of diagnosis × age2 in the prediction of intracortical myelin signal. Larger partial η2 values suggest that diagnosis × age2 explains a greater degree of variance in T1-weighted signal related to intracortical myelin. Thus, increased partial η2 could be highlighting areas of strongest difference between healthy controls (n = 60) and participants with bipolar disorder (n = 45) in this age range. We observed the largest effect sizes in motor and premotor regions. Left prefrontal regions also showed notable effect sizes, although we observed small to medium effect sizes throughout the medial and lateral cortices. Regions with the greatest effect size corresponded with regions of greatest difference between participants with bipolar disorder and controls (Fig. 2). L = left hemisphere; R = right hemisphere.](image-url)
abnormalities. For example, functional imaging studies using both positron emission tomography and single-photon emission computed tomography have shown increased activation in the left hemisphere in the frontal, prefrontal and temporal lobes, and in the cingulate cortex, in bipolar disorder.

Further, many studies have highlighted hemispheric asymmetries in bipolar disorder in relation to white-matter structures specifically. In a recent study of the lateralization of white-matter abnormalities in psychotic disorders, Ho and colleagues showed that compared to healthy controls, participants with bipolar disorder displayed a greater left-biased laterality index of fractional anisotropy in several regions of frontal, subcortical and fronto-occipital white-matter tracts. Further, a study of twin pairs showed significantly decreased white-matter volume in the left hemispheres of participants with bipolar disorder and their unaffected twins, highlighting a potential etiological relationship between white-matter damage in the left hemisphere and bipolar disorder. Our finding of age-related deficits in ICM development that were biased toward the left hemisphere illustrate a potential new microstructural property that may underlie some previously described observations, such as altered cortical function and structure. Our results also support the findings of many postmortem studies, which show deficits in cortical oligodendrocyte count and density in bipolar disorder. Future studies into cortical changes in bipolar disorder might also benefit from multiparametric MRI to more specifically identify brain pathology. Examples include combining R1 and magnetization transfer-weighted mapping to increase specificity for ICM, and diffusion-weighted MRI to assess changes in axonal and dendritic densities in the cortex.

Recent animal studies have demonstrated the dynamic plasticity of ICM and its relation to behaviour. For instance, an optogenetic study in mice showed that neuronal activity stimulates oligodendrocyte precursor cell proliferation in the cortex, promoting oligodendrogenesis and leading to an increased thickness of the myelin sheath. Ultimately, these microstructural changes were associated with behavioural modulation in these mice. As well, reduced thickness of the myelin sheath in the medial prefrontal cortex caused by early-life social isolation has been linked with long-standing deficits in cognitive performance in adult mice. It is conceivable that the age-related ICM deficits in bipolar disorder that we saw in the current study may be associated with emotional dysregulation and cognitive dysfunction, both of which are commonly observed in the course of bipolar disorder. Future studies should investigate the relation between ICM and cognitive/emotional processing in bipolar disorder.

Interestingly, the strongest effect sizes for age-related ICM deficits in our study were in the premotor and motor areas. The motor cortex is one of the most heavily myelinated regions, among other myelin-rich areas such as the...
primary visual, auditory and somatosensory cortices. It is unique, however, because motor regions continue to myelinate for up to 4 years past other regions, peaking around 38 years of age. Notably, motor-speed dysfunction has been shown in people with bipolar disorder and their first-degree relatives, and has recently been identified as an endophenotype of bipolar disorder. Motor-speed deficits have also been seen in children of women with bipolar disorder as young as 1 year of age. Deficits in ICM in these brain regions may account, at least in part, for these behavioural observations.

Our exploratory analyses suggest that illness characteristics such as earlier age of onset, longer duration of illness and more manic/hypomanic/mixed episodes may be associated with decreased ICM. However, these results should be considered preliminary and must be confirmed in future studies, ideally with a longitudinal design. Participants with bipolar disorder in the current study reported illness onset during adolescence, and this is also commonly reported in the literature. Several lines of evidence have implicated abnormal myelination in the onset of psychiatric disorders, because the peak onset of many psychiatric disorders coincides with a period of intense myelin development. As well, abnormalities in white matter have routinely been identified during the first episode of bipolar disorder and in offspring and first-degree relatives of people with bipolar disorder. Our results were consistent with studies showing that longer duration of illness and the number of mood episodes were associated with declining grey-matter and white-matter integrity. Another preliminary finding of the present study was that the total medication load and the use of antipsychotics and anticonvulsants in particular may affect ICM. Unfortunately, we were unable to determine whether this finding was a direct effect of medication use or an effect of illness severity, because people who are more ill tend to take more medications. Longitudinal studies are needed to examine the potential effects of psychotropics on myelination in vivo.

Limitations

Limited by the cross-sectional design of the current study, we were precluded from examining the progressive impact of illness burden on ICM. Longitudinal studies are needed to confirm our results; help determine whether the effects seen here were due to dysmyelination or demyelination in bipolar disorder; and understand the relationship between ICM, risk of mood relapse and medication use. We did not assess other clinical factors that may affect ICM, such as childhood trauma, medical and psychiatric comorbidities, and lifetime alcohol or substance use. Further, our study sample consisted of young adults between the ages of 17 and 45 years. Samples of children and older adults may display distinct ICM trajectories over age. For instance, a recent study investigated cortical thickness from childhood to the mid-30s in people with autism-spectrum disorder and found an initial period of delayed cortical thinning, followed by a period of rapid cortical thinning that seemingly overcompensated for the initial delay. As previously mentioned, cortical thickness and ICM are likely intertwined, and the trajectory of ICM over age may vary depending on the age range in question. It also remains to be determined whether ICM changes are accompanied by changes in the integrity and strength of axonal signal propagation. Investigation of ICM in relation to cognitive performance will be important for determining the functional consequences of aberrant ICM maturation over age in bipolar disorder.

Conclusion

Our study provides the first evidence of widespread age-related deficits in ICM maturation throughout the cerebral cortex in young adults with bipolar disorder. Considering the potential role of ICM in establishing and maintaining neuronal circuitry and its ability to affect behavioural performance, future work should investigate the role of ICM in behavioural, cognitive and emotional processing in bipolar disorder.

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Contributors: L. Minuzzi, N. Bock and B. Frey designed the study. M. Sehmbi acquired and analyzed the data, which C. Rowley, F. Kapczinski, J. Kwiecien, N. Bock and B. Frey also analyzed. M. Sehmbi wrote the article, which all authors reviewed. All authors approved the final version to be published and can certify that no other individuals not listed as authors have made substantial contributions to the paper.
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Brain grey-matter volume alteration in adult patients with bipolar disorder under different conditions: a voxel-based meta-analysis

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Introduction

Bipolar disorder is a common chronic psychiatric disorder that often results in permanent disability. It affects more than 2% of the general population and is characterized by mood disturbances with recurrent episodes of mania, hypomania and depression, interspersed with euthymic periods of absent or subsyndromal mood symptoms; it may or may not include psychotic symptoms.1,2 Not only is bipolar disorder associated with premature death, significant disability and impaired psychosocial functioning, it also results in significant societal costs, driven mainly by poor work adjustment and lost productivity.3 However, only 20% of patients with bipolar disorder who seek treatment during a depressive episode are correctly diagnosed within the first year.4 From onset to diagnosis, it takes an average of 5 to 10 years for appropriate treatment to begin.5,6 Affective disorders are frequently misdiagnosed and inappropriately treated;7 research into the neuropathological underpinnings of bipolar disorder to identify objective biomarkers such as structural or functional brain abnormalities may improve diagnosis and treatment in clinical practice.

A large number of studies have been published that assess the volume of regional grey-matter structures in the brains of people with bipolar disorder, but the results have been inconsistent, showing both smaller and larger grey-matter volumes. Markedly conflicting findings have been reported for some structures, such as the prefrontal and temporal regions. Some studies, including meta-analyses, have identified decreased grey-matter volume in the prefrontal regions8–10 and in the bilateral middle and superior temporal gyri9,11 in patients with bipolar disorder compared to controls. McDonald and colleagues12 also noted significant heterogeneity across studies for several brain structures, including the subgenual

Background: The literature on grey-matter volume alterations in bipolar disorder is heterogeneous in its findings. Methods: Using effect-size differential mapping, we conducted a meta-analysis of grey-matter volume alterations in patients with bipolar disorder compared with healthy controls. Results: We analyzed data from 50 studies that included 1843 patients with bipolar disorder and 2289 controls. Findings revealed lower grey-matter volumes in the bilateral superior frontal gyri, left anterior cingulate cortex and right insula in patients with bipolar disorder and in patients with bipolar disorder type I. Patients with bipolar disorder in the euthymic and depressive phases had spatially distinct regions of altered grey-matter volume. Meta-regression revealed that the proportion of female patients with bipolar disorder or bipolar disorder type I was negatively correlated with regional grey-matter alteration in the right insula; the proportion of patients with bipolar disorder or bipolar disorder type I taking lithium was positively correlated with regional grey-matter alterations in the left anterior cingulate/paracingulate gyri; and the proportion of patients taking antipsychotic medications was negatively correlated with alterations in the anterior cingulate/paracingulate gyri. Limitations: This study was cross-sectional; analysis techniques, patient characteristics and clinical variables in the included studies were heterogeneous. Conclusion: Structural grey-matter abnormalities in patients with bipolar disorder and bipolar disorder type I were mainly in the prefrontal cortex and insula. Patients’ mood state might affect grey-matter alterations. Abnormalities in regional grey-matter volume could be correlated with patients’ specific demographic and clinical features.
prefrontal cortex (PFC), the amygdala and the thalamus. Studies using linear mixed-effects regression models or anatomic likelihood estimation maps also found that compared with controls, patients with bipolar disorder type I had higher grey-matter volumes in the left temporal lobe, cingulate gyrus, parahippocampal gyrus and paracentral lobule, along with lower volumes in the bilateral frontal, cingulate, temporal and cerebellar regions and the left parietal areas.

Using the anisotropic effect size version of seed-based d-mapping (AES-SDM), a recent meta-analysis of 30 studies found both lower and higher grey-matter volumes in the cortical brain regions of patients with bipolar disorder, and also extracted data for original statistical maps.

Measures of brain volume in patients with bipolar disorder may also be influenced by heterogeneity in variables such as age, illness duration, mood state and psychotropic medication use, as well as by variability in imaging methodology, which may contribute to inconsistencies across studies. For instance, some psychotropic medications that are recommended to treat established bipolar disorder (such as lithium and valproate) have neuroprotective or neurotrophic effects, while others (such as antipsychotics) are associated with smaller brain volumes, although those associations are less pronounced than those in people taking lithium.

However, studies have also found no significant associations between grey-matter alterations and sex, mood, lithium use, antipsychotic medication use or methodological variables. The included studies may have contained extensive heterogeneity in features such as the clinical characteristics of disease subtype, mood state and medication use in patients with bipolar disorder, as well as in the methodology used. As a relatively new quantitative, coordinate-based meta-analysis approach, AES-SDM allows the results of individual studies to be weighted and controlled for several moderator variables, including demographic, clinical and imaging factors. Importantly for the present application, AES-SDM has been successfully used in neuropsychiatric populations.

Because many studies have been published since the most recent meta-analysis, it is an opportune time to perform an updated meta-analysis assessing grey-matter volumes in patients with bipolar disorder, using the latest version of AES-SDM. Our meta-analysis included larger studies — especially those with negative findings — and gave the study the power to detect at least moderate differences. As well, we were able to obtain more homogeneous findings than previous reports by exploring grey-matter volume alterations under different conditions of bipolar disorder. The latest version of AES-SDM includes some new features, such as the ability to combine repeated measures (e.g., from several contrasts of the same sample) and create funnel plots and Egger tests automatically.

Using AES-SDM, our aim was to perform a coordinate-based meta-analysis of MRI voxel-based morphometry studies to identify regional grey-matter volumetric differences in patients with bipolar disorder under different conditions, compared with healthy controls. We also aimed to explore the demographic and clinical variables that could affect grey-matter volume, focusing on the associations between regional grey-matter structures and concomitant medications (particularly mood stabilizers) given the reported neurotrophic properties of these drugs.

**Methods**

**Search and inclusion of studies**

We conducted a systematic search of PubMed, Embase and Web of Science for studies that compared patients with bipolar disorder and healthy controls and were published in English up to August 2017. We used the following keywords: magnetic resonance imaging OR MRI AND bipolar affective disorder OR bipolar disorder OR BD OR mania OR mood disorders. We used broad search terms to minimize the likelihood of missing relevant studies. We cross-referenced all relevant original research, reviews and meta-analyses, including the reference lists of eligible articles, to identify studies that had been missed in the literature searches.

To be considered for inclusion, studies had to meet the following criteria: (1) compared brain volumetric differences between patients with bipolar disorder and healthy controls using structural MRI and published as an original paper in a peer-reviewed journal; (2) reported a grey-matter volume comparison (including “grey-matter density” or “grey-matter concentration”) between patients with bipolar disorder and healthy controls aged 18–65 years (to minimize the effect of neurodevelopment and neurodegeneration, respectively); (3) used a whole-brain voxel-based morphometry (VBM) approach; (4) reported stereotactic coordinates (Talairach space or Montreal Neurological Institute [MNI] space); (5) enrolled patients with bipolar disorder only (studies that enrolled patients with schizoaffective disorder, schizophrenia or unipolar depression were excluded); and (6) set significance for differences between groups at a threshold of \( p < 0.05 \) (corrected for multiple comparisons) or \( p < 0.001 \) (uncorrected for multiple comparisons). When multiple studies used the same patient group, we selected the study with the largest sample size. If the same control group was used in several subgroup comparisons, we included a combined summary result in the meta-analysis. For studies that used longitudinal treatment designs, we included only baseline pretreatment data.

A study was excluded if (1) bipolar disorder was secondary to a somatic condition such as temporal lobe epilepsy or multiple sclerosis and was investigated solely as a comorbid psychiatric condition; (2) it included fewer than 10 patients; or (3) data were insufficient (e.g., missing neuroanatomical coordinates) even after the author(s) had been contacted by email. Each study was assessed by 2 reviewers (X. W. and Q. L.) to ensure that all inclusion and exclusion criteria were met. For each study in the meta-analysis, we extracted peak coordinates for grey-matter volume differences that were significant at the whole-brain level (no small-volume correction). To minimize errors, coordinate data were independently extracted by 2 authors (X. W. and Q. L.) according to the AES-SDM method, and inconsistencies were resolved by a third independent assessor.
Voxel-based meta-analysis

We conducted a meta-analysis of regional grey-matter differences between patients with bipolar disorder and healthy controls using AES-SDM version 5.141 (www.sdmproject.com/). The software uses a voxel-based meta-analytic approach that is based on (and improves on) other methods such as anatomic likelihood estimation. In summary, AES-SDM allows peak coordinates and statistical parametric maps to be combined to create whole-brain effect size and variance maps, which can then be used to perform voxel-wise random-effects meta-analyses. This method has been thoroughly described elsewhere and is briefly summarized here. First, we used peak coordinates and effect sizes (derived, for example, from t values) of grey-matter differences to recreate a map for each study of the effect size of the grey-matter volume differences between patients with bipolar disorder and healthy controls. This recreation was based on anisotropic kernels that estimated the effect size of the voxels close to a peak, based on the correlation between each voxel and the peak. Second, we recreated a separate standard MNI map of the differences in grey matter for each study using an anisotropic Gaussian kernel, which assigned higher effect sizes to the voxels that were more correlated with peaks. This anisotropic kernel optimized recreation of effect-size maps and was robust because it did not depend on a full width at half maximum. Third, we derived a map of the effect-size variance for each study using its effect-size map and its sample size. Fourth, we obtained a mean map with voxel-wise calculation of the random-effects mean of the study maps, weighted by sample size, within-study variance and between-study heterogeneity. Division of meta-analytic effect sizes by their standard errors yields z-values, but these are not normally distributed, so we assessed statistical significance using a permutation test. For all main analyses, it has been shown that \( p < 0.005 \) (uncorrected) with a cluster-level extent threshold of \( k > 10 \) optimally balances false positives and negatives. For each cluster that was significantly different between patients and controls, we used the Egger test to assess potential publication bias.

We used complementary analyses, such as jackknife and meta-regression analyses, to test the robustness of the results and potential confounders, respectively. We conducted a jackknife sensitivity analysis to assess the robustness of the results by iteratively repeating the same analysis, excluding one data set at a time, to establish whether the results remained significant. We conducted a heterogeneity analysis to determine whether there was significant unexplained between-study variability in the results. Considering the relatively small number of studies in our analysis, we set the cutoff for inclusion of potential confounders in meta-regressions to \( \geq 20 \) studies to minimize the occurrence of false positives. We conducted meta-regression analyses to account for potential demographic and clinical confounders, such as handedness, mean age, proportion of each sex, age of onset, illness duration, severity of manic symptoms, antipsychotic medication use, lithium use, antidepressant medication use, magnetic field strength and image smoothing level within patient groups. We used a more conservative voxel-level threshold of \( p < 0.0005 \) (uncorrected) in accordance with previous meta-analyses, including only regions found in the main analyses. Studies that did not report these measures were excluded from these analyses. We could not study the severity of depressive symptoms or the proportion of patients taking valproate, because data were available for fewer than 20 studies.

Finally, we performed subgroup analyses for studies that included only patients with bipolar disorder type I, studies that included only patients in the euthymic or depressive phase, and studies that included only patients with bipolar disorder and psychotic symptoms, followed by jackknife and meta-regression analyses as described above. However, we did not perform meta-regression analyses for the subgroups of patients in the euthymic or depressive phase, or in patients with psychotic symptoms, because fewer than 20 studies were available.

We submitted peak coordinates to MRIcron (www.nitrc.org/projects/mricron/), which provided templates for visualizing the results.

Results

Included studies and sample characteristics

We identified a total of 17034 records. After duplicates and obviously irrelevant articles (e.g., animal studies, case reports and functional MRI studies) had been removed, the search resulted in a total of 391 abstracts that were examined by 2 researchers for relevance and other qualifications for inclusion. Eventually, 50 studies with a total of 58 comparisons between patients with bipolar disorder and healthy controls met the inclusion criteria and were included in the meta-analysis (Fig. 1).

In general, demographic details were well reported in 49 studies (98%). Overall, 28 studies (56%) provided age of onset, and 37 studies (74%) provided illness duration. Sixteen studies (32%) reported on depressive symptoms, and 24 studies (48%) reported on manic symptoms. Information on medication treatment was incomplete: 4 studies (8%) provided no medication-related information for patients with bipolar disorder, and 39 (78%) did not report adequate treatment details. For studies that provided information about medications at the time of MRI acquisition, 37 (74%) included patients who were prescribed at least 1 mood-stabilizing agent (lithium or valproate), 36 (72%) reported the number of lithium users, 16 (32%) included people who were prescribed valproate, 37 (74%) included people who were prescribed an antipsychotic medication, and 29 (58%) included people who were prescribed an antidepressant. Table 1 shows the demographic and/or clinical information for patients with bipolar disorder under different conditions, including subgroup and regression analyses. Details of these studies are presented in Appendix 1, Table S1 and Table S2, available at jpn.ca/180002-a1.
Regional differences in grey-matter volume: pooled analysis

Areas with smaller grey-matter volumes in patients with bipolar disorder relative to healthy controls are shown in Table 2 and Fig. 2A; we found no larger grey-matter volumes. These findings were robust; the clusters that did not meet the criteria for robustness are shown in Appendix 1, Table S3. The largest area showing lower grey-matter volume in patients with bipolar disorder relative to controls was in the left superior frontal gyrus (SFG), extending into the bilateral SFG, the left anterior cingulate/paracingulate gyri (ACPG) and the bilateral median cingulate/paracingulate gyri. Another large cluster was located in the right insula, extending into the right inferior frontal gyrus. We found smaller clusters showing lower volume in the left insula, extending into the left superior temporal gyrus (STG).

Regional differences in grey-matter volume: subgroup analyses

Bipolar disorder type I

Relative to healthy controls, patients with bipolar disorder type I had significantly smaller grey-matter volumes in a few regions (Table 3 and Fig. 2B), but no regions with larger grey-matter volume. Clusters that did not meet the criteria for robustness are shown in Appendix 1, Table S4. The largest area showing smaller grey-matter volumes in patients with bipolar disorder type I was in the right insula. Another large cluster with smaller volumes was in the left SFG, extending into the bilateral SFG. We also found 2 small clusters with smaller volumes in the left inferior frontal gyrus and the left ACPG.

Bipolar disorder, euthymic phase

The grey-matter volume differences in euthymic bipolar disorder relative to healthy controls are shown in Table 3 and Figure 2C. The clusters that did not meet the criteria for robustness are shown in Appendix 1, Table S5. Compared with controls, patients with bipolar disorder in the euthymic phase had smaller grey-matter volumes in the right inferior parietal gyri and the right thalamus, and larger volumes in the left insula.

Bipolar disorder, depressive phase

In patients with depressive bipolar disorder, we found smaller grey-matter volumes in a large cluster in the right insula, extending into the right STG, and in the right median cingulate/paracingulate gyri. We found 1 region with larger grey-matter volume in the left supplementary motor area (Table 3 and Fig. 2D). The clusters that did not meet the criteria for robustness are shown in Appendix 1, Table S6.
Grey-matter volume in adults with BD

Bipolar disorder with psychotic symptoms

Patients who had bipolar disorder with psychotic symptoms had significantly smaller grey-matter volumes in several regions (Table 3), but no regions with larger grey-matter volume relative to healthy controls. The clusters that did not meet the criteria for robustness are shown in Appendix 1, Table S7. We found grey-matter deficits in a cluster of the right SFG, extending into the left ACPG and left SFG, and in 2 regions of the right precentral gyrus and left STG.

Bipolar disorder type II

Only 3 studies directly compared grey-matter volume differences between patients with bipolar disorder type II and controls. As a result, we did not conduct a subgroup analysis of grey matter volumes in patients with bipolar type II.

Meta-regression analyses

With a stringent threshold of \( p < 0.0005 \) to minimize spurious findings, meta-regression analyses indicated that studies with a higher proportion of female patients found greater volume deficits in the right insula in patients with bipolar disorder relative to controls (Brodmann areas [BA] 48; peak MNI: \( x, y, z = 44, -8, 10; Z = -2.687; p < 0.001; 309 \) voxels; Appendix 1, Fig. S1), and in patients with bipolar disorder type I relative to controls (BA 47; peak MNI: \( x, y, z = 42, 18, -10; Z = -3.328; p < 0.001; 284 \) voxels; Appendix 1, Fig. S2).

Studies with higher proportions of patients taking lithium found larger grey-matter volume in the right insula (BA 47; peak MNI: \( x, y, z = 40, 18, -12; Z = 3.301; p < 0.001; 124 \) voxels) and left ACPG (BA 32; peak MNI: \( x, y, z = -6, 46, 8; Z = 2.571; p < 0.001; 68 \) voxels) in patients with bipolar disorder relative to healthy controls (Fig. 3A and B). They also found larger grey-matter volume in the right insula (BA 47; peak MNI: \( x, y, z = 42, 18, -10; Z = 3.857; p < 0.001; 207 \) voxels) in patients with bipolar disorder type I relative to healthy controls (Fig. 3D and E).

Studies with a higher proportion of patients taking antipsychotic medications found greater volume deficits in the left ACPG in patients with bipolar disorder relative to healthy controls (BA 32; peak MNI: \( x, y, z = -4, 50, 12; Z = 3.526; p < 0.001; 207 \) voxels) in patients with bipolar disorder type I relative to healthy controls (Fig. 3D and E). Studies with a higher proportion of patients taking antipsychotic medications found greater volume deficits in the left ACPG in patients with bipolar disorder relative to healthy controls (BA 32; peak MNI: \( x, y, z = -4, 50, 12; Z = 3.526; p < 0.001; 207 \) voxels) in patients with bipolar disorder type I relative to healthy controls (Fig. 3D and E).

We found no significant associations between grey-matter volume and handedness, mean age, age of onset, Young Mania Rating Scale score, antidepressant medication or methodological variables, including MRI field strength.

Analysis of heterogeneity and publication bias

Analyses of heterogeneity revealed that a number of regions with altered grey-matter volumes showed significant statistical heterogeneity among studies (Appendix 1, Table 1: Demographic and clinical characteristics of patients with bipolar disorder).

Table 1: Demographic and clinical characteristics of patients with bipolar disorder

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Total analyses</th>
<th>Subgroup analyses</th>
<th>Regression analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Studies, ( n ) (%)</td>
<td>Patients, ( n )</td>
<td>Controls, ( n )</td>
</tr>
<tr>
<td>All BD</td>
<td>50 (100)</td>
<td>1843</td>
<td>NA</td>
</tr>
<tr>
<td>BDI</td>
<td>47 (94)</td>
<td>1558</td>
<td>NA</td>
</tr>
<tr>
<td>BDII or others</td>
<td>9 (18)</td>
<td>188</td>
<td>NA</td>
</tr>
<tr>
<td>BD, euthymic phase</td>
<td>27 (54)</td>
<td>799</td>
<td>NA</td>
</tr>
<tr>
<td>BD, depression phase</td>
<td>16 (32)</td>
<td>514</td>
<td>NA</td>
</tr>
<tr>
<td>BD, mania phase</td>
<td>10 (20)</td>
<td>136</td>
<td>NA</td>
</tr>
<tr>
<td>BD, mixed phase</td>
<td>1 (2)</td>
<td>5</td>
<td>NA</td>
</tr>
<tr>
<td>BD, psychosis</td>
<td>11 (22)</td>
<td>381</td>
<td>NA</td>
</tr>
<tr>
<td>BD = bipolar disorder I</td>
<td>1008 (54.69)</td>
<td>822</td>
<td>1531</td>
</tr>
<tr>
<td>Lithium users, ( n ) (%)</td>
<td>24 (48)</td>
<td>21 (43.75)</td>
<td>NA</td>
</tr>
<tr>
<td>Antipsychotic users, ( n ) (%)</td>
<td>603 (32.72)</td>
<td>503 (33.60)</td>
<td>NA</td>
</tr>
<tr>
<td>Antidepressant users, ( n ) (%)</td>
<td>307 (45.06)</td>
<td>207 (30.18)</td>
<td>NA</td>
</tr>
<tr>
<td>YMRS</td>
<td>1008 (54.69)</td>
<td>822</td>
<td>1531</td>
</tr>
<tr>
<td>BD, euthymic phase</td>
<td>27 (54)</td>
<td>799</td>
<td>NA</td>
</tr>
<tr>
<td>BD, depression phase</td>
<td>16 (32)</td>
<td>514</td>
<td>NA</td>
</tr>
<tr>
<td>BD, mania phase</td>
<td>10 (20)</td>
<td>136</td>
<td>NA</td>
</tr>
<tr>
<td>BD, mixed phase</td>
<td>1 (2)</td>
<td>5</td>
<td>NA</td>
</tr>
<tr>
<td>BD, psychosis</td>
<td>11 (22)</td>
<td>381</td>
<td>NA</td>
</tr>
</tbody>
</table>

BD = bipolar disorder; BDI = bipolar disorder type I; BDII = bipolar disorder type II; NA = not available; YMRS = Young Mania Rating Scale.
Tables S8 to S12). As indicated by nonsignificant Egger tests of funnel plot asymmetry, we found no evidence of publication bias or detectable small-study effects in the SFG (t = 1.26; p = 0.21; 95% confidence interval [CI] −0.774 to 3.424), the right insula (t = 1.45; p = 0.15; 95% CI −0.492 to 3.050) or the left insula (t = 0.18; p = 0.86; 95% CI −1.506 to 1.799).

**Discussion**

This meta-analysis found that patients with bipolar disorder in general or bipolar disorder type I in particular had lower grey-matter volumes in the PFC, the temporal cortex, the insula and the anterior cingulate cortex (ACC), which may be implicated in the pathophysiology of bipolar disorder.

**Table 2: Clusters showing grey-matter differences between patients with bipolar disorder and controls**

<table>
<thead>
<tr>
<th>Cluster peak/regions</th>
<th>Peak MNI coordinates, x, y, z</th>
<th>SDM Z value</th>
<th>p value</th>
<th>Voxel size</th>
<th>Brodmann area</th>
<th>Cluster breakdown</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD v. controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BD &gt; controls</td>
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<tr>
<td>None</td>
<td>—</td>
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</tr>
<tr>
<td>BD &lt; controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior frontal gyrus, medial part</td>
<td>−2, 54, 12</td>
<td>−2.704</td>
<td>&lt; 0.001</td>
<td>4369</td>
<td>9, 10, 23, 32</td>
<td>Bilateral superior frontal gyrus, medial part, left anterior cingulate/paracingulate gyri, bilateral median cingulate/paracingulate gyri</td>
</tr>
<tr>
<td>Right insula</td>
<td>44, 16, 0</td>
<td>−3.614</td>
<td>&lt; 0.001</td>
<td>2562</td>
<td>47, 48</td>
<td>Right insula, right inferior frontal gyrus, opercular part</td>
</tr>
<tr>
<td>Left insula</td>
<td>−36, 16, −14</td>
<td>−2.679</td>
<td>&lt; 0.001</td>
<td>1113</td>
<td>48</td>
<td>Left insula, left temporal pole, superior temporal gyrus</td>
</tr>
</tbody>
</table>

BD = bipolar disorder; MNI = Montreal Neurological Institute; SDM = seed-based d-mapping.
*Clusters that met our criteria for robustness.

**Fig. 2:** Brain regions differed significantly among groups. Areas of smaller (blue) and greater (red) grey-matter volume in patients with bipolar disorder compared with healthy controls in the meta-analyses. Images are presented in radiological orientation. (A) All patients with bipolar disorder. (B) Patients with bipolar disorder type I. (C) Patients with bipolar disorder, euthymic phase. (D) Patients with bipolar disorder, depressive phase. Statistical inferences were made with a voxel-level statistical threshold of p < 0.005 and a minimum cluster size of > 10 voxels. ACPG = anterior cingulate/paracingulate gyri; B = bilateral; IFG = inferior frontal gyrus; IPG = inferior parietal gyrus; L = left; mCPG = median cingulate/paracingulate gyri; MFG = middle frontal gyrus; R = right; SFG = superior frontal gyrus; SMA = supplementary motor area; STG = superior temporal gyrus.
Meanwhile, patients in the euthymic and depressive phases of bipolar disorder had spatially distinct regions of altered grey-matter volumes relative to healthy controls. Moreover, smaller grey-matter volume in the right insula may be the mood-related structural pathological marker of bipolar disorder. In addition, alteration of grey-matter volume in the right insula in some studies was negatively associated with the proportion of female patients. Larger grey-matter volume in the right insula and the left ACPG was positively correlated with a higher percentage of patients taking lithium (bipolar disorder or bipolar disorder type I), while smaller grey-matter volume in the left ACPG was negatively correlated with a higher percentage of patients taking antipsychotic drugs (bipolar disorder or bipolar disorder type I). These findings suggest between-group abnormalities (both disorder-related and treatment-related) in regional brain grey-matter volumes in bipolar disorder pathology, in bipolar disorder subtypes and in different states.

**Grey-matter volume: findings**

**Smaller grey-matter volumes in bipolar disorder and bipolar disorder type I**

Largely consistent with our findings of smaller grey-matter volumes in the PFC, left ACC and right insula in patients with bipolar disorder and bipolar disorder type I relative to healthy controls, 1 meta-analysis also reported that only smaller grey-matter volumes in the left ACC and right insula

### Table 3: Clusters showing grey matter differences in subgroup analyses*

<table>
<thead>
<tr>
<th>Cluster peak/regions</th>
<th>Peak MNI coordinates, x, y, z</th>
<th>SDM Z value</th>
<th>p value</th>
<th>Voxel size</th>
<th>Brodmann area</th>
<th>Cluster breakdown</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDI v. controls</td>
<td></td>
<td></td>
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<td>BDI &gt; controls</td>
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<tr>
<td>None</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>BDI &lt; controls</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Right insula</td>
<td>42, 18, –10</td>
<td>–3.577</td>
<td>&lt; 0.001</td>
<td>1954</td>
<td>47</td>
<td>Right insula</td>
</tr>
<tr>
<td>Left superior frontal gyrus, medial part</td>
<td>2, 48, 32</td>
<td>–2.698</td>
<td>&lt; 0.001</td>
<td>1369</td>
<td>9, 10, 32</td>
<td>Bilateral superior frontal gyri, medial part</td>
</tr>
<tr>
<td>Left inferior frontal gyrus, orbital part</td>
<td>–38, 18, –14</td>
<td>–2.583</td>
<td>&lt; 0.001</td>
<td>809</td>
<td></td>
<td>Left inferior frontal gyrus</td>
</tr>
<tr>
<td>Left anterior cingulate/ paracingulate gyrus</td>
<td>0, 10, 26</td>
<td>–1.997</td>
<td>&lt; 0.001</td>
<td>62</td>
<td></td>
<td>Left anterior cingulate/ paracingulate gyrus</td>
</tr>
<tr>
<td>BD, euthymic phase v. controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BD, euthymic phase &gt; controls</td>
<td></td>
<td></td>
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<tr>
<td>Left insula</td>
<td>–36, –4, 18</td>
<td>1.177</td>
<td>&lt; 0.001</td>
<td>20</td>
<td>48</td>
<td>Left insula</td>
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<tr>
<td>BD, euthymic phase &lt; controls</td>
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<td></td>
<td></td>
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<tr>
<td>Right inferior parietal gyrus</td>
<td>58, –50, 38</td>
<td>–1.400</td>
<td>&lt; 0.001</td>
<td>66</td>
<td>40</td>
<td>Right inferior parietal gyrus</td>
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<tr>
<td>Right thalamus</td>
<td>4, –10, 12</td>
<td>–1.484</td>
<td>&lt; 0.001</td>
<td>39</td>
<td></td>
<td>Right thalamus</td>
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<td>BD, depressive phase v. controls</td>
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<td></td>
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<td></td>
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<tr>
<td>Left supplementary motor area</td>
<td>–6, –8, 76</td>
<td>1.150</td>
<td>&lt; 0.001</td>
<td>70</td>
<td>6</td>
<td>Left supplementary motor area</td>
</tr>
<tr>
<td>BD, depressive phase &lt; controls</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Right insula</td>
<td>42, 14, –4</td>
<td>–3.523</td>
<td>&lt; 0.001</td>
<td>1602</td>
<td>38, 47, 48</td>
<td>Right insula, right temporal pole, superior temporal gyrus</td>
</tr>
<tr>
<td>Right median cingulate/ paracingulate gyr</td>
<td>2, 6, 38</td>
<td>–2.250</td>
<td>&lt; 0.001</td>
<td>793</td>
<td>24</td>
<td>Right median cingulate/ paracingulate gyr</td>
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<td>BD, psychosis v. controls</td>
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<tr>
<td>None</td>
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<td></td>
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<tr>
<td>BD, psychosis &lt; controls</td>
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</tr>
<tr>
<td>Right superior frontal gyrus, medial part</td>
<td>6, 56, 20</td>
<td>–2.148</td>
<td>&lt; 0.001</td>
<td>106</td>
<td>10</td>
<td>Right superior frontal gyrus, medial part, left anterior cingulate/paracingulate gyr, left superior frontal gyrus, medial orbital</td>
</tr>
<tr>
<td>Right precentral gyrus</td>
<td>46, –14, 44</td>
<td>–2.253</td>
<td>&lt; 0.001</td>
<td>98</td>
<td>6</td>
<td>Right precentral gyrus</td>
</tr>
<tr>
<td>Left temporal pole, superior temporal gyrus</td>
<td>–34, 8, –22</td>
<td>–2.284</td>
<td>&lt; 0.001</td>
<td>70</td>
<td>38</td>
<td>Left temporal pole, superior temporal gyrus</td>
</tr>
</tbody>
</table>

BD = bipolar disorder; BDI = bipolar disorder type I; MNI = Montreal Neurological Institute; SDM = seed-based d-mapping.

*Clusters that met our criteria for robustness.
Frontoinsular cortex were associated with bipolar disorder and bipolar disorder type I patients. Lower grey-matter volumes in the PFC, ACC and insula were the most consistently reported findings in patients with bipolar disorder relative to healthy controls. Studies also consistently found disorder-related grey-matter deficits and progressive grey-matter loss in bipolar disorder relative to healthy controls, primarily in frontal regions such as the PFC and ACC, consistent with the frequently observed deficits in executive function in bipolar disorder. The ACC, widely thought to play a role in cognitive control, forms an anterior component of the default mode network. A review of structural and functional neuroimaging studies has suggested that a disruption of the neural circuitry in the ventrolateral PFC and the medial PFC (including the ACC) mediates both voluntary and automatic emotion processing and regulation. The ACC (the most frequently affected region, a representative of the anterior limbic system) and the insula are considered paralimbic regions and are implicated in multiple functions, including reward, punishment and emotional processing. Structural deficits in this region might suggest impaired emotional processing in bipolar disorder.

Furthermore, brain network abnormality is a useful framework for considering the varied presentations of bipolar disorder. Structural and functional imaging studies have reported disrupted brain networks in the default mode and salience networks involving the regions mentioned above, particularly the frontoinsular cortex, ACC and medial PFC in bipolar disorder. Using Granger causal fMRI analysis, Palaniyappan and colleagues found significant differences in effective connectivity in the triple-network system (the default mode, salience and central executive networks) between people with schizophrenia-spectrum disorders and bipolar disorder. Such a model-based approach can be used to separate patients with schizophrenia-spectrum disorders from those with bipolar disorder by effective connectivity (static or

![Fig. 3: Results of the meta-regression analyses of regional grey-matter volume studies in patients with bipolar disorder (A, B, C) and patients with bipolar disorder type I (D, E, F) against the percentages of patients taking lithium and antipsychotic medications. (A, B) The percentage of patients with bipolar disorder taking lithium was positively associated with grey-matter volumes in the right insula and left ACPG. (C) The percentage of patients with bipolar disorder taking antipsychotic medications was negatively associated with grey-matter volume in the left ACPG. (D, E) The percentage of patients with bipolar disorder type I taking lithium was positively associated with grey-matter volumes in the right insula and left ACPG. (F) The percentage of patients with bipolar disorder type I taking antipsychotic medications was negatively associated with grey-matter volumes in the left ACPG.](image-url)
Grey-matter volume in adults with BD

Grey-matter alterations in different mood states

Our study revealed smaller grey-matter volumes in the left inferior frontal cortex, the right inferior parietal cortex and the thalamus, as well as larger grey-matter volumes in the left insula in euthymic patients with bipolar disorder. We also observed smaller grey-matter volumes in the right insula, right superior temporal region and median cingulate cortex, as well as larger grey-matter volume in the left supplementary motor area in patients with bipolar disorder during the depressive phase compared with healthy controls, although these alterations did not appear to be diagnostically specific. In line with our findings, studies also reported smaller volumes in the left hippocampus or lesser cortical thickness in the prefrontal areas, the left superior temporal cortex and the right ACC in euthymic patients with bipolar disorder relative to healthy controls. Some within-subject MRI studies of patients with bipolar disorder revealed that, compared to patients in the euthymic phase, those in the depressive phase exhibited lower grey-matter density/volume in the PFC, ACC and left inferior parietal lobe, and greater grey-matter density in the subcortical PFC, left inferior temporal gyrus and parahippocampal gyrus. In our meta-analysis, we also found right-sided grey-matter volume deficits in different mood states. Many hypothesis-driven morphometric studies in bipolar disorder have reported lateralized findings, mostly right-sided, but these findings did not fulfill specific predictions and therefore attracted limited attention. Although many structural imaging studies have explored patients with bipolar disorder regardless of mood state, our preliminary findings have provided evidence that the mood state of patients with bipolar disorder could be correlated with grey-matter changes as measured by VBM, and/or alterations in symptomatic patients might reflect illness traits for lower treatment response, further demonstrating that the mood state of patients cannot be ignored in neuroimaging studies of bipolar disorder. The lack of consensus among previous structural neuroimaging studies of bipolar disorder might reflect, in part, the varied mood state of the patients. However, the criteria for euthymia of bipolar disorder varied across the included studies and might have affected some of our findings, especially for patients with bipolar disorder in the euthymic phase. Our findings should be viewed as preliminary. A more stringent definition of euthymia should be considered in future research. Further prospective longitudinal studies that include bipolar disorder in different mood states are necessary to comprehensively evaluate this concept.

Consistent with a large number of structural neuroimaging studies in bipolar disorder, our study identified disease-related neuroanatomical abnormalities in the frontal–cingulo–thalamic circuit, although the direction and
intensity of the alterations may be a matter of debate. Structural deficiency in these and associated regions could underlie bipolar disorder.54–56

Potential state-related grey-matter alteration

Similar to other published reviews and meta-analyses,9,11,30,57,58 our meta-analysis revealed that smaller right insular grey-matter volume was the consistent pathological alteration in all patients with bipolar disorder and in the subgroups, except for patients in the euthymic phase (although results from patients with bipolar mania were unavailable). One study found volumetric reductions in the right insula before the onset of first-episode mania, compared to healthy controls or people at ultra-high risk of psychosis who had no psychiatric diagnosis after follow-up; it also found no effect of prescribed mood stabilizers or antipsychotic drugs.59 A meta-analysis of VBM studies found that only 2 regions had consistently reduced volume across studies of patients with bipolar disorder: the left ACC and the right anterior insula.60 If the findings in the depressive- and manic-phase bipolar disorder groups were different from those in the euthymic group, these differences might be inferred to be state-related or reflect a healing process. Structural brain abnormalities in bipolar disorder may provide some indication of accompanying brain dysfunction. Functional network analysis also indicates that the insular cortex processes information through high-level cognitive control and attentional processes, mediating “salience switching” between other large functional networks involved in externally oriented attention and internally oriented cognition, some of which are impaired in patients with bipolar disorder.60 Our findings suggest that the abnormal right insula structure might be the structural pathological marker related to mood state that could serve as a specific region of interest for further studies in bipolar disorder.

Effects of demographic and clinical variables on grey-matter volume

Our meta-regression analysis revealed that alteration of grey-matter volume in patients with bipolar disorder and bipolar disorder type I was correlated with some indication of accompanying brain dysfunction. Functional network analysis also indicates that the insular cortex processes information through high-level cognitive control and attentional processes, mediating “salience switching” between other large functional networks involved in externally oriented attention and internally oriented cognition, some of which are impaired in patients with bipolar disorder.60 Our findings suggest that the abnormal right insula structure might be the structural pathological marker related to mood state that could serve as a specific region of interest for further studies in bipolar disorder.8

Largely consistent with other published studies,5,21 our findings suggested that, with the proportion of patients taking lithium, the grey-matter volumes increased in the right insula and the left ACPG in overall bipolar disorder and bipolar disorder type I patients. Lithium administration had a volume-enhancing effect on grey-matter structures in the right frontal lobe, particularly in the paralimbic regions associated with emotional processing,62–64 and was correlated with treatment response in patients with bipolar disorder,52,65,66 possibly by counteracting grey-matter volume deficits in critical cortical areas.67 Meta-analyses have also suggested that lithium-treated patients had greater global grey-matter volume and hippocampus volumes than those who did not take lithium, highlighting the therapeutic potential of lithium in conditions characterized by abnormal changes in brain structure.68,69 In a partially overlapping sample, untreated patients with bipolar disorder had lower left ACC volumes, but lithium-treated patients were not significantly different from healthy controls.45 A longitudinal imaging study of a medication-free bipolar disorder cohort that was naive to mood stabilizers and antipsychotic medications suggested that lithium treatment induced sustained greater grey-matter volume in patients with bipolar disorder, possibly mediating the long-term efficacy of lithium.62 Lithium-induced increases in grey-matter volume might be related to the neurotrophic–neuroprotective effects of lithium as a possible etiology for observed neuroanatomical differences.70,71 The possible mechanisms of action for lithium are increasing inhibitory and reducing excitatory neurotransmission, increasing protective proteins such as brain-derived neurotrophic factor and B-cell lymphoma 2, reducing oxidative stress and reducing apoptotic processes through inhibition of glycogen synthase kinase 3 and autophagy (for a review, see Mahli and colleagues72). In addition, functional neuroimaging studies offered preliminary evidence that mood-stabilizing medications might normalize functional abnormalities within frontotemporal neural systems in bipolar disorder.73 Some of the inconsistencies between neuroanatomical studies of patients with bipolar disorder might be attributable to competing processes, with disorder-related atrophy and/or tissue reduction pitted against the possible neurotrophic or neuroprotective effects of mood-stabilizing medication.

Although the effect of lithium is fairly established, there is some debate about the effect of antipsychotic medication on the brain structure. Our findings support the understanding that the degree of exposure to an antipsychotic agent is related to the decrease in grey-matter volume. This finding was in line with those of previous studies74,75 that had reported an association between greater brain-volume loss and higher dosages of antipsychotics in psychosis. In rat models, chronic administration of both classes of antipsychotic drugs resulted in a reduction in grey-matter volume, primarily in the frontal cortices.76,77 Studies also suggested that antipsychotic treatment was one of the most significant contributors to observed cognitive impairment.78,79

Notably, the influence of medication on changes in grey-matter volume remains contentious. Our study indicated that
psychotropic medication use might be an important contributor to heterogeneity across studies. One review suggested that mood stabilizers, antipsychotics and antidepressants could be neuroprotective in patients and animal models of psychiatric disorders, whereas other reviews reported little to no effect of psychopharmacological treatment on structural and functional brain findings in patients with bipolar disorder. For instance, a follow-up study of patients with bipolar disorder found that insula volume was not affected by lithium or valproate.264 No evidence suggested a relationship between antipsychotics, antipsychotics or mood stabilizers and grey-matter-volume changes in the anterior insula.245,246 These data further raised the possibility that psychotropic medications could be a major confounding factors in both cross-sectional and longitudinal volume comparisons and might account for at least some of the inconsistency and wide variability of volumetric findings in affective psychosis studies, particularly for bipolar disorder.

As well, existing evidence has suggested that psychosis in bipolar disorder may represent a distinct phenotype. Our study also found a volume-weakening effect of the psychotic dimension on the grey matter of the bilateral medial SFG, right precentral gyrus and left STG in patients with bipolar disorder. A recent review reported grey-matter volume deficits mainly in the frontal cortex, and increased subcortical grey-matter volume mainly in the basal ganglia in patients with psychotic bipolar disorder compared with healthy controls.137 However, there was significant heterogeneity (e.g., current psychotic symptoms v. lifetime psychotic symptoms) in the definition of the psychotic dimension across the included studies. Future studies will be needed to further explore the neuropathological basis of psychosis in patients with bipolar disorder.

Limitations

There were some limitations to this study. First, we could not determine whether structural alterations were part of the pathogenesis of bipolar disorder or a consequence of the illness, because all of the included studies were cross-sectional group comparisons. Second, our subgroup analysis did not examine grey-matter alteration in patients with bipolar disorder in the manic phase because of insufficient data. Imaging studies also lent some support to the proposition that manic episodes are related to brain structural abnormalities, and their association with clinical features and specific medications might account for at least some of the inconsistency and wide variability of volumetric findings in affective psychosis studies, particularly for bipolar disorder.

Conclusion

Our findings suggest that disorder-related structural grey-matter abnormalities in patients with bipolar disorder and bipolar disorder type I were found mainly in the prefrontal and temporal cortex, the ACC and the insula, and that patients’ mood state could be associated with grey-matter alterations. In particular, the right insula might be the site of state-related structural grey-matter alteration. Furthermore, abnormalities in grey-matter volume might correlate with specific clinical features such as sex, lithium (often resulting in increased grey-matter volumes in patients) and antipsychotic medications (often resulting in decreased grey-matter volumes in patients). Prospective and longitudinal studies involving homogeneous samples in different phases of drug-naive bipolar disorder are needed to elucidate the underlying mechanisms of bipolar disorder and further clarify trajectories of neurobiological changes and their association with clinical features and specific medication exposure (e.g., lithium) over time.

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Competing interests: None declared.

Contributors: X. Wang and Z. Jia designed the study. F. Tian and H. Wang acquired the data, which X. Wang, Q. Luo, F. Tian, B. Cheng, L. Qiu, S. Wang, M. He, M. Duan and Z. Jia analyzed. X. Wang and Q. Luo wrote the article, which all authors reviewed. All authors approved the final version to be published and can certify that no other individuals not listed as authors have made substantial contributions to the paper.

References


Grey-matter volume in adults with BD


Cerebral blood flow in striatal regions is associated with apathy in patients with schizophrenia

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Introduction

For decades, it has been hypothesized that striatal dysfunction is a fundamental mechanism underlying symptoms of schizophrenia. Robust findings in the literature have shown increased striatal dopamine synthesis in schizophrenia as measured by positron emission tomography (PET) and single-photon emission computed tomography (SPECT). Studies using functional MRI (fMRI) to assess striatal response to rewards have shown a decreased signal in unmedicated patients with schizophrenia. Results in medicated patients have been heterogeneous, indicating a complex relationship between dopamine dysregulation and fMRI findings.

More recently, it has been suggested that an increase in dopamine turnover could be accompanied by an increased perfusion of striatal areas. Arterial spin labelling (ASL) imaging allows for an absolute measure of regional cerebral blood flow (rCBF). Previous studies have suggested an increase in striatal rCBF in patients with schizophrenia and a high risk for psychosis, but these findings were not fully consistent. Further research is clearly needed.

Schizophrenia is a disorder with heterogeneous symptom expression along its course, and negative symptoms have a strong effect on long-term morbidity and poor functional outcome. There is now consensus that negative symptoms can be divided into 2 dimensions: apathy, which consists of anhedonia, avolition and asociality, and diminished expression, which combines the symptoms of blunted affect and alogia. In fMRI studies, an association between ventral striatal hypoactivation and negative symptoms (in particular, apathy) has repeatedly been reported. A recent study also found dorsal striatal hypoactivation in response to reward and apathy.

These fMRI results do not reflect absolute hypoactivation in the striatum; rather, they represent a decreased signal difference between rewarding and nonrewarding stimuli.

Background: Striatal dysfunction has been proposed as a pathomechanism for negative symptoms in schizophrenia. There is consensus that negative symptoms can be grouped into 2 dimensions: apathy and diminished expression. Recent studies suggest that different neural mechanisms underlie these dimensions, but the relationship between regional resting-state cerebral blood flow (rCBF) and negative symptom dimensions has not been investigated. Methods: This study included 29 patients with schizophrenia and 20 healthy controls. We measured rCBF in the striatum using arterial spin labelling (ASL) MRI. We assessed negative symptoms using the Brief Negative Symptom Scale. Results: In the ventral and dorsal striatum, rCBF was not different between patients with schizophrenia and controls. However, we did find a positive association between the severity of apathy and increased rCBF in the ventral and dorsal striatum in patients with schizophrenia. This effect was not present for diminished expression. Limitations: All patients were taking atypical antipsychotics, so an effect of antipsychotic medication on rCBF could not be excluded, although we did not find a significant association between rCBF and chlorpromazine equivalents. Conclusion: The main finding of this study was a specific association between increased striatal rCBF and the negative symptom dimension of apathy. Our results further support the separate assessment of apathy and diminished expression when investigating the neural basis of negative symptoms. The ASL technique can provide a direct and quantitative approach to investigating the role of rCBF changes in the pathophysiology of negative symptoms.
Therefore, the absolute measure of rCBF provided by ASL can offer additional information about the neural basis of symptoms. Most studies investigating ASL have not focused specifically on the striatum; they have used whole-brain analysis. Nevertheless, a few have reported associations between striatal rCBF and symptoms. Kindler and colleagues showed a positive correlation between striatal rCBF and positive symptoms in patients with treatment-resistant auditory hallucinations. Zhuo and colleagues found increased rCBF in striatal and auditory areas in patients with auditory verbal hallucinations.

In addition to these ASL studies on rCBF in the striatum, earlier PET studies have reported an association between negative symptoms and reduced rCBF at rest and during an attentional task in frontal and parietal regions. However, these studies did not address the distinction between apathy and diminished expression. Liemburg and colleagues found apathy to be related to abnormal activation in parietal and thalamic regions during a planning task, but did not specifically investigate striatal rCBF. Overall, negative symptoms seem to be associated with reduced rCBF, particularly in frontal regions, but regional specificity has yet to be determined.

The main goal of this study was to investigate the association between striatal rCBF and negative symptoms. There is evidence that apathy and diminished expression show different associations with behavioural and neurobiological correlates, suggesting differences in pathophysiology. Therefore, this distinction is of high relevance when investigating striatal rCBF. The paucity of reported associations between striatal rCBF and negative symptoms could result from the fact that until now apathy and diminished expression have not been addressed separately, even though the striatum might play very different roles in their pathophysiology.

Based on the extant (albeit limited) evidence for increased striatal resting-state rCBF in patients with schizophrenia, our first hypothesis was that these patients would show increased rCBF in the ventral and dorsal striatum compared with controls. Our second and main hypothesis was that apathy would be associated with altered rCBF in the ventral and dorsal striatum. Because no study has previously reported striatal rCBF in relation to specific dimensions of negative symptoms, we could not make predictions about the directionality of the effects.

Methods

Participants

Twenty-nine patients with schizophrenia and 20 healthy controls, matched at a group level for age and sex, were included in the present study. We recruited patients from outpatient and inpatient units of the Psychiatric Hospital of the University of Zurich and affiliated institutions. We recruited healthy controls from the community via advertisement.

We conducted the Mini-International Neuropsychiatric Interview to confirm diagnosis. Patients were clinically stable and had been on a stable dose of medication for at least 2 weeks before testing. Inpatients were at the end of their hospitalization, engaging in a multimodal therapy program and activities outside the hospital. The average duration of hospitalization for patients with schizophrenia in Switzerland is longer than in most other countries, so the majority of patients would have been treated as outpatients in other health care systems.

Exclusion criteria for patients were any other DSM-IV axis I disorder; acute psychotic symptoms (i.e., scores higher than 4 on the positive subscale of the Positive and Negative Syndrome Scale [PANSS]); extrapyramidal adverse effects (i.e., a total score higher than 2 on the Modified Simpson–Angus Scale [MSAS]); and lorazepam dosage higher than 1 mg/d. If patients met the criteria for cannabis abuse or dependency, they were also excluded from the study. Participants were excluded if they had any alcohol use disorder based on lifetime criteria. Smoking was not an exclusion criterion, but participants did not smoke for 2 hours before the ASL scans.

Controls were excluded if any neuropsychiatric diagnosis was present in the structured Mini-International Neuropsychiatric Interview. Any participants with a neurologic disorder were excluded.

The Ethics Committee of the Canton of Zurich approved the project, and participants gave written informed consent to participate in the study. The ability of each participant with schizophrenia to provide informed consent was evaluated by the treating psychiatrist.

Clinical and neuropsychological assessment

We assessed negative symptoms using the Brief Negative Symptom Scale. We calculated the 2 dimensions of negative symptoms as follows: the apathy dimension consisted of the anhedonia, avolition and asociality items; the diminished expression dimension included the blunted affect and alogia items. Other assessment instruments used were the PANSS, the Calgary Depression Scale for Schizophrenia, the Global Assessment of Functioning scale and the Personal and Social Performance scale. We assessed cognition using a brief neurocognitive test battery (see our previous studies for details) to compute a composite cognitive ability score for each participant. The following domains were included in the battery: verbal learning, verbal and visual short-term and working memory, processing speed, planning, and semantic and phonemic fluency.

MRI data acquisition

We acquired MRI data on a Philips Achieva 3.0 T whole-body scanner (Best). We employed resting-state pseudo-continuous ASL (pCASL) perfusion-weighted scans. Owing to superior signal-to-noise ratio, pCASL is considered to be a more reliable method than other ASL sequences. We based the imaging parameters for pCASL on the sequence developed by Dai and colleagues. The plane was positioned parallel to the imaging volume, with a 20 mm labeling gap between the imaging volume and the labelling volume. The ASL parameters for the single-shot, gradient-echo,
echo planar imaging sequence were as follows: repetition time 4400 ms, echo time 20 ms, flip angle 90°, field of view 240 × 161 × 240 mm, spacing 3 mm, matrix size 80 × 80, 23 slices with a slice thickness of 7 mm and no gap, SENSE 2.5, postlabelling delay 1525 ms, label duration 1650 ms, number of dynamics 75 (duration 667.9 s). One dynamic consisted of a control and a labelled image. We also acquired high-resolution anatomic images (repetition time 8.1 ms, echo time 3.7 ms, field of view 240 × 240 mm², voxel size 1 × 1 × 1 mm) using a standard T₁-weighted 3D magnetization-prepared rapid gradient echo sequence.

Calculation of cerebral blood flow

We performed image data processing and analysis using the ASLtoolbox running in MATLAB (MathWorks, Inc.) and compatible with SPM12 statistical parametric mapping software (Wellcome Trust Centre for Neuroimaging, implemented in MATLAB). For each participant, we conducted image preprocessing, including independent realignment for labelled and unlabelled images, spatial smoothing (6 × 6 × 14 mm kernel), perfusion-weighted image construction and calculation, and normalization to the Montreal Neurological Institute template (for ASL data, rCBF calculations should be performed before spatial normalization). We recorded equilibrium brain tissue magnetization (M0) images in a separate run for each participant using the same parameters as for the pCASL sequence, apart from repetition time (10 s). Next, we calculated unique cerebral spinal fluid M0 values per participant for each session (corrected for T₂* decay using a T₂* value of 74.9 ms); we took the relevant H₂O partition coefficient from the literature and considered it in the calculation of each perfusion-weighted image. We generated perfusion-weighted image series by simple subtraction of the label and control images, and then conversion to absolute mean rCBF image series.

Region-of-interest image analysis

We derived predefined regions of interest (ROIs) for the ventral and dorsal striatum from previous key publications that used fMRI (Fig. 1). Yip and colleagues defined ROI coordinates (Montreal Neurological Institute) for the ventral striatum according to a meta-analysis by Knutson and Greer (left: x = −12, y = 10, z = −2; right: x = 10, y = 8, z = 0; both 9 mm spheres); we have also used these coordinates in previous studies. We also adopted coordinates for the dorsal striatum ROI from Yip and colleagues (left: x = −9, y = 3, z = 15; right: x = 9, y = 3, z = 15; both 9 mm spheres). We generated the ROIs using the Wake Forest University Toolbox. For each ROI (ventral or dorsal striatum) we extracted mean rCBF using the MarsBaR toolbox (http://marsbar.sourceforge.net).

To compare the mean cortical (grey matter masked) cerebral blood flow (CBF) between groups, we extracted the mean CBF for each group from 90 cortical brain regions (AAL atlas, http://neuro.imm.dtu.dk/wiki/Automated_Anatomical_Labeling) and applied an unpaired 2-tailed t-test.

Statistical analysis

We conducted statistical analyses using IBM SPSS Statistics version 22. We tested demographic comparisons using a χ² test, t tests and Mann–Whitney U tests if the criterion of normal distribution was not met. To test our first hypothesis, we calculated t tests for group comparisons of rCBF between patients and controls for each ROI (ventral and dorsal striatum). We confirmed normal distribution with a Shapiro–Wilk test, and tested homogeneity of variance using a Levene test. Both assumptions were met in the current sample. We also calculated analyses of covariance to control for potential sociodemographic differences between the patient and control groups.

To test the main hypothesis, we calculated Spearman correlation coefficients (r) between negative symptoms (apathy and diminished expression) and rCBF in the ventral and dorsal striatum. We used a Steiger z test to calculate the difference between the 2 negative symptom dimensions as dependent variables and rCBF as the common independent variable.

To address potentially confounding factors in the patient group, we calculated Spearman correlation coefficients between rCBF in the ventral and dorsal striatum and age, positive symptoms, chlorpromazine equivalents, depressive symptoms and cognitive impairments via the composite cognitive ability score. Only age showed a significant association with rCBF, as well as apathy. Therefore, we calculated non-parametric partial correlations to account for the effects of age on the correlation between negative symptoms and rCBF.

All primary analyses described above related to bilateral striatal regions; we had no a priori hypotheses about differences between the left and right striatum.

Fig. 1: Regions of interest of the (A) ventral and (B) dorsal striatum.
Results

Demographic and clinical characteristics

Demographic and clinical characteristics are summarized in Table 1. The groups showed no significant differences with respect to age or sex. Controls had a higher educational level and higher cognitive scores.

Patients took the following antipsychotic monotherapies: clozapine (2 patients), olanzapine (1 patient), quetiapine (2 patients), amisulpride (2 patients), risperidone (7 patients), paliperidone (2 patients) and aripiprazole (2 patients). Several patients took a combination of antipsychotic medications: clozapine and aripiprazole (1 patient), clozapine and amisulpride (1 patient), olanzapine and aripiprazole (1 patient), olanzapine and quetiapine (1 patient), olanzapine and risperidone (1 patient), amisulpride and quetiapine (1 patient), risperidone and quetiapine (3 patients), risperidone and aripiprazole (1 patient), and aripiprazole and quetiapine (1 patient).

CBF differences between groups

To test our first hypothesis, we compared rCBF in the ventral and dorsal striatum between groups and found that patients and controls did not differ significantly (Table 2). Thus, we could not confirm our hypothesis that patients with schizophrenia would show altered rCBF in the striatum.

After we controlled for educational level and cognitive score, patients with schizophrenia showed a trend toward higher rCBF in the ventral striatum ($F_{1,45} = 3.37, p = 0.07$), but not in the dorsal striatum ($F_{1,45} = 1.89, p = 0.18$).

To control for differences in total grey matter CBF between patients (mean ± SD 41.4 ± 8.8 mL/100 mg/min) and controls (mean ± SD 41.7 ± 8.7 mL/100 mg/min) we used a t test, which showed no significant differences between groups ($t_{47} = 0.11, p = 0.91$). We also addressed potential differences in total grey matter volume between patients (mean ± SD 671.8 ± 51.4 mm$^3$) and controls (mean ± SD 677.7 ± 46.23 mm$^3$), and found no significant differences between groups ($t_{47} = 0.41, p = 0.68$).

Table 1: Demographic, psychopathological and clinical characteristics of study participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients, mean ± SD*</th>
<th>Healthy controls, mean ± SD*</th>
<th>Statistical test‡</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>27.7 ± 7.2</td>
<td>30.6 ± 6.6</td>
<td>$t_{47}$ = 1.45</td>
<td>0.15</td>
</tr>
<tr>
<td>Sex, female: male</td>
<td>7:22</td>
<td>6:14</td>
<td>$\chi^2_{1} = 0.21$</td>
<td>0.65</td>
</tr>
<tr>
<td>Education, yr</td>
<td>11.4 ± 2.8</td>
<td>14.2 ± 2.5</td>
<td>$U = 101.5$</td>
<td>≤ 0.001</td>
</tr>
<tr>
<td>Smoking, pack-years</td>
<td>5.7 ± 13.7</td>
<td>2.4 ± 4.8</td>
<td>$U = 239.5$</td>
<td>0.25</td>
</tr>
<tr>
<td>Duration of illness, yr</td>
<td>7.2 ± 7.1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Age of onset, yr</td>
<td>21.0 ± 4.8</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Chlorpromazine equivalents, mg/d</td>
<td>497.7 ± 407.4</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>BNSS score</td>
<td>15.5 ± 6.9</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Diminished expression</td>
<td>8.6 ± 7.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>SANS score§</td>
<td>11.9 ± 5.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Apathy</td>
<td>5.4 ± 2.4</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>47.7 ± 10.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CDSS total score</td>
<td>2.1 ± 2.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>GAF score</td>
<td>58.1 ± 9.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PSP total score</td>
<td>58.2 ± 9.1</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cognition (composite cognitive ability)**</td>
<td>−0.85 ± 0.80</td>
<td>0 ± 0.58</td>
<td>$t_{47} = 4.07$</td>
<td>≤ 0.001</td>
</tr>
<tr>
<td>MWT IQ</td>
<td>24.5 ± 6.0</td>
<td>28.7 ± 2.8</td>
<td>$t_{47} = 2.89$</td>
<td>0.006</td>
</tr>
</tbody>
</table>

BNSS = Brief Negative Symptom Scale; CDSS = Calgary Depression Scale for Schizophrenia; GAF = Global Assessment of Functioning; MWT IQ = Multiple Word Test Intelligence Quotient; PANSS = Positive and Negative Syndrome Scale; PSP = Personal and Social Performance scale; SANS = Scale for the Assessment of Negative Symptoms; SD = standard deviation.

*Unless otherwise indicated.

†All patients were receiving atypical antipsychotics at the time of testing.

‡We investigated potential group differences using 2-sample t tests for continuous data and $\chi^2$ tests for categorical data. For data with non-normal distribution, we applied Mann–Whitney U tests.

§Apathy includes avolition/apathy and anhedonia/asociality; diminished expression includes affective flattening or blunting and alogia.

¶Positive factor = P1, P3, P5, P9; negative factor = N1, N2, N3, N4, N6, G7; disorganized factor = P2, G5, N11; excited factor = P4, P7, G8, G14; depressed factor = G2, G3, G6.

**Cognition data have been z-transformed based on the data of the control group for each test separately. The composite cognitive ability score was computed as the mean of the z-transformed test scores at the participant level.
No participants were excluded because of excessive motion. Patients and controls did not differ significantly with respect to motion parameters.

**Correlation between CBF and negative symptoms**

To test our main hypothesis, we calculated Spearman correlations between rCBF in the striatum and the 2 negative symptom dimensions in the patient group (Table 3). We found a significant positive correlation between ventral striatal rCBF and apathy (Fig. 2a and Table 3), and between dorsal striatal rCBF and apathy (Fig. 2b and Table 3). This finding provides evidence that patients with more apathy show higher rCBF in the striatum. We found no significant correlation between ventral striatal rCBF and diminished expression (Fig. 2c and Table 3) or between dorsal striatal rCBF and diminished expression (Fig. 2d and Table 3). The results of the Steiger z test were nearly significant (ventral striatum: \( z = 1.88, p = 0.06 \); dorsal striatum: \( z = 1.73, p = 0.08 \)). In other words, the correlation between rCBF and apathy was stronger than that between rCBF and diminished expression. For the Brief Negative Symptom Scale total score, we observed a trend-level correlation between rCBF and diminished expression. For the Brief Negativistic Symptom Scale total score, we observed a trend-level correlation between rCBF and diminished expression. For the Brief Negativistic Symptom Scale total score, we observed a trend-level correlation between rCBF and diminished expression.

We found that rCBF did not correlate significantly with the following potentially confounding variables: PANSS positive factor, Calgary Depression Scale for Schizophrenia for depressive symptoms, chlorpromazine equivalents and composite cognitive ability score (Table 4). However, we did find significant positive correlations between age and rCBF of the ventral and dorsal striatum. Therefore, we calculated nonparametric partial correlations to control for the effects of age on the correlation of rCBF with apathy. The association between apathy and rCBF in the dorsal striatum was at trend level (\( r = 0.34, p = 0.07 \)), but not in the ventral striatum (\( r = 0.20, p = 0.29 \)).

Regarding our main hypothesis, we found a significant association between the severity of apathy and rCBF in both the left and right ventral striatum, and found the same pattern as in the bilateral analysis (Table 3).

In an exploratory analysis, we evaluated the associations with apathy and diminished expression in the left and right striatum, and found the same pattern as in the bilateral analysis.

We also performed an exploratory voxel-wise analysis of rCBF in the prefrontal cortex and anterior cingulate cortex. The statistical threshold was set at a peak-level family-wise error rate correction of \( p = 0.05 \). No voxels were significantly associated with apathy in the patient group.

**Discussion**

We observed no significant differences in striatal rCBF between patients with schizophrenia and healthy controls. Importantly, apathy — but not diminished expression — was associated with dorsal striatal rCBF and (to a lesser extent) ventral striatal rCBF.

For this reason, our first hypothesis concerning group differences could not be confirmed. This was at odds with some studies, which reported increased striatal rCBF in patients with schizophrenia, but other studies did not observe these effects. Potential explanations for these differences between studies include variations in image acquisition and data analysis. Most importantly, the patient populations differed in numerous ways. In our study, we specified inclusion and exclusion criteria to assess primarily for negative symptoms. In contrast, patients in the study by Kindler and colleagues had treatment-resistant auditory hallucinations. Another important factor might be the type of antipsychotic medication used for treatment.

In our study, all patients were treated with atypical antipsychotic medication, which in fMRI studies has been shown to attenuate group differences in striatal activation.

Regarding our main hypothesis, we found a significant association between the severity of apathy and rCBF in both the

### Table 2: Mean rCBF values by region of interest and group

<table>
<thead>
<tr>
<th>Region of interest</th>
<th>Patients, mean ± SD</th>
<th>Healthy controls, mean ± SD</th>
<th>Statistical test</th>
<th>( p ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventral striatum</td>
<td>33.8 ± 7.9 mL/100 g/min</td>
<td>31.5 ± 9.3 mL/100 g/min</td>
<td>( t_9 = -0.91 )</td>
<td>0.37</td>
</tr>
<tr>
<td>Dorsal striatum</td>
<td>28.4 ± 9.1 mL/100 g/min</td>
<td>26.1 ± 7.8 mL/100 g/min</td>
<td>( t_9 = -0.95 )</td>
<td>0.35</td>
</tr>
</tbody>
</table>

rCBF = regional cerebral blood flow; SD = standard deviation.

### Table 3: Spearman correlations between apathy and diminished expression and the left and right ventral and dorsal striatum in patients with schizophrenia

<table>
<thead>
<tr>
<th>Mean rCBF</th>
<th>Apathy</th>
<th>Diminished expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r_s )</td>
<td>( p ) value</td>
</tr>
<tr>
<td>Left and right ventral striatum</td>
<td>0.38</td>
<td>0.040</td>
</tr>
<tr>
<td>Left and right dorsal striatum</td>
<td>0.48</td>
<td>0.008</td>
</tr>
<tr>
<td>Left ventral striatum</td>
<td>0.32</td>
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</tr>
<tr>
<td>Right ventral striatum</td>
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<td>0.030</td>
</tr>
<tr>
<td>Left dorsal striatum</td>
<td>0.44</td>
<td>0.017</td>
</tr>
<tr>
<td>Right dorsal striatum</td>
<td>0.51</td>
<td>0.005</td>
</tr>
</tbody>
</table>

rCBF = regional cerebral blood flow.
ventral and dorsal striatum, a relationship that we did not find for diminished expression. This differential effect for the 2 negative symptom dimensions might account at least in part for the lack of consistent previous findings for the rCBF correlates of negative symptoms. In our study, an aggregation of overall negative symptoms would have led to a non-significant finding. The only other ASL study that specifically assessed apathy evaluated rCBF during planning-task performance, during which the authors observed reduced parietal and thalamic perfusion. However, they did not report striatal perfusion, and comparison with our resting-state approach is difficult. It seems to be important for future ASL studies to assess both dimensions of negative symptoms separately, because different neural mechanisms may underlie these symptoms.

While the distinction between apathy and diminished expression (the 2 negative symptom dimensions) has received very limited interest in previous ASL studies, the blood-oxygen-level-dependent (BOLD) fMRI literature has provided evidence for dissociation of their neural correlates. For instance, Kirschner and colleagues reported reduced activity in the ventral striatum during reward anticipation that correlated with apathy, but not with diminished expression. For the dorsal striatum, an association between reduced activity and avolition — but not anhedonia — has been shown. Importantly, reduced activity in these fMRI studies reflects attenuated signal differences between rewarding and nonrewarding stimuli. Therefore, an association of apathy with both a reduced task-related fMRI signal and increased resting-state rCBF in the striatum is not contradictory.

At this point, a mechanistic explanation for the association of apathy with striatal rCBF remains speculative. Several studies have found increased rCBF in the striatum to be

**Fig. 2:** Spearman correlation, including significance test, of (A) mean rCBF of the left and right ventral striatum with apathy; (B) mean rCBF of the left and right dorsal striatum with apathy; (C) mean rCBF of the left and right ventral striatum with diminished expression; (D) mean rCBF of the left and right dorsal striatum with diminished expression. BNSS = Brief Negative Symptom Scale; DS = dorsal striatum; rCBF = regional cerebral blood flow; VS = ventral striatum.
related to higher dopaminergic activity.\textsuperscript{53,56} In addition, a PET study reported an association between increased dorsal striatal dopamine release and negative symptoms,\textsuperscript{57} which might seem at odds with the observation that decreased dopamine availability can lead to apathy in neurologic patients and in animal models.\textsuperscript{57–59} However, the hypothesis of aberrant salience attribution in schizophrenia proposes that increased dopaminergic activity in the striatum leads to difficulties in distinguishing between relevant and irrelevant stimuli.\textsuperscript{54,60} This inability to differentiate relevant and (in particular) rewarding stimuli could lead to a decrease in goal-directed behaviour and promote apathy.\textsuperscript{62}

We found no significant correlation between positive symptoms or cognition and striatal rCBF. For positive symptoms, it needs to be kept in mind that the aim of the study was to investigate the neural correlates of negative symptoms, and patients with significant positive symptoms were excluded, considerably reducing variance. Cognitive deficits were not an exclusion criterion, but patients had to be able to take part in this relatively demanding study, and the overall cognitive performance of the patient group was less than 1 standard deviation below the control group.

Surprisingly, we did not find a significant group difference in rCBF of the striatum, despite the relationship between apathy and striatal rCBF. Striatal rCBF was slightly higher in patients than in controls, although this difference was not significant. This type of pattern can best be explained by a difference between patients and controls that is present only for younger patients than in controls, although this difference was not significant. This type of pattern can best be explained by a difference between age and relevant stimuli.\textsuperscript{54,60} This inability to differentiate relevant and (in particular) rewarding stimuli could lead to a decrease in goal-directed behaviour and promote apathy.\textsuperscript{62}

We observed that greater age was associated with reduced striatal rCBF and lower apathy, although effects of age have not been reported in previous studies of patients with schizophrenia. However, our finding was consistent with previous reports of reduced rCBF with increasing age in healthy individuals.\textsuperscript{64,65} Interestingly, Bijanki and colleagues\textsuperscript{66} found a negative relationship between age and negative symptoms, as well as white-matter integrity measured by diffusion tensor imaging, emphasizing the need to include age as a confounding variable. Our finding that younger patients showed stronger apathy than older participants might seem surprising, but it was consistent with the study by Bijanki and colleagues.\textsuperscript{66} Overall, the inclusion of age in partial correlations of apathy and striatal rCBF attenuated the association, but the effect in the dorsal striatum remained at trend level.

### Limitations

This study provides evidence for a positive association between increased striatal rCBF and the negative symptom dimension of apathy. However, several limitations need to be taken into account in the interpretation of these findings. First, our sample size was moderate; these findings require replication in a larger sample, which would also allow for further evaluation of the effect of age on the observed associations. Second, our sample was recruited with the aim of investigating the neural correlates of negative symptoms, and is thus not representative of the entire population of patients with schizophrenia. Different associations between symptoms and striatal rCBF could be found in patients with higher levels of positive or depressive symptoms. Third, all patients in our study took second-generation antipsychotic medication. Previous research has suggested an influence of antipsychotic medication on striatal rCBF.\textsuperscript{52,63,67,68} While we did not observe an association between striatal rCBF and antipsychotic dose, we cannot exclude a potential effect of antipsychotic medication. Thus, future studies should include nonmedicated patients and patients taking first-generation antipsychotics to generalize the relationship between apathy and striatal activity to these populations.

### Conclusion

The association between increased striatal rCBF and the negative symptom dimension of apathy, but not diminished expression, provides further evidence for the assumption of different underlying neural bases. These dimensions should be considered separately in future research on negative symptoms. Furthermore, ASL seems to provide a direct and quantitative technique for investigating negative symptoms, circumventing the limitations of task-based measures often employed for BOLD-fMRI and the invasiveness of PET and SPECT. This may qualify ASL as an alternative technique for developing biomarkers that reflect the pathomechanisms of negative symptoms.

<table>
<thead>
<tr>
<th>Correlation factor</th>
<th>Mean rCBF ventral striatum</th>
<th>Mean rCBF dorsal striatum</th>
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<tr>
<td></td>
<td>$r_{p}$</td>
<td>p value</td>
</tr>
<tr>
<td>PANSS positive factor</td>
<td>−0.15</td>
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</tr>
<tr>
<td>PANSS negative factor</td>
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<td>0.39</td>
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<td>CDSS total score</td>
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<tr>
<td>Chlorpromazine equivalents</td>
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</tr>
<tr>
<td>Composite cognitive ability score</td>
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<td>0.17</td>
</tr>
<tr>
<td>Age</td>
<td>−0.39</td>
<td>0.04</td>
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</tbody>
</table>

CDSS = Calgary Depression Scale for Schizophrenia; PANSS = Positive and Negative Syndrome Scale; rCBF = regional cerebral blood flow.
Association of apathy and rCBF in striatal regions

Acknowledgements: This study was supported by the Swiss National Science Foundation (grant no. 105314_140351 to S. Kaiser). P.N. Tobler was supported by the Swiss National Science Foundation (PP00P1_128574, PP00P1_150739, CRSII3_141965 and 00014_165988). The authors thank M. Bischof for his support in data acquisition, A. Manoliu for his support with layout of the figures, and all patients and healthy volunteers for their participation.

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Competing interests: P. Tobler has received grant support from Pfizer. E. Seifritz has received grant support from H. Lundbeck and has served as a consultant and/or speaker for AstraZeneca, Otsuka, Takeda, Eli Lilly, Janssen, Lundbeck, Novartis, Pfizer, Roche and Servier. S. Kaiser has received speaker honoraria from Roche, Lundbeck, Janssen and Takeda. He receives royalties for cognitive test and training software from Schuhfried. None of these activities is related to the present study. All other authors declare no biomedical financial interest or potential conflicts of interest.

Contributors: M. Kirschn er, M.N. Hartmann-Riemer, E. Seifritz, P. Stämpfli, P.N. Tobler and S. Kaiser designed the study: M. Kirschn er and M.N. Hartmann-Riemer acquired the data, which K. Schneider, L. Michels, M. Kirschn er, A. Burrer and S. Kaiser analyzed. K. Schneider, L. Michels, E. Seifritz and S. Kaiser wrote the article, which all authors reviewed. All authors approved the final version to be published and can certify that no other individuals not listed as authors have made substantial contributions to the paper.

References


Research Paper

GABA levels and TSPO expression in people at clinical high risk for psychosis and healthy volunteers: a PET-MRS study

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Introduction

γ-Aminobutyric acidergic (GABAergic) dysfunction and immune activation have been implicated in the pathophysiology of schizophrenia. Preclinical evidence suggests that inflammation-related abnormalities may contribute to GABAergic alterations in the brain, but this has never been investigated in vivo in humans. In this multimodal imaging study, we quantified cerebral GABA plus macromolecule (GABA+) levels in antipsychotic-naive people at clinical high risk for psychosis and in healthy volunteers. We investigated for the first time the association between GABA+ levels and expression of translocator protein 18 kDa (TSPO; a marker of microglial activation) using positron emission tomography (PET).

Methods: Thirty-five people at clinical high risk for psychosis and 18 healthy volunteers underwent 3 T proton magnetic resonance spectroscopy to obtain GABA+ levels in the medial prefrontal cortex (mPFC). A subset (29 people at clinical high risk for psychosis and 15 healthy volunteers) also underwent a high-resolution [18F]FEPPA PET scan to quantify TSPO expression. Each participant was genotyped for the TSPO rs6971 polymorphism. Results: We found that GABA+ levels were significantly associated with TSPO expression in the mPFC ($F_{1,40} = 10.45$, $p = 0.002$). We found no significant differences in GABA+ levels in the mPFC ($F_{1,51} = 0.00$, $p > 0.99$) between people at clinical high risk for psychosis and healthy volunteers. We found no significant correlations between GABA+ levels or residuals of the association with TSPO expression and the severity of prodromal symptoms or cognition.

Limitations: Given the cross-sectional nature of this study, we could determine no cause-and-effect relationships for GABA alterations and TSPO expression. Conclusion: Our findings suggest that TSPO expression is negatively associated with GABA+ levels in the prefrontal cortex, independent of disease status.

Background: γ-Aminobutyric acidergic (GABAergic) dysfunction and immune activation have been implicated in the pathophysiology of schizophrenia. Preclinical evidence suggests that inflammation-related abnormalities may contribute to GABAergic alterations in the brain, but this has never been investigated in vivo in humans. In this multimodal imaging study, we quantified cerebral GABA plus macromolecule (GABA+) levels in antipsychotic-naive people at clinical high risk for psychosis and in healthy volunteers. We investigated for the first time the association between GABA+ levels and expression of translocator protein 18 kDa (TSPO; a marker of microglial activation) using positron emission tomography (PET). Methods: Thirty-five people at clinical high risk for psychosis and 18 healthy volunteers underwent 3 T proton magnetic resonance spectroscopy to obtain GABA+ levels in the medial prefrontal cortex (mPFC). A subset (29 people at clinical high risk for psychosis and 15 healthy volunteers) also underwent a high-resolution [18F]FEPPA PET scan to quantify TSPO expression. Each participant was genotyped for the TSPO rs6971 polymorphism. Results: We found that GABA+ levels were significantly associated with TSPO expression in the mPFC ($F_{1,40} = 10.45$, $p = 0.002$). We found no significant differences in GABA+ levels in the mPFC ($F_{1,51} = 0.00$, $p > 0.99$) between people at clinical high risk for psychosis and healthy volunteers. We found no significant correlations between GABA+ levels or residuals of the association with TSPO expression and the severity of prodromal symptoms or cognition. Limitations: Given the cross-sectional nature of this study, we could determine no cause-and-effect relationships for GABA alterations and TSPO expression. Conclusion: Our findings suggest that TSPO expression is negatively associated with GABA+ levels in the prefrontal cortex, independent of disease status.
changes underlying schizophrenia, without the confounding influence of antipsychotic medication. Only 3 studies have investigated GABA levels in people at CHR for psychosis. Wang and colleagues reported unaltered GABA levels in the mPFC of antipsychotic-naive people at CHR (21 people at CHR and 23 healthy volunteers). Similarly, a recent study also failed to detect differences in GABA levels in the mPFC of antipsychotic-naive people at CHR (21 people at CHR and 20 healthy volunteers), whereas another study reported increased GABA levels in the mPFC (23 people at CHR and 24 healthy volunteers). Converging evidence from preclinical, genetic and peripheral studies have implicated immune-related abnormalities in the pathophysiology of schizophrenia. Microglia play a critical role in both healthy and diseased states of the central nervous system. In the developing and mature central nervous system, microglia are involved in synaptic pruning and maturation, and in maintaining synaptic plasticity. On the other hand, microglia also function as inflammatory cellular mediators in response to tissue damage or brain insult. Neuroinflammation is characterized (at least in part) by microglial activation. Microglial activation is associated with elevated expression of a mitochondrial protein, translocator protein 18 kDa (TSPO), making TSPO a suitable marker for quantifying microglial activation in vivo. Currently, we can quantify TSPO expression in vivo using positron emission tomography (PET). The most replicated finding across TSPO PET studies in schizophrenia suggests no differences in TSPO expression using second-generation radioligands, although a recent study reported a significant reduction in patients with schizophrenia compared with healthy volunteers, also supported by a recent meta-analysis. Three other studies reported elevated TSPO expression in people with schizophrenia and first-episode psychosis, but these studies used the first-generation radioligand [11C]PK11195, which has several known limitations. Only 2 studies have investigated TSPO expression in people at CHR for psychosis, and both reported a lack of group differences between people at CHR and healthy volunteers using total distribution volume (Vt), a validated outcome measure for second-generation TSPO radioligands.

Accumulating evidence suggests that GABAergic signaling can modulate inflammatory processes, and that immune processes can induce reciprocal changes in the GABAergic system, providing a potential link between immune activation and GABAergic alterations in schizophrenia. Preclinical studies have consistently shown alterations in gene expression across GABAergic pathways and reductions in GABAergic parvalbumin-positive interneurons following prenatal exposure to inflammatory stimuli. Activation of GABA receptor activity attenuates immune activation, while inhibition of receptor activity increases inflammatory responses. In fact, studies have shown that microglial activation is negatively regulated by GABAergic neurotransmission. As well, GABA receptors are present on immune cells, including astrocytes and microglia, suggesting a potential role for GABA in neuroimmune responses. Moreover, GABAergic deficits can lead to glutamatergic hyperactivity, resulting in toxicity-mediated neuronal death, excessive glial activation and neuroinflammation. Although there is a close relationship between GABAergic and immune dysfunction in schizophrenia, the in vivo relationship between these 2 systems has yet to be investigated in humans. In the present study, we investigated GABA plus macromolecule (GABA+) in the mPFC of antipsychotic-naive people at CHR for psychosis, making this the largest GABA+ 1H-MRS study in this population (35 people at CHR and 18 healthy volunteers). We also investigated, to our knowledge for the first time, the in vivo association between GABA+ levels and TSPO expression using 3 T 1H-MRS and high-resolution PET, respectively. Based on previous evidence, we hypothesized that there would be a negative association between GABA+ levels and TSPO expression in the brain.

Methods

Participants

We initially enrolled 36 people at CHR for psychosis and 18 healthy volunteers. Most of the people in the CHR group were antipsychotic-naive (n = 30).

People in the CHR group were included if they met diagnostic criteria for prodromal risk syndrome as assessed by the Criteria of Prodromal Syndromes. They were excluded if they had a current axis I disorder as determined by the Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I). Healthy volunteers were included if they did not have a history of past psychoactive drug use and/or first-degree relatives with a major mental disorder. All participants were excluded if they had a clinically significant medical illness, had a current diagnosis of alcohol or substance use/dependence, were pregnant or breastfeeding, or had metal implants that precluded an MRI scan. In the CHR group, we assessed clinical status and the severity of prodromal symptoms using the Structured Interview for Psychosis-Risk Syndromes, the Scale of Psychosis-Risk Symptoms, the Calgary Depression Scale for Schizophrenia for depression symptoms and the Apathy Evaluation Scale for apathy. We assessed neurocognitive performance using the Repeatable Battery for the Assessment of Neuropsychological Status.

This study was approved by the research ethics board at the Centre for Addiction and Mental Health in Toronto, Canada. All participants provided written informed consent after procedures had been explained thoroughly.

Data acquisition and analysis: 1H-MRS

All participants underwent a 1H-MRS scan on a 3 T MR-750 scanner (General Electric Medical Systems) equipped with an 8-channel head coil. Head position was fixed at the centre of the head coil with tape strapped across the forehead and soft padding to minimize head motion. A 24 mL (20 × 40 × 30 mm3) MRS voxel was positioned as shown in Figure 1A. The voxel called mPFC was composed of 65% cingulate gyrus and 28% superior frontal gyrus (Appendix 1, Fig. 2S, available at jpn.ca/170201-a1). We performed shimming using the
manufacturer-supplied automated shimming routine (AUTOSHIM) to adjust magnet homogeneity. We measured GABA levels using a modification of standard single-voxel, double-spin echo data acquisition by inserting a pair of frequency-selective radiofrequency (RF) pulses before and after the second volume-selective, 180° RF pulse (MEGA-PRESS).\textsuperscript{13,55–59} This J-editing approach used frequency-selective RF pulses to alter the temporal evolution of the strongly coupled spins in the GABA C2, C3 and C4 multiplets. The frequencies of these editing RF pulses were centred to suppress the C3 resonance of GABA at 1.9 ppm in the “on” condition and at 7.5 ppm in the “off” condition (Fig. 1B). This editing RF pulse pair inhibited and allowed J-modulation of the coupled GABA spin system, such that subtraction of the 2 subspectra yielded the J-edited GABA resonance free from the overlapping creatine and aspartate resonances. However, our data acquisition did not exclude the underlying macromolecule, so the GABA observed in this study was GABA+. We averaged 8 excitations for each “on” and “off” frequency cycle, resulting in 66 data frames. We combined these multichannel raw MRS data sets in the time domain based on coil sensitivity derived from unsuppressed water signal, weighted by the sum of the squares of the signal intensities from each channel (Fig. 1B).\textsuperscript{60} Subtraction of the “on” from the “off” condition yielded only the J-edited GABA C4 resonance (Fig. 1C).

Acquisition parameters for the measurements were as follows: echo time 68 ms, repetition time 1500 ms, bandwidth = 5 kHz, number of excitations = 528 (512 water-suppressed, 16 water-unsuppressed), data points = 4096. The data acquisition implementation used in this study has been validated in a large-scale, multivendor, multisite study, with the aim of understanding the factors that impact GABA measurement outcome with a coefficient of variation of the entire healthy volunteer cohort of 12% for GABA.\textsuperscript{61}

All postprocessing and analysis were performed with Gannet\textsuperscript{62,63} using Gaussian line shape fitting, with modifications to allow the output of GABA+ area (Fig. 1D). The data were zero-padded to 32 000 points, and we used line-broadening of 3 Hz. The ratio of GABA+ to unsuppressed water peak areas has been reported.\textsuperscript{64} Spectra that exceeded a full width at half maximum of unsuppressed water resonance greater than 10 Hz were excluded from further analysis.

To estimate the fractions of grey matter, white matter and cerebrospinal fluid in the voxel, we created volume images and a segmentation mask for the mPFC from the MRS raw data file and 3D T\textsubscript{1}-weighted images using an in-house MATLAB-based code. We then performed segmentation using statistical parametric mapping software (SPM8; Welcome Centre for Human Neuroimaging) to determine fractional tissue composition in the voxel.

**Data acquisition and analysis: PET and structural MRI**

Data from PET and MRI were available for a subset of our sample (29 people at CHR and 15 healthy volunteers). As well, 26 people at CHR and 13 healthy volunteers were available for analysis. We used statistical parametric mapping software (SPM8) to analyze the relationship between GABA levels and structural brain measures. The results showed a significant negative correlation between GABA levels and grey matter volume in the mPFC, suggesting that decreased GABA levels may be associated with structural brain changes in this region.
included in our previous cohorts, including 1 manuscript in submission. The PET and MRI scans were obtained on average 16.09 days apart. The PET data acquisitions have been described in detail elsewhere. Briefly, we obtained proton-density-weighted and T1-weighted brain magnetic resonance images for each participant using a 3 T MR-750 scanner. We performed all [18F]FEPPA scans using a high-resolution research tomograph (CPS/Siemens). Each participant was given an intravenous bolus injection of 186.53 ± 11.01 MBq of [18F]FEPPA for 125 min. Arterial and manual blood samples were taken to measure radioactivity in blood and the relative proportion of radiolabelled metabolites. We collected arterial blood for the first 22.5 min at a rate of 2.5 mL/min after radioligand injection, using an automatic blood sampling system (Model PBS-101, Veenstra Instruments). We performed all [18F]FEPPA scans using a high-resolution research tomograph (CPS/Siemens). Each participant was given an intravenous bolus injection of 186.53 ± 11.01 MBq of [18F]FEPPA for 125 min. Arterial and manual blood samples were taken to measure radioactivity in blood and the relative proportion of radiolabelled metabolites. We collected arterial blood for the first 22.5 min at a rate of 2.5 mL/min after radioligand injection, using an automatic blood sampling system (Model PBS-101, Veenstra Instruments). We took manual blood samples at −5, 2.5, 7, 12, 15, 20, 30, 45, 60, 90 and 120 min relative to the time of injection. Dispersion and metabolite-corrected plasma input function were generated as previously described. We extracted time–activity curves for the mPFC using a validated in-house imaging pipeline regions of mental interest. The region of interest was delineated using proton-density MRIs. We derived V_t in the mPFC from the time–activity curve and plasma input function using a 2-tissue compartment model, which has been validated for [18F]FEPPA quantification.

**Genotyping: rs6971 polymorphism**

A polymorphism in the TSPO gene (rs6971) affects the binding affinity of second-generation radioligands, including [18F]FEPPA. We genotyped all participants on the basis of this polymorphism as high-affinity (C/C), mixed-affinity (C/T) or low-affinity (T/T) binders, as described elsewhere.

**Statistical analysis**

We tested differences in participant characteristics between the CHR group and healthy volunteers using χ² tests for categorical variables (e.g., sex) and analysis of variance for continuous variables (e.g., age). To test for differences in GABA+ levels in mPFC between groups, we used univariate analysis of variance, with GABA+ levels as the dependent variable and group (CHR v. healthy volunteers) as a fixed factor. To test our hypothesis regarding the association between [18F]FEPPA V_t and GABA+ levels in mPFC, we used a general linear model with [18F]FEPPA V_t in mPFC as the dependent variable; group and TSPO genotype (categorical variable, high-affinity binders or mixed-affinity binders) as fixed factors; and GABA+ levels as a covariate. We tested the main effects of group, TSPO genotype and GABA+ levels, and the interaction between GABA+ levels and group. We removed the GABA+ × group interaction from the final model because it was not significant. Owing to the large-scale difference between GABA+ levels (10⁻⁵ range) and [18F]FEPPA V_t (10⁵ range), we standardized GABA+ levels before the analysis by subtracting the mean and dividing the difference by the standard deviation. To test for potential confounding effects of sex and age on GABA+ levels and association with TSPO expression, we added both factors as covariates to the model. We also explored the associations between the residuals of the general linear model and clinical and neuropsychological measures in people at CHR for psychosis using bivariate correlations. We performed statistical analyses using SPSS version 23.0 (IBM Inc.). We set the significance level at p < 0.05.

**Results**

The demographic and clinical characteristics of the participants are shown in Table 1. One member of the CHR group was excluded because of motion during the ¹H-MRS scan that could not be corrected. We found no significant differences in sex and age between the CHR group and the healthy volunteers (Table 1), or in TSPO genotype or PET parameters (Appendix 1, Table S1). Five members of the CHR group were on low-dose antipsychotic treatment with risperidone (1 taking 0.5 mg and 2 taking 1 mg), quetiapine (75 mg) or aripiprazole (5 mg) at the time of the PET scan. All participants had had a negative urine drug screen, except for 1 member of the CHR group, who had a positive urine drug screen for benzodiazepines (1-time use) and 4 members of the CHR group who had a positive urine drug screen for cannabis, but no other drugs. The averaged unsuppressed water line width for GABA+ data acquisition, full width at half maximum, was 7.39 ± 0.85 Hz for healthy volunteers and 7.57 ± 1.14 Hz for the CHR group. We observed no significant differences in full width at half maximum between groups (Appendix 1, Table S2).

**GABA+ levels in the mPFC**

We found no significant differences in GABA+ levels in mPFC between the CHR group and healthy volunteers (35 CHR and 18 healthy volunteers; F_{1,38} = 9.42, p = 0.004). Results remained the same when controlling for sex and age (F_{1,38} = 0.00, p > 0.99). Results remained the same when excluding members of the CHR group who were tobacco users, or were positive for cannabis, benzodiazepine or antipsychotic use. We found no significant differences in grey matter, white matter or cerebrospinal fluid fractions in the mPFC voxel between the CHR group and the healthy volunteers (Appendix 1, Table S2). We also found no significant correlations between GABA+ levels and symptom severity or clinical and neuropsychological measures (Appendix 1, Table S3).

**GABA+ and [18F]FEPPA V_t in the mPFC**

We observed a significant negative association between GABA+ levels and [18F]FEPPA V_t in the mPFC (β = −1.47, SE = 0.45; F_{1,51} = 10.45, p = 0.002; Fig. 3). We obtained similar results after controlling for sex and age (β = −1.43, SE = 0.46; F_{1,51} = 9.42, p = 0.004). Results remained the same when excluding members of the CHR group who were tobacco users, or were positive for cannabis, benzodiazepine or antipsychotic use. We found no significant correlations between residuals of the model and symptom severity or clinical and...
neuropsychological measures. The lack of group differences in \([^{18}F]\)FEPPA VT between CHR and healthy volunteers has been reported elsewhere (Appendix 1, Table S1).

**Discussion**

This is, to our knowledge, the first in vivo study to investigate GABA+ levels in the mPFC of people at CHR for psychosis and their association with TSPO expression. As previously reported, we observed no significant differences in GABA+ levels or \([^{18}F]\)FEPPA VT in the mPFC between members of the CHR group and healthy volunteers. We reported a significant negative association between GABA+ levels and TSPO expression in the mPFC. We found no correlations between GABA+ levels in the mPFC or residuals of the model and severity of prodromal symptoms or neuropsychological scores.

**GABA+ levels in the mPFC**

We found no significant differences in GABA+ levels in the mPFC between the CHR group and the healthy volunteers (35 people at CHR and 18 healthy volunteers). We also found no associations between GABA+ levels and the severity of prodromal symptoms or clinical and neuro-psychological measures. Of the 3 \(^3\)H-MRS GABA studies in people at CHR for psychosis, 2 studies reported unaltered GABA levels in the mPFC, and the other reported elevated GABA levels in people at CHR compared with healthy volunteers. Such discrepancies in the findings are most likely due to differences in the positioning of the \(^3\)H-MRS voxel in the mPFC. A recent study using a smaller sample and a voxel that overlapped with ours (i.e., it was more dorsally placed in the mPFC) reported unaltered GABA levels in antipsychotic-naive people at CHR compared with healthy controls. Of the 2 studies with the \(^3\)H-MRS voxel more ventrally placed in the mPFC, 1 reported unaltered GABA levels, but the other reported increased GABA levels in people at CHR compared with healthy volunteers. This discrepancy could be attributable to methodological rigour, given that the MEGA-PRESS spectrum shown in the former study was of poorer quality (a very broad GABA resonance) than the spectrum shown in the study that reported mPFC GABA elevations. Future studies should consider standardizing voxel placement and size, naming voxels consistently and (if possible) quantifying GABA levels in both dorsal and ventral brain regions to clarify whether regional differences exist in the mPFC in people at CHR for psychosis. However, consistent with our findings, a recent

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**Table 1: Demographic and clinical characteristics of study participants**

| Measure                  | Healthy volunteers
<table>
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<td>RBANS, total, mean ± SD</td>
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AES = Apathy Evaluation Scale; CDSS = Calgary Depression Scale for Schizophrenia; CHR = clinical high risk for psychosis; LSD = lysergic acid diethylamide; MDMA = 3,4-methylenedioxymethamphetamine (ecstasy); SOPS = Scale of Psychosis-Risk Symptoms; RBANS = Repeatable Battery for the Assessment of Neuropsychological Status; SD = standard deviation.

*All participants underwent a urine drug screen at baseline for cannabis, ethanol, methadone and cocaine. Baseline results were negative, except for 1 member of the CHR group with a positive result for benzodiazepine, and 4 members of the CHR group with a positive result for cannabis.

†Participants at clinical high risk for psychosis who were currently on antipsychotic treatment: 0.5 mg risperidone (n = 1), 1.0 mg risperidone (n = 2), 75 mg quetiapine (n = 1), and 5 mg aripiprazole (n = 1).

‡Score missing for 2 participants.
meta-analysis of $^{1}$H-MRS GABA in schizophrenia failed to reveal group differences in the mPFC. Longitudinal clinical follow-up revealed that 7 of the 35 people at CHR in this study (20%) converted to psychosis. Although this was a cross-sectional study and conclusions were limited by small sample size, we found no significant differences in GABA+ levels in the mPFC between converters at CHR and nonconverters ($F_{1,35} = 1.53, p = 0.23$; Appendix 1, Figure S1).

**GABA+ and $[^{18}F]$FEPPA VT in the mPFC**

We found a significant negative association between GABA+ levels and $[^{18}F]$FEPPA $V_t$ suggesting that regardless of clinical diagnosis, higher TSPO expression is associated with lower GABA levels. Although this is the first in vivo study to investigate the link between immune activation and the GABAergic system in the brain, accumulating evidence from preclinical studies suggests a potential link between these 2 systems. Preclinical studies have consistently shown that immune activation alters components of the GABAergic system in the brain, including reductions in GABA receptor expression and function, neuronal density and the expression of GAD65 and GAD67, 2 major GABA-synthesizing enzymes. Similarly, reduced GABA levels in the prefrontal cortex of mice have been reported following prenatal exposure to inflammatory stimuli. Further, supporting our results, overexpression of interleukin-6, specifically in glial cells, decreased GABA-positive and parvalbumin-positive neurons in adult mouse hippocampus. In addition, GABA$_A$ and GABA$_B$ receptor agonists can reduce microglial activation and attenuate the release of proinflammatory cytokines, while inhibition of GABA receptor activity increases inflammatory responses. Our results provide, to our knowledge, the first in vivo evidence linking GABA levels and TSPO expression, but future studies should investigate the mechanisms underlying this association.

**Limitations**

Interpretation of the results of this study should consider the following limitations. First, the proton MRS voxels are relatively large because of the low concentration of GABA in the human brain. Our mPFC voxel contained approximately 65% cingulate gyrus and 28% superior frontal gyrus (Appendix 1, Figure S2). In addition, the GABA quantification reported in this study was a combination of GABA molecule and mobile macromolecule (up to 50% of total GABA signal), termed GABA+. The contribution of this macromolecule to the total GABA signal in healthy participants has been documented using the same J-editing approach. However, it is not known whether this mobile macromolecule might differ in people at CHR for psychosis.

Second, there have been inconsistencies in the placement of $^{1}$H-MRS voxels across GABA studies in schizophrenia, particularly in the mPFC. We acknowledge that the failure of this study to detect group differences in GABA+ levels between people at CHR for psychosis and healthy volunteers may be due to the more dorsal placement of our $^{1}$H-MRS voxel in the mPFC — consistent with Modinos and colleagues — compared with a more ventral placement.

Third, in neurochemical brain-imaging studies, relatively small sample sizes represent a potential limitation; however, to our knowledge, this is the largest GABA $^{1}$H-MRS study in people at CHR for psychosis. In addition, given the novelty of our findings in linking TSPO expression and GABA levels, this study can serve as a framework for future studies.

Fourth, although we acquired scans on 2 separate days, both $[^{18}F]$FEPPA PET and GABA $^{1}$H-MRS have satisfactory
test–retest reliability, and no changes in PET and MRI machine stability and CHR pathophysiology are expected in our time interval (about 16 days).

Fifth, the voxel used for $^{1}$H-MRS in mPFC was larger than the region of interest used with PET and also included the dorsal part of the anterior cingulate cortex, but this was unlikely to affect the results presented.

Sixth, although an increase in $^{[18]}$F-FEPPA binding was mostly attributed to microglial activation, studies show that astrocytes and vascular endothelial cells also express TSPO. However, this does not affect the overall conclusion of our study, given that astrocytes are also involved in the immune response. Further studies are needed to evaluate the contribution of these components to the TSPO signal. In addition, the link between TSPO expression and microglial activation is not yet well understood. Several studies have shown marked elevations in TSPO expression in activated microglia, including a study by Sandiego and colleagues that reported robust increases in $^{[14]}$C]PBR28 binding (a second-generation TSPO radioligand) following a lipopolysaccharide challenge, confirming that TSPO is upregulated during immune activation; however, other studies have shown a decrease in TSPO in proinflammatory states.

Seventh, the $^{[18]}$F-FEPPA outcome measure, $V_r$, is a composite measure that includes both specific and nonspecific-plus-free binding, and to date, it is unknown whether the nonspecific-plus-free binding is different between groups. Further, in this study we did not correct $^{[18]}$F-FEPPA $V_r$ for the plasma-free fraction of the radioligand ($f_p$), because it has been shown to substantially increase the variability.

Finally, we acknowledge that GABA does not provide a full picture of excitatory and inhibitory balance in the brain. However, exploratory analysis in our data set revealed no significant group differences between mPFC glutamate + glutamate dehydrogenase and inhibitory index, or associations with TSPO expression in the mPFC (Appendix 1, Table S4, Table S5, Figure S3, Figure S4). Similarly, we found no group differences in mPFC N-acetylaspartate/creatine or choline/creatine ratios between people at CHR for psychosis and healthy volunteers, or associations with TSPO expression in the mPFC (Appendix 1, Table S4, Table S5).

Conclusion

The results of this study suggest a link between GABA+ levels and TSPO expression in the mPFC, independent of disease status.

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Contributors: R. Mizrahi designed the study. All authors acquired and analyzed the data. N. Sailasuta and R. Mizrahi wrote the article, which all authors reviewed. All authors approved the final version to be published and can certify that no other individuals not listed as authors have made substantial contributions to the paper.

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Lateral orbitofrontal dysfunction in the Sapap3 knockout mouse model of obsessive–compulsive disorder

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Background: Obsessive–compulsive disorder (OCD) is a common psychiatric disorder that affects about 2% of the population, but the underlying neuropathophysiology of OCD is not well understood. Although increasing lines of evidence implicate dysfunction of the orbitofrontal cortex (OFC) in OCD, a detailed understanding of the functional alterations in different neuronal types in the OFC is still elusive. Methods: We investigated detailed activity pattern changes in putative pyramidal neurons and interneurons, as well as local field potential oscillations, in the lateral OFC underlying OCD-relevant phenotypes. We applied in vivo multichannel recording in an awake OCD mouse model that carried a deletion of the Sapap3 gene, and in wild type littermates. Results: Compared with wild type mice, the lateral OFC of Sapap3 knockout mice exhibited network dysfunction, demonstrated by decreased power of local field potential oscillations. The activity of inhibitory and excitatory neurons in the lateral OFC showed distinct perturbations in Sapap3 knockout mice: putative interneurons exhibited increased activity; putative pyramidal neurons exhibited enhanced bursting activity; and both putative pyramidal neurons and interneurons exhibited enhanced discharge variability and altered synchronization. Limitations: To exclude motor activity confounders, this study examined functional alterations in lateral OFC neurons only when the mice were stationary. Conclusion: We provide, to our knowledge, the first direct in vivo electrophysiological evidence of detailed functional alterations in different neuronal types in the lateral OFC of an OCD mouse model. These findings may help in understanding the underlying neuropathophysiology and circuitry mechanisms for phenotypes relevant to OCD, and may help generate and refine hypotheses about potential biomarkers for further investigation.

Introduction

Obsessive–compulsive disorder (OCD) is a debilitating neuropsychiatric condition with a lifetime prevalence of 2%. It is characterized by persistent intrusive thoughts (obsessions) and repetitive actions (compulsions). Although dysfunction of the cortico–striato–thalamo–cortical circuitry has been implicated in the pathogenesis of OCD and is supported by neuroimaging studies in patients, the underlying neuropathological changes are still not well understood. The orbitofrontal cortex (OFC) may be central to our understanding of OCD, because it is the most frequently reported region of structural, functional and connectivity alterations in patients with OCD. The OFC is thought to update outcome expectations when rules linking stimuli to outcomes are changed. Therefore, the OFC is essential for behaviour flexibility and goal-directed behaviours, both of which are impaired in people with OCD. For this reason, the OFC is well suited as a neural substrate for OCD pathogenesis.

Although a large number of functional neuroimaging studies have shown altered metabolic activity in the OFC of people with OCD, a detailed understanding of the functional alterations is still elusive. For example, a majority of studies have reported increased resting metabolic activity in the OFC of people with OCD, which is exacerbated by symptom provocation and alleviated after successful treatment. However, these studies can only measure metabolic levels to indirectly reflect general neuronal activity levels. The noninvasive methods used in clinical studies also have limited spatial and temporal resolution. Direct electrophysiological evidence of detailed activity change of different neuronal types in the OFC of people with OCD or animal models is still lacking. Furthermore, there are discrepancies in the directionality of findings in clinical neuroimaging studies that may be due...
to the heterogeneity of the disorder, comorbidities, medication history or different subregions of the OFC analyzed.

In the present study, we investigated detailed functional change in different neuronal types in the lateral OFC (lOFC) that underlie OCD-relevant phenotypes by applying in vivo multichannel recording in an awake OCD mouse model that carried a deletion of the Sapap3 gene. These Sapap3 knockout (KO) mice demonstrate several OCD-like behaviours, including excessive and pathological self-grooming and increased anxiety-like behaviours, suggesting potential relevance to OCD. As well, the entire constellation of OCD-like behaviours in Sapap3 KO mice is alleviated by chronic fluoxetine, a first-line treatment for OCD. Human genetics studies also support a role for Sapap genes in OCD. Therefore, a detailed understanding of functional alterations in the OFC of Sapap3 KO mice could help identify potential circuit mechanisms for behaviours relevant to OCD. The OFC consists of lateral and medial subregions. The lateral and medial OFC may perform different functions, such as processing negative versus positive valence. A previous study from our group found that selective stimulation of the lOFC suppressed overexpression of both spontaneous and conditioned repetitive grooming behaviours, suggesting involvement of the lOFC in these behaviours in Sapap3 KO mice. To investigate functional alterations in the lOFC in Sapap3 KO mice, we focused on the lOFC subregion in the current study. Using single-unit and local field potential (LFP) recording in the lOFC, we studied activity pattern changes in different neuronal populations and alterations in LFP oscillations in Sapap3 KO mice. Our goal was to shed light on the neuropathophysiology underlying OCD-like behaviours and advance our circuit-level understanding of phenotypes relevant to OCD. Our findings may help to generate and refine hypotheses for further investigation. For example, LFP alterations and increased burst firing in lOFC may be useful biomarker candidates for further examination in people with OCD.

Methods

Animal use

All experiments were conducted according to protocols approved by the Institutional Animal Care and Use and Institutional Biosafety committees of the Capital Medical University (Beijing, China) and the Massachusetts Institute of Technology (Cambridge, Massachusetts). Our group had previously found that from age 2 to 3 months, Sapap3 KO mice exhibited significantly increased self-grooming that resembled compulsive OCD behaviours. Therefore, we performed all of our experiments on adult Sapap3 KO mice aged 3 to 10 months, and on age-matched, wild type (WT) littermates of either sex.

Surgery

Our method of electrophysiological recording in head-restrained, mobile mice was based on previous studies with modifications. Briefly, for head-plate implantation, mice were anesthetized by intraperitoneal injection of Avertin solution (20 mg/mL, 0.5 mg/g body weight) and then mounted in a stereotactic holder and kept warm (37°C) with an electric heating pad (BrainKing Biotech). A small skull region (~1 mm in diameter) located posterior to the lOFC based on stereotactic coordinates (anterior–posterior = 2.3 mm, medial–lateral = 1.3 mm) was thinned but not broken with a high-speed drill. A custom-made head plate with a hole 2 mm in diameter was placed on the skull, with the hole centred over the thinned region above the lOFC. The head plate was affixed to the skull with Metabond (Parkell Inc.), and the thinned skull and hole in the head plate were then covered with Kwik-sil (World Precision Instruments) for protection. Mice were individually housed after surgery and allowed to recover for 3 to 5 days before habituation training. To minimize potential stress effects, mice were trained to habituate to a head-fixed spherical treadmill for 2 to 4 hours each day for 4 consecutive days before recording. Mice quickly learned to balance and walk on the apparatus and stayed quiet for most of the time during recording, indicating low stress.

Electrophysiological recording

During electrophysiological recording, the mouse’s head was restrained by a head plate, and the mouse was able to manoeuvre on the top surface of an air-supported floating styrofoam ball. Immediately before recording, we opened a small craniotomy in the thinned skull area above the lOFC. We detected extracellular spiking signals and LFP using a 32-channel silicon probe (A4×8–5mm–50–200–413–A32–15; NeuroNexus) arranged in a 4 × 8 pattern (4 shanks with 8 recording sites in each shank), lowered to the lOFC (anterior–posterior = 2.5–2.8 mm; medial–lateral = 1.0–1.6 mm, dorsal–ventral = 1.3–2 mm) and tilted rostrally at an angle of 15° to the vertical plane. Based on the above coordinates, we discarded neural activities recorded outside the lOFC from analysis. We sampled unit activity at 30 kHz and high-pass filtered it at 250 Hz using a Blackrock Cerebus data acquisition system (Blackrock Microsystems LLC). We sampled LFP at 1 kHz and low-pass-filtered it at 250 Hz. To avoid possible noise contamination in low-frequency oscillations, we discarded LFP data below 1.5 Hz.

Spike sorting and single-unit classification

We sorted unit activity containing spikes of multiple neurons manually offline using Offline Sorter (Plexon Inc.) and a combination of template-matching and principal-components analyses. A total of 362 single units were well isolated. Units with a trough half width within 100–200 μs, a peak half width within 467–700 μs and a trough:peak ratio within 1.2–2.8 were classified as putative pyramidal neurons. Units with a trough half width within 67–167 μs, a peak half width within 100–300 μs and a trough:peak ratio within 1.1–1.8 were classified as putative interneurons. Using these criteria, we identified 294 units as putative pyramidal neurons and 51 units as putative interneurons.
Detailed explanations of these cell-type classification criteria are provided in Appendix 1, available at jpn.ca/180032-a1.

Statistical analysis

All analyses used custom Matlab software (H.L.). For LFP oscillation power, the mean baseline firing rate of putative pyramidal neurons and interneurons, the percentage of spikes in the bursting mode per neuron and the number of bursts per minute per neuron, we determined statistical significance between WT and Sapap3 KO mice using the Wilcoxon rank sum test. For LFP oscillation power, n was the number of animals. For single-unit activity, n was the number of neurons. We measured the correlation between firing rate and the depth of the neurons using the Spearman rank correlation coefficient.

We measured firing variability using \( CV_2 \). We defined \( CV_2 \) for spike i as the standard deviation of 2 adjacent interspike intervals (ISIs) divided by their mean and multiplied by \( \sqrt{2} \).

\[
CV_2 = \frac{2|\Delta t_{i+1} - \Delta t_i|}{\Delta t_{i+1} + \Delta t_i}
\]

Each \( CV_2 \) corresponds to an ISI value that is the mean of the 2 adjacent ISIs used to compute \( CV_2 \). We computed mean \( CV_2 \) by averaging all \( CV_2 \) values corresponding to ISIs between a certain range. The ISI boundaries were logarithmically spaced with a ratio of 1.3. Because of the refractory period, we set the minimum ISI boundary at 1.69 ms. To access the spaced with a ratio of 1.3. Because of the refractory period, certain range. The ISI boundaries were logarithmically 2 adjacent ISIs used to compute \( CV_2 \). We computed mean \( CV_2 \) by averaging all \( CV_2 \) values corresponding to ISIs between a certain range. The ISI boundaries were logarithmically spaced with a ratio of 1.3. Because of the refractory period, we set the minimum ISI boundary at 1.69 ms. To access the significant difference of \( CV_2 \) for different ISI ranges between WT and KO mice, we applied a Wilcoxon rank sum test to compare the mean \( CV_2 \) values that corresponded to each ISI range. We then calculated the family-wise error rate to correct for multiple comparisons.

We calculated the spike-triggered average (STA) of the LFP at an interval of −5 s to 5 s, with LFP resampled at 200 Hz, so the bin size of the STA was 5 ms. We deemed STA fluctuation to be statistically significant when more than 10 consecutive bins (equal to a 50 ms time window) within an interval of −1 s to 1 s lay outside the minimum/maximum bound of its values at intervals of −5 s to −1 s and 1 s to 5 s. To access the significant difference for STA between WT and Sapap3 KO mice, we applied the Wilcoxon rank sum test to the data points within the same corresponding bins of STA. If 20 or more consecutive bins had \( p < 0.05 \), we considered the STA during that time window to be significantly different.

Histology

To confirm recording location, mice were deeply anesthetized at the end of each recording (Nembutal, 50–100 mg/kg) and intracardially perfused with 50 mL 1 × PBS, followed by 50 mL 4% paraformaldehyde in PBS. Mouse brains were then postfixed in 4% paraformaldehyde/PBS overnight at 4°C and cryoprotected with 30% sucrose. Coronal sections were cut at 50 mm using a freezing microtome and reacted with Hoechst.

Results

Recording neuronal activity from the IOFC of awake mice

To identify changes in individual neuronal activity, we recorded extracellular single units and LFP from the IOFC of head-fixed, awake adult Sapap3 KO mice (n = 24, 20 males and 4 females) and their age-matched, WT littermates (n = 21, 19 males and 2 females; Fig. 1A and B). Two-way analysis of variance (ANOVA) analysis showed a significant effect of genotype but no effect of sex (Appendix 1), so we pooled the data for male and female mice. To minimize stress, the mice were allowed to behave on an air-supported, frictionless spherical treadmill. Because the activity of the IOFC is modulated by movement (Fig. 1C and D), we analyzed only the stationary epochs of the recordings to exclude movement or motor-directed activity confounders.

Reduced LFP oscillation power in the IOFC of Sapap3 KO mice

Brain rhythms are critical to coordinating the activity of neuronal populations across multiple spatial and temporal scales, and are involved in a wide range of cognitive and perceptual processes. To assess the rhythmic alterations of Sapap3 KO mice, we recorded LFP at 64 sites, evenly distributed in a rectangular plane in the IOFC that was 600 μm in the medial–lateral dimension and 700 μm in the dorsal–ventral dimension, from a depth of 1300 μm to 2000 μm. We calculated the LFP power for each mouse by averaging the recordings across the 64 sites. The IOFC LFP oscillations in Sapap3 KO mice exhibited reduced power at all frequency bands compared with their WT littermates (Fig. 2A and B). Specifically, the δ (1.5–4 Hz), θ (4–11 Hz), β (11–30 Hz) and γ (30–100 Hz) bands all had reduced power in Sapap3 KO mice compared with WT mice (δ power normalized to WT mean: KO = 0.57 ± 0.05, WT = 1 ± 0.09, p < 0.001; θ power normalized to WT mean: KO = 0.59 ± 0.07, WT = 1 ± 0.09, p < 0.001; β power normalized to WT mean: KO = 0.54 ± 0.06, WT = 1 ± 0.11, p < 0.001; γ power normalized to WT mean: KO = 0.56 ± 0.06, WT = 1 ± 0.20, p = 0.03; Wilcoxon rank sum test; Fig. 2C, D, E and F). This is, to our knowledge, the first report of LFP alterations in the OFC in an OCD animal model. Because brain-rhythm alterations have been associated with several neuropsychiatric disorders including OCD, alterations in OFC LFP oscillations may serve as a candidate biomarker to be further examined in people with OCD.

Increased activity of IOFC putative interneurons in Sapap3 KO mice

Brain rhythms are generated by the coordinated activity of multiple neuronal populations. The disrupted LFP oscillations found in the IOFC of Sapap3 KO mice may indicate altered activity of multiple neuronal types. To dissect the role of individual neurons, we recorded a total of 362 single units that were isolated unambiguously using high spike-sort
quality. Among them, we recorded 215 single units from Sapap3 KO mice: 182 were classified as putative pyramidal neurons and 30 were classified as putative interneurons, based on action potential waveforms. We recorded 147 single units from WT littermates: 112 were classified as putative pyramidal neurons and 21 were classified as putative interneurons (Fig. 3A and B).

The firing rate of putative interneurons while the mouse was at rest increased in Sapap3 KO mice (WT mean ± SEM = 18.07 ± 1.50 Hz, median = 17.62 Hz; KO mean ± SEM = 22.56 ± 1.31 Hz, median = 23.30 Hz; \( p = 0.02 \) Wilcoxon rank sum test; Fig. 3C). Interestingly, however, the firing rate of putative pyramidal neurons at rest was unchanged between WT and Sapap3 KO mice (WT mean ± SEM = 2.75 ± 0.30 Hz, median = 1.62 Hz; KO mean ± SEM = 2.78 ± 0.23 Hz, median = 1.50 Hz; \( p = 0.63 \) Wilcoxon rank sum test; Fig. 3D), suggesting intricate network imbalances in this mouse model with OCD-like behaviours. For both putative pyramidal neurons and interneurons, there was no correlation between firing rate and depth in either WT or Sapap3 KO mice (Spearman rank correlation coefficient: WT putative interneurons \( r = 0.02, p = 0.94 \); KO putative interneurons \( r = 0.29, p = 0.12 \); WT putative pyramidal neurons \( r = -0.15, p = 0.11 \); KO putative pyramidal neurons \( r = -0.04, p = 0.57 \); Fig. 3E and F).

**Increased bursting activity of lOFC putative pyramidal neurons in Sapap3 KO mice**

The overall firing pattern (not merely the firing rate) determines neuronal function. Although we did not see any changes in the firing rate of pyramidal cells, we sought to compare their spike patterns between WT and Sapap3 KO mice. In Sapap3 KO mice, lOFC putative pyramidal neurons showed a notable enhancement of bursting activity compared with WT littermates (Fig. 4). Putative pyramidal neurons in Sapap3 KO mice fired more bursts of doublet or triplet spikes with very short intra-burst ISI (<10 ms). The number of bursts per minute per neuron was significantly increased.

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**Fig. 1:** Recording position and movement modulation of neuronal activity in lOFC. (A) Left: the red shadow summarizes the recording region, which was largely in the lOFC and sometimes also included the very medial portion of the dOFC. Right: a histology example showing the tracks of the 4 electrode shanks reviewed by DiI (a fluorescent lipophilic cationic indocarbocyanine dye; red). (B) Electrode map. The recording electrodes had 4 shanks spaced by 200 \( \mu \)m. Each shank had 8 recording sites spaced by 50 \( \mu \)m. (C) An example of movement modulation of the spike activity of a putative pyramidal neuron in the lOFC of a WT mouse. This neuron decreased firing rate during locomotion. Horizontal bars indicate movement bouts. (D) An example of movement modulation of the LFP in the lOFC of a WT mouse. Black trace in the upper panel shows LFP when stationary. Grey trace in the lower panel shows LFP during locomotion. AOD = anterior olfactory area, dorsal part; AOL = anterior olfactory area, lateral part; AOM = anterior olfactory area, medial part; AOV = anterior olfactory area, ventral part; dOFC = dorsolateral orbitofrontal cortex; FrA = frontal association cortex; LFP = local field potential; lOFC = lateral orbitofrontal cortex; mOFC = medial orbitofrontal cortex; PrL = prelimbic cortex; vOFC = ventral orbitofrontal cortex; WT = wild type.
in *Sapap3* KO mice (WT mean ± SEM = 12.4 ± 1.9 times/min, median = 5.8 times/min; KO mean ± SEM = 18.2 ± 2.0 times/min, median = 10.7 times/min; *p* = 0.001, Wilcoxon rank sum test; Fig. 4C). The percentage of spikes per neuron in the bursting mode was also significantly increased in *Sapap3* KO mice (WT mean ± SEM = 22.9 ± 2.1%, median = 16.1%; KO mean ± SEM = 32.3 ± 1.8%, median = 29.0%; *p* < 0.001, Wilcoxon rank sum test; Fig. 4D). The intra-burst ISI was significantly shorter in *Sapap3* KO mice compared with WT mice (WT mean ± SEM = 6.60 ± 0.13 ms; KO mean ± SEM = 6.23 ± 0.09 ms; *p* = 0.016, Wilcoxon rank sum test). Bursts with short intra-burst ISI are more reliable and efficient for eliciting synaptic transmission than tonic firing. Therefore, the increased bursting activity seen in *Sapap3* KO mice may enable IOFC pyramidal neurons to provide a stronger output and drive increased activity in the downstream structures of the orbito-fronto–striatal circuit in this OCD mouse model, reflecting specific pathologic neural processes in IOFC that underlie phenotypes relevant to OCD.

**Increased firing variability for both neuronal types in Sapap3 KO mice**

Both IOFC putative pyramidal neurons and interneurons in *Sapap3* KO mice exhibited enhanced discharge variability compared to WT littermates. To measure firing variability,
Orbitofrontal dysfunction in an OCD mouse model

Fig. 3: The mean firing rate of IOFC putative interneurons increased in Sapap3 KO mice, but the mean firing rate of putative pyramidal neurons did not change. (A, B) Isolation and classification of the recorded single units in IOFC. (A) Top panel: an example of an isolated putative pyramidal single unit in IOFC. Bottom panel: an example of an isolated putative interneuron single unit in IOFC. Left to right: overlay of the waveforms of the isolated single unit (yellow) and the noise waveforms (grey). Interspike interval histogram. Projection of the clusters correspondent to the unit and the noise (x axis: PC1, y axis: PC2, z axis: nonlinear energy). (B) Three-dimensional scatter plot illustrating spike characteristics of all 362 single units recorded in the IOFC of WT and Sapap3 KO mice. Each unit is represented as a dot for peak width at half-peak amplitude (x axis), trough width at half trough amplitude (y axis) and ratio of trough to peak amplitude (z axis). We identified 2 major clusters. Putative pyramidal neurons are shown in red. Putative interneurons are shown in black. Units that did not meet the criteria for these classifications are shown in blue. (C) The mean firing rate of putative interneurons increased in Sapap3 KO mice (red) compared to WT mice (black); *p = 0.02, Wilcoxon rank sum test. (D) The mean firing rate of putative pyramidal neurons was similar between WT (black) and Sapap3 KO (red) mice; p = 0.63, Wilcoxon rank sum test. The whiskers in the box plots cover 95% of the data. (E) We found no correlation between interneuron firing rate and depth. Spearman rank correlation coefficient: WT, r = 0.02, p = 0.94; KO, r = 0.29, p = 0.12. (F) We found no correlation between pyramidal neuron firing rate and depth. Spearman rank correlation coefficient: WT, r = −0.15, p = 0.11; KO, r = −0.04, p = 0.57. KO = knockout; IOFC = lateral orbitofrontal cortex; PC = principal component; WT = wild type.
we adopted a method that was less sensitive to firing rate fluctuation over time than the coefficient of variation of ISIs. This method compared only adjacent ISIs by calculating $C_{V^2}$ for adjacent ISIs (see Methods).

Neurons cannot fire as variably at a high rate as at a low rate because of the refractory period. To avoid comparing the periods when the neuron fires quickly with periods when the neuron fires slowly, we did not compute the mean $C_{V^2}$ over the entire recording period. Instead, we computed the mean $C_{V^2}$ for different ISI values. Both IOFC putative pyramidal neurons and interneurons in Sapap3 KO mice exhibited enhanced discharge variability for ISIs from 10–190 ms compared to WT mice ($p_{FWE} < 0.001$ for both putative pyramidal neurons and putative interneurons, Wilcoxon rank sum test for each ISI range; family-wise error rate was calculated to correct for multiple comparisons; Fig. 5). Cortical neurons typically fire action potentials with high temporal precision. Change of the spike timing influences information coding in several sensory modalities, such as olfaction, gustation, audition and vision. The increased discharge variability of pyramidal neurons and interneurons interferes with normal information coding and processing in OFC and may reflect circuitry abnormalities for OCD-like behaviours.

**Altered synchronization of IOFC putative pyramidal neurons and interneurons in Sapap3 KO mice**

Temporal precision of firing and a tightly maintained balance between excitation and inhibition is critical to normal neural synchronization. Because we observed altered firing variability

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**Fig. 4:** The IOFC putative pyramidal neurons showed increased bursting activity in Sapap3 KO mice. (A) A representative example of bursty pyramidal neurons in Sapap3 KO mice: a1 shows the raw recording trace showing 2 bursts with short intra-burst ISI (intra-burst ISI < 10 ms, shown by arrows); a2 shows ISI distribution (bin size 1 ms); a3 shows an enlarged view of the ISI distribution from 0–20 ms to better demonstrate the short intra-burst ISI. (B) A representative example of non-bursty pyramidal neurons in Sapap3 KO mice. (C) The number of bursts per minute per neuron increased in Sapap3 KO mice; ***$p = 0.001$, Wilcoxon rank sum test. (D) The percentage of spikes per neuron in the bursting mode increased in Sapap3 KO mice; ***$p < 0.001$; Wilcoxon rank sum test. The whiskers in the box plots cover 95% of the data. KO = knockout; ISI = interspike interval; IOFC = lateral orbitofrontal cortex; WT = wild type.
and distinct perturbations of excitatory and inhibitory neurons, we then sought to investigate whether the levels of synchronous activity in IOFC would change in Sapap3 KO mice by calculating the STA of LFP. Because the LFP averages over many neurons, STA is more sensitive than cross-correlation in detecting local neuronal synchronization. Troughs in STA of LFP correspond to depolarization of intracellularly measured membrane potential, reflecting summed excitatory events in a pool of neurons. Upward deflections in STA of LFP correspond to a drop in membrane potential. In both WT and Sapap3 KO mice, IOFC putative pyramidal neurons and interneurons all fired preferentially at the lowest point of the trough. The percentage of cells entrained to the LFP oscillations as measured by significant fluctuations in the STA around the time of spike was similar between WT and Sapap3 KO mice (WT putative pyramidal neurons 70.6 ± 9.5%, mean ± SEM, KO putative pyramidal neurons 72.0 ± 7.0%, p = 0.9, Wilcoxon rank sum test; WT putative interneurons 96.4 ± 3.9%, KO putative interneurons 100%, p = 0.9, Wilcoxon rank sum test). However, the shape of averaged STA of both putative pyramidal neurons and interneurons differed in Sapap3 KO mice. The central trough of the STA of both putative pyramidal neurons and interneurons was reduced in Sapap3 KO mice (Fig. 6). This reduction may have been due to the reduced power of LFP oscillation in Sapap3 KO mice. In WT mice, the averaged STA of putative interneurons exhibited a broad second peak after the central trough (Fig. 6A). This peak was significantly reduced in Sapap3 KO mice, indicating that interneurons experienced less synchronized inhibition after firing a spike. The peak ahead of the central trough of the STA of putative pyramidal neurons was eliminated in Sapap3 KO mice (Fig. 6B), indicating that synchronized inhibition on membrane potential, which sculpts the time window when an action potential can occur, was reduced in Sapap3 KO mice. As a result, in Sapap3 KO mice, the spike timing of individual IOFC pyramidal neurons may become less accurate. This was consistent with our finding that the firing variability of IOFC putative pyramidal neurons increased in Sapap3 KO mice. Taken together, the changes in synchrony, along with the spike activity pattern and LFP changes reported in previous sections, point to the IOFC as a malfunctioning neural substrate for behavioural phenotypes relevant to OCD.

Discussion

Cognitive and executive function requires the coordinated activity of large-scale networks. Deficits in temporal coordination in the OFC can lead to disruption of its normal function and be involved in the pathophysiology of OCD. In the present study, we have reported alterations in LFP oscillations in the IOFC of an OCD mouse model, to our knowledge, for the first time. Specifically, we found that Sapap3 KO mice exhibited reduced power in δ, θ, β and γ oscillations at rest. The neural substrates contributing to the different frequency bands of LFP oscillations and the mechanisms by which these oscillations are generated are not well understood. Therefore, a mechanistic interpretation of how the altered activity pattern of IOFC pyramidal neurons and interneurons contributes to decreased LFP oscillations in multiple frequency bands is challenging. Nevertheless, changes in neuronal activity synchronization do contribute to LFP oscillation alterations. We found that putative interneurons experienced less synchronized inhibition after firing an action potential and putative pyramidal neurons experienced less synchronized inhibition before firing an action potential. This reduced synchronous activity in the IOFC may contribute to decreased LFP oscillation power in multiple frequency bands. Consistent with our results, animal studies have shown that deep-brain stimulation of the nucleus accumbens, which can effectively alleviate OCD symptoms, elevated spontaneous LFP oscillation power in the δ, β and γ frequency bands in the OFC in rats. In contrast, low-frequency deep-brain stimulation of the nucleus accumbens, which is ineffective in OCD, exerted no effect on LFP in the OFC. Given that LFP oscillation power in the OFC was decreased in an OCD mouse model and increased with deep-brain stimulation of the nucleus accumbens in rats, alterations in LFP may serve as a potential neurophysiological biomarker to be further examined in people with OCD.

Inhibitory interneurons form reciprocal connections broadly with pyramidal neurons, and so are well positioned to coordinate the timing of pyramidal cell activity, regulate information processing and gate information flow. Compromised cortical inhibitory interneurons have been implicated in multiple psychiatric and neurologic disorders, including schizophrenia, autism and epilepsy. However, little research on inhibitory interneurons has been done in people with OCD or in animal models. It has been reported that Sapap3 KO mice have a decreased number of PV-expressing interneurons in the centromedial striatum. For the first time, our work found elevated spontaneous activity and enhanced discharge variability of putative inhibitory interneurons in the IOFC of Sapap3 KO mice. Because the IOFC directly projects to the striatum, inhibition is disrupted in both parts of the cortical–striatal circuit in this OCD mouse model. Inhibitory interneurons are critical for the normal function of the OFC. The activity of inhibitory interneurons in the OFC showed strong behaviour correlates. Compromised inhibitory interneurons in the OFC altered pyramidal neuron activity correlations with decision and reward, and impaired reversal learning. The activity alterations of interneurons we found may disrupt the normal function of the IOFC and help identify one aspect of malfunction in this region for behaviours relevant to OCD. Interneurons also play a fundamental role in rhythmogenesis. The elevated spontaneous activity and discharge variability of interneurons may be causally involved in the LFP alterations in IOFC, as we found in Sapap3 KO mice. Alternatively, the increased spontaneous activity of interneurons could represent adaptive, homeostatic or unrelated processes to compensate for other primary abnormalities of the IOFC in Sapap3 KO mice.

Although the OFC is thought to play a critical role in OCD, there are discrepancies in the directionality of findings about how the baseline activity of the OFC is altered in people with OCD or animal models. These discrepancies may be due to several factors, including the heterogeneity of the disorder,
comorbidities, medication history and the different subdivisions of OFC analyzed. Our study excluded these confounders by focusing on the IOFC in an OCD mutant mouse model, and with clear classification of different cell types. Two studies have assessed OFC activity change in OCD mouse models. One found that the baseline activity of IOFC putative pyramidal neurons measured by electrophysiological recording was similar between WT and Sapap3 KO mice, consistent with our results. The other reported upregulated baseline activity in the OFC in Slitrk5 KO mice measured by FosB expression. Because this study relied on molecular markers of cell activity, we do not know the details of activity pattern change for

![Fig. 5: The IOFC putative pyramidal neurons and interneurons exhibited enhanced discharge variability in Sapap3 KO mice (red) compared with WT littermates (black). (A) Mean C_o of IOFC putative pyramidal neurons plotted against the mean of the 2 adjacent ISIs used to compute C_o. (B) Mean C_o of IOFC putative interneurons plotted against the mean of the 2 adjacent ISIs used to compute C_o. The x axis is the mean of the 2 adjacent ISIs used to compute C_o. The lines are the mean C_o values in logarithmically spaced bins. The ratio between bin boundaries was 1.3. We chose logarithmic binning because the upper limit of C_o at shorter ISIs changes much more rapidly than at longer ISIs. Shading represents standard error of the mean. KO = knockout; ISI = interspike interval; IOFC = lateral orbitofrontal cortex; WT = wild type.](image)

![Fig. 6: Comparisons of IOFC ensemble synchronization in WT (black) and Sapap3 KO (red) mice. (A) Averaged STA of LFP of IOFC putative interneurons. (B) Average STA of LFP of IOFC putative pyramidal neurons. Black horizontal bars indicate ranges with significant difference between WT and Sapap3 KO mice. Shading represents standard error of the mean. KO = knockout; LFP = local field potential; IOFC = lateral orbitofrontal cortex; STA = spike-triggered average; WT = wild type.](image)
specific neuronal types. The increased bursting activity of putative pyramidal neurons we found could cause this increase in FosB expression. The discrepancy may also result from the different OCD animal models used (Sapap3 KO v. Slitrk5 KO), different cell types (putative pyramidal neurons v. all cells) and different subregions of the OFC examined (IOFC v. the entire OFC). The medial and lateral OFC perform different functions, such as processing positive versus negative valence.35,36 The activity of these 2 subregions may be differentially affected in OCD mouse models, giving rise to inconsistent results when examining the IOFC versus the entire OFC.

Although the mean firing rate of IOFC pyramidal neurons was similar between WT and Sapap3 KO mice, their activity pattern changed dramatically in Sapap3 KO mice. Specifically, IOFC pyramidal neurons exhibited significantly increased bursting activity with short intra-burst ISI (< 10 ms). Overall activity pattern determines neuronal function, not merely firing rate. Bursts with short intra-burst ISI have special importance in brain function. Compared with tonic firing, burst firing is more reliable for eliciting synaptic transmission, provides stronger output, enhances signal-to-noise ratio and facilitates synaptic plasticity.41 The increased bursting activity of IOFC pyramidal neurons may provide pathologic stronger output through the OFC–striatal circuit and drive increased activity in the striatum of Sapap3 KO mice, as reported in previous studies.37,58 Another study suggested that sustained increase in synaptic strength from the OFC pyramidal neurons to ventral striatum synapses led to increased repetitive behaviour in mice.59 Many clinical and animal studies have suggested that hyperactivity in the cortico–striato–thalamo-cortical circuit is associated with OCD pathology.2,3,10,11 The enhanced bursting activity of IOFC pyramidal neurons may drive hyperactivity in the cortico–striato–thalamo-cortical circuit and contribute to OCD-like behaviours in Sapap3 KO mice. A recent study reported that a depression-like state depended critically on a bursting mode of firing in the lateral habenula in rats and mice.60 The bursting activity of neurons in the lateral habenula was greatly enhanced in rat and mouse models of depression, and reducing their bursting activity elicited antidepressant effects. Increasing bursting activity by optogenetics was sufficient to induce depression-like behaviours. This study suggested that abnormal bursting activity in a single nucleus could lead to symptoms relevant to a psychiatric disorder. Abnormal bursting activity has not been studied in people with OCD or in animal models. In a future study, we plan to investigate whether fluoxetine can suppress the abnormal bursting activity of IOFC pyramidal neurons, accompanied by alleviation of OCD-like behaviours in Sapap3 KO mice. We also plan to investigate whether artificially increasing the bursting activity of IOFC pyramidal neurons can induce symptoms relevant to OCD.

**Limitations**

One limitation of this study was that we examined activity pattern alterations of IOFC neurons only when the mice were resting; we did not examine grooming-related activity. As an association cortex, the OFC performs complex cognitive and executive brain functions. Its neuronal activity is modulated by many behaviours, including grooming-associated movements themselves. To exclude such confounders, we compared neuronal activity between WT and Sapap3 KO mice, recorded only when the mice were stationary. To collect enough data during stationary periods, we applied a head-fixed configuration instead of a free-behaviour configuration, because mice stayed stationary for most of the time during head-fixed recording (percent of time spent stationary, mean ± SEM: WT 90.1 ± 2.3%, KO 90.8 ± 3.5%). In contrast, mice usually moved most of the time during free-movement recording (including both locomotion and fine movement), leaving few stationary periods for effective data analysis. Comparing resting neuronal activity between WT and Sapap3 KO mice required very high recording and spike-sorting quality. We applied very strict criteria for spike sorting and included only very well isolated single units to ensure the accuracy of our results. Specifically, the cluster of the isolated single unit had to be well separated from other clusters without any overlapping of the edge, because edge overlapping in spike sorting results in contamination by other units or loss of spikes of the isolated unit. Either situation can significantly affect the measurement of firing rate and firing variability. Another limitation was that we investigated functional abnormalities without establishing causality for these functional changes and the OCD-like behaviours. Our reasons were as follows. First, this was a pioneering in vivo electrophysiological study of OFC dysfunction in an OCD animal model; characterizing the functional abnormalities was the first step and can serve an important foundation for future work. Second, artificially generating bursts with very short intra-burst ISI without changing the mean firing rate is very challenging; we have not found an appropriate way to manipulate the oscillation power of broad frequency bands in the LFP without changing the mean firing rate of excitatory neurons. Nevertheless, causal manipulation is definitely a key future experiment that may require us to develop new manipulation techniques.

**Conclusion**

Here, we have provided the first direct in vivo electrophysiological evidence of detailed functional alterations in different neuronal types and local network dysfunction in the IOFC in phenotypes relevant to OCD. These findings advance our understanding of the neuropathophysiology and circuitry mechanisms that underlie OCD-like behaviours, and may help generate and refine hypotheses for further investigation. For example, the LFP alterations and increased bursting activity may be useful biomarker candidates for further examination in people with OCD.

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Spontaneous low-frequency fluctuations in the neural system for emotional perception in major psychiatric disorders: amplitude similarities and differences across frequency bands

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Introduction

Psychiatric nosology arose in central Europe toward the end of the 19th century. In particular, Kraepelin made the foundational distinction between dementia praecox (schizophrenia) and manic depression.1 The distinction between bipolar illness and unipolar (major) depression was first proposed in the 1950s2 and has since become more widely accepted. More recently, DSM-5 divided mood disorders into 2 classifications: bipolar disorder and major depressive disorder. Over the past several decades, the majority of studies have focused on identifying differences between these conditions to establish clear boundaries for psychiatric diagnosis. However, previous findings indicate important commonalities among schizophrenia, bipolar disorder and major depressive disorder in terms of clinical features,3 genetic and environmental risk factors,4 neuropathophysiology5 and neural alterations,6 suggesting that they may have shared neurobiological features. Direct comparisons are needed to more closely examine the common and distinct neural systems across all 3 disorders.

Impaired emotional perception is a common endophenotype in schizophrenia, bipolar disorder and major depressive disorder. A set of regions in the striatum, thalamus, limbic system (amygdala and hippocampus), paralimbic system and heteromodal areas (prefrontal cortex) have

Background: Growing evidence indicates both shared and distinct features of emotional perception in schizophrenia, bipolar disorder and major depressive disorder. In these disorders, alterations in spontaneous low-frequency fluctuations have been reported in the neural system for emotional perception, but the similarities and differences in the amplitude of low-frequency fluctuation (ALFF) across the 3 disorders are unknown. Methods: We compared ALFF and its signal balance in the neural system for emotional perception at 2 frequency bands (slow-5 and slow-4) in 119 participants with schizophrenia, 100 with bipolar disorder, 123 with major depressive disorder and 183 healthy controls. We performed exploratory Pearson partial correlation analyses to determine the relationship between ALFF signal balance and clinical variables. Results: We observed commonalities in ALFF change patterns across the 3 disorders for emotional perception neural substrates, such as increased ALFF in the anterior cerebrum (including subcortical, limbic, paralimbic and heteromodal cortical regions) and decreased ALFF in the posterior visual cortices. Schizophrenia, bipolar disorder and major depressive disorder showed significantly decreased ALFF signal balance in the neural system for emotional perception at both slow-5 and slow-4 frequency bands, with the greatest alterations for schizophrenia, followed by bipolar disorder and major depressive disorder. We found a negative correlation between ALFF signal balance and negative/disorganized symptoms in slow-4 across the 3 disorders. Limitations: The relatively broad age range in our sample and the cross-sectional study design may not account for our findings. Conclusion: The extent of the commonalities we observed further support the concept of core neurobiological disruptions shared among the 3 disorders; ALFF signal balance could be an important neuroimaging marker for the diagnosis and treatment of schizophrenia, bipolar disorder and major depressive disorder.
Amplitude across frequency bands in major psychiatric disorders

long been thought to be involved in emotional perception, and abnormalities in these regions have consistently been reported in schizophrenia, bipolar disorder and major depressive disorder. More recently, the importance of the visual cortices in emotional perception has been raised. altered activity and connectivity of the visual regions during emotional processing have also been observed in schizophrenia, bipolar disorder and major depressive disorder. Interestingly, baseline and post-treatment blood oxygenation level–dependent (BOLD) response change in the visual cortex has been correlated with clinical response to scopolamine as measured by the Montgomery-Åsberg Depression Rating Scale in major depressive disorder, further supporting the involvement of the visual cortices in emotion. Taken together, a range of neural components appear to be involved in emotional perception in schizophrenia, bipolar disorder and major depressive disorder, including abnormalities in the neural system for emotional perception (striatum, thalamus, limbic system, paralimbic system and prefrontal cortex), and in the primary visual and associated cortices.

Convergent evidence suggests that the balance or interaction of the primary cortex and heteromodal areas, and the limbic and paralimbic regions, appears to be dynamically influenced by neurodevelopmental processes and the neurotransmitter milieu. Histological studies in primates demonstrate selective pruning of the excitatory synapses (glutamatergic) in the primary cortex and relative preservation of inhibitory synapses (GABAergic) in heteromodal areas during development. Excessive synaptic pruning during early development, heavily influenced by glutamate in the primary visual cortices, could lay the foundation for disrupted higher-order function in later developmental stages. Altered sensory processing provides inaccurate input to higher-order regions, such as the prefrontal cortex, and results in inappropriate or maladaptive learning and adaptation in neural circuits. Maladaptations in higher-order regions may then feed back onto sensory processing, setting up a vicious circle for progressive and persistent disruptions in neural networks, potentially leading to the development of schizophrenia, bipolar disorder or major depressive disorder. Understanding how the conventional regions of emotional perception (striatum, thalamus, limbic/paralimbic systems and heteromodal cortex) and the primary visual regions interact in schizophrenia, bipolar disorder and major depressive disorder could help to further elucidate the neuropathophysiology of the 3 disorders.

Resting-state functional MRI (rsfMRI) can probe the brain–behaviour relation between different aspects of the BOLD signal and behavioural traits, and has been widely used in the field. Functional connectivity analyses assess associations between BOLD time series of voxels in different regions, but amplitude of low-frequency fluctuation (ALFF) analyses measure voxel-wise fluctuations in the amplitude of BOLD signal at very low frequencies (typically 0.01–0.08 Hz). The ALFF signal is correlated with baseline cerebral blood flow and is thought to reflect spontaneous, intrinsic neuronal activity. Although the exact neural substrate for ALFF is unclear, ALFF signal is mostly reliable in grey matter, not white matter, suggesting that ALFF derives from neural activity. Recent evidence suggests that the BOLD activity measured by ALFF accurately reflects neural activity that underlies the glutamate–glutamine cycle in neurons and astrocytes, and coupled glutamatergic and GABAergic systems. Because different frequency bands may reflect different physiological mechanisms, subsequent studies have examined ALFF at more refined neural oscillation frequencies to improve precision: slow-5 (0.01–0.027 Hz) and slow-4 (0.027–0.073 Hz).

Studies of ALFF in people with schizophrenia, bipolar disorder and major depressive disorder have shown significant differences compared with healthy controls, most prominently in regions involved in emotional perception, such as the subcortical (thalamus and striatum), limbic (amygdala and hippocampus), paralimbic (orbitofrontal cortex, insula, temporal pole, cingulate gyrus and parahippocampal gyrus) and heteromodal (ventral prefrontal cortex, dorsolateral prefrontal cortex structures and the visual cortices [fusiform gyrus, lingual gyrus, precuneus and cuneus]) regions. Similar findings were observed in a recent multicentre transdiagnostic study of people with schizophrenia, schizoaffective disorder and bipolar disorder and their unaffected family members, which focused on the slow-5 and slow-4 bands. The authors found ALFF in the frontal, subcortical and temporal regions and in the visual regions (precuneus and cuneus) of people with schizophrenia, schizoaffective disorder and bipolar disorder. Furthermore, they found increased ALFF primarily in anterior brain regions (including prefrontal, temporal and subcortical regions) and decreased ALFF mainly in posterior regions (such as the precuneus, cuneus and posterior cingulate). Taken together with findings for the potential neural mechanisms underlying the interaction between the primary visual/associated cortices and high-order, limbic/striatal areas described above, the balance of ALFF between the conventional regions of emotional perception and visual cortices may contribute to emotion-related symptoms in schizophrenia, bipolar disorder and major depressive disorder. Further supporting the importance of ALFF balance between brain regions in mental disorders, a recent study in bipolar disorder found that the ALFF balance between the default mode network and the sensorimotor network in slow-5 was associated with mood states.

In this study, we examined ALFF in slow-5 and slow-4 bands in people with schizophrenia, bipolar disorder and major depressive disorder, and evaluated how the ALFF balance between conventional regions of emotional perception and visual cortices related to symptom measures. We hypothesized that we would observe increased ALFF in conventional regions (subcortical regions, limbic, paralimbic and heteromodal cortices) and decreased ALFF in visual cortices across the 3 disorders. We also predicted that ALFF signal balance between conventional regions and visual cortices would correlate with specific symptom measures across the 3 disorders.
Methods

Participants

This study included 525 participants aged 13 to 45 years: 119 with schizophrenia, 100 with bipolar disorder, 123 with major depressive disorder and 183 healthy controls. All participants provided written informed consent after receiving a detailed description of the study. The study was approved by the institutional review board of China Medical University. Participants with schizophrenia, bipolar disorder and major depressive disorder were recruited from inpatient and outpatient services at Shenyang Mental Health Centre and the Department of Psychiatry, First Affiliated Hospital of China Medical University, Shenyang, China. Healthy control participants were recruited from the local community by advertisement. Participants were excluded for substance or alcohol abuse/dependence, a concomitant major medical disorder, any MRI contraindication, history of head trauma with loss of consciousness for ≥ 5 minutes, or any neurologic disorder. Two trained psychiatrists determined the presence or absence of Axis I psychiatric disorders using the Structured Clinical Interview for DSM-IV Axis I disorders \(^2^9\) in participants 18 years and older, and the Schedule for Affective Disorders and Schizophrenia for School-Age Children–Present and Lifetime version \(^3^0\) in participants younger than 18 years. Participants with schizophrenia, bipolar disorder or major depressive disorder met DSM-IV diagnostic criteria and had no comorbid Axis I disorders. Healthy control participants had no current or lifetime DSM-IV Axis I disorder or history of a psychotic, mood or other DSM-IV Axis I disorder in first-degree relatives (as determined by detailed family history). We obtained symptom measures using the Brief Psychiatric Rating Scale (BPRS) \(^3^1\) and Hamilton Rating Scale for Depression (HAMD). \(^3^0\) Cognitive function was evaluated using the Wisconsin Card Sorting Test (WCST). \(^3^2\)

MRI data acquisition and processing

See Appendix 1, available at jpn.ca/170226-a1, for details on MRI data acquisition and processing.

Statistical analysis

Demographic and clinical data

We performed analyses of demographic and clinical characteristics and cognitive measures using analysis of variance and \(\chi^2\) tests. Results were significant at \(p < 0.05\). On 342 patients, we performed exploratory factor analysis using the principal component factor method to identify a parsimonious list of factors using BPRS and HAMD items. We determined the number of factors to be extracted according to the scree-plot method. We performed orthogonal rotation using the varimax method. We identified 5 interpretable and clinically relevant factors that captured 62.35% of the rotated variance, with loadings at 0.4. We then used the resulting factors of BPRS and HAMD (Appendix 1, Table S1) from the exploratory factor analysis in the correlation analyses described below.

Voxel-wise analyses of ALFF across diagnostic groups

We performed 4 group analyses (schizophrenia, bipolar disorder, major depressive disorder and healthy controls) of ALFF values in each band in SPM8 (Wellcome Center for Human Neuroimaging). We used analysis of covariance (ANCOVA) with diagnostic group as an independent factor, and age and sex as covariates. We set statistical significance at a corrected \(p < 0.05\). We corrected for multiple comparisons by combining individual voxel \(p < 0.001\), uncorrected, with a cluster size of > 25 voxels for slow-5 and > 27 voxels for slow-4, as determined by Monte Carlo simulation (AlphaSim, Analysis of Functional NeuroImages). \(^3^1\) We performed post hoc pair-wise \(t\) contrasts (schizophrenia v. healthy controls, bipolar disorder v. healthy controls and major depressive disorder v. healthy controls) to visualize differences between each patient group and healthy controls in regions that showed significant differences in the 4 groups for slow-5 and slow-4. We set significance at \(p < 0.05\) by Monte Carlo simulation. Voxel-wise analyses of functional ALFF values across the diagnostic groups can be found in Appendix 1, Supplemental Materials.

ALFF balance ratios across diagnostic groups

To examine the balance between the conventional regions of emotional perception (cEP) and the visual cortices (VC), we calculated the following ratio for each participant: \((\text{ALFF}_{cEP} - \text{ALFF}_{VC})/(\text{ALFF}_{cEP} + \text{ALFF}_{VC})\). The balance ratio represented the proportion of total ALFF that was due to the difference in ALFF between the conventional regions of emotional perception and the visual cortices. We extracted ALFF values from the conventional regions of emotional perception \(^9\) (amygdala, hippocampus, insula, ventral striatum, ventral and dorsal anterior cingulate cortex, prefrontal cortex) and the visual cortices that showed shared significant differences compared with healthy controls across schizophrenia, bipolar disorder and major depressive disorder. We performed a 4-group ANCOVA for each frequency band. We performed post hoc pair-wise comparisons (schizophrenia v. healthy controls, bipolar disorder v. healthy controls and major depressive disorder v. healthy controls). Statistical significance was corrected for false discovery rate (FDR) and set at \(p_{\text{FDR}} < 0.05\).

ALFF balance ratios and clinical variables

After we determined normal distribution of data using the Kolmogorov–Smirnov Test, we performed exploratory Pearson partial correlation analyses to investigate the relationship between ALFF balance using the above ratio and clinical variables for each frequency band, including illness duration, exploratory factor analysis factors and WCST scores in schizophrenia, bipolar disorder and major depressive disorder, controlling for age and sex. We performed additional exploratory ANCOVA analyses to determine the effects of medication status and first-episode status on the ALFF ratio for each band. Statistical significance was set at \(p_{\text{FDR}} < 0.05\).
Results

Demographic and clinical data

Demographic and clinical details are presented in Table 1. We found no significant differences in age or handedness among the schizophrenia, bipolar disorder, major depressive disorder or healthy control groups. We did observe significant differences in sex and WCST score among the 4 groups (p < 0.05). We also noted significant differences in illness duration, medication status, first-episode status, and HAMD and BPRS total scores among the 4 groups (p < 0.05, Table 1).

ALFF balance ratios across diagnostic groups

The 4-group analysis showed significant ALFF differences in the slow-5 and slow-4 bands in the bilateral striatum (including the caudate nuclei and putamen), the limbic and paralimbic regions (including the bilateral amygdala, bilateral hippocampus, left temporal pole, left insular cortex, left orbitofrontal cortex, bilateral parahippocampal gyri and left anterior cingulate cortex) and the heteromodal cortices (including the bilateral ventral prefrontal cortex, dorsolateral prefrontal cortex, right frontal pole, left inferior temporal gyri and left middle temporal gyrus). We also saw significant differences in both bands in the visual cortices, specifically in the bilateral cuneus, precuneus, lingual gyri and calcarine cortex, as well as in the bilateral posterior cingulate gyri and bilateral primary somatosensory cortices and left primary motor cortex (Fig. 1A, Fig. 2A, Table 2, Table 3). We found significant ALFF differences specific to the frequency bands of interest (details in Appendix 1, Supplemental Materials).

In post hoc analyses we found increased ALFF in the striatal, limbic, paralimbic and heteromodal regions and decreased ALFF in the visual cortex in people with schizophrenia, bipolar disorder and major depressive disorder compared with healthy controls (Fig. 1B–D and Fig. 2B–D). Most interestingly, alterations were graded across groups, with the greatest alterations in schizophrenia, followed by bipolar disorder and then major depressive disorder.

See Appendix 1, Supplemental Materials, for functional ALFF values across the diagnostic groups.

ALFF balance ratios and clinical variables

We found significant differences in balance ratios across groups in slow-5 (F = 27.481, p_{FDR} < 0.001) and slow-4 (F = 27.138, p_{FDR} < 0.001; Fig. 3). Post hoc pair-wise comparisons showed significant decreases in balance ratios for slow-5 and slow-4 in the schizophrenia, bipolar disorder and major depressive disorder groups compared with the healthy control group. These decreases were graded across groups, such that the lowest ratios were in the schizophrenia group, followed by bipolar disorder and then major depressive disorder (Fig. 3).

Balance ratios and clinical variables for each frequency band were normally distributed. Correlation analyses

| Table 1: Participant demographic characteristics, clinical characteristics and cognitive function |
|-------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|-----------------|-----------------|
| Characteristic                                 | Group; mean ± SD or no. (%)    |                                   |                                   |                                   | F    | p value         |
| Age at scan, yr                                | Control (n = 183)              | Schizophrenia (n = 119)           | Bipolar disorder (n = 100)       | Major depressive disorder (n = 123) | F    | p value         |
|                                                | 26.62 ± 8.00                   | 24.77 ± 9.07                     | 25.72 ± 7.88                     | 27.74 ± 9.76                     | 2.596 | 0.052           |
| Male                                           | 73 (40)                        | 54 (45)                          | 48 (48)                          | 34 (28)                          | 10.932 | 0.012           |
| Right-handed                                   | 175 (96)                       | 107 (90)                         | 97 (97)                          | 116 (94)                         | 9.121 | 0.17            |
| Duration, mo                                   | 22.09 ± 36.42                  | 41.48 ± 56.18                    | 21.10 ± 39.24                    | 6.605                            | 0.002           |
| First episode                                   | 84 (71)                        | 52 (52)                          | 97 (79)                          | 27.221                           | < 0.001          |
| Medication                                      | 71 (60)                        | 65 (65)                          | 49 (40)                          | 20.746                           | < 0.001          |
| Antidepressant                                  | 9 (8)                          | 28 (28)                          | 43 (35)                          | 27.007                           | < 0.001          |
| Antipsychotic                                   | 65 (55)                        | 32 (32)                          | 4 (3)                            | 77.108                           | < 0.001          |
| Mood stabilizer                                 | 4 (3)                          | 52 (52)                          | 0                                | 131.489                          | < 0.001          |
| Hamilton Rating Scale for Depression*          | 1.17 ± 1.68                    | 8.01 ± 6.98                      | 11.74 ± 9.54                     | 21.37 ± 8.72                     | 198.298 | < 0.001          |
| Brief Psychiatric Rating Scale†                | 18.31 ± 0.82                   | 36.14 ± 14.09                    | 25.68 ± 8.43                     | 25.59 ± 6.17                     | 65.772 | < 0.001          |
| Cognitive function (Wisconsin Card Sorting Test)‡ | 32.31 ± 10.66                  | 17.39 ± 12.17                    | 25.44 ± 11.52                    | 25.03 ± 11.48                    | 11.100 | < 0.001          |
| Corrected responses                            | 4.32 ± 1.97                    | 1.72 ± 1.90                      | 2.00 ± 2.03                      | 14.756                           | < 0.001          |
| Categories completed                            | 16.59 ± 10.66                  | 30.62 ± 12.22                    | 22.56 ± 11.52                    | 10.569                           | < 0.001          |
| Total errors                                    | 5.76 ± 6.68                    | 14.70 ± 13.02                    | 9.22 ± 9.37                      | 9.67 ± 9.31                      | 5.126 | 0.002           |
| Nonperseverative errors                        | 9.98 ± 5.58                    | 15.93 ± 8.64                     | 13.16 ± 7.04                     | 13.64 ± 6.04                     | 6.033 | 0.001           |

SD = standard deviation.
*Control, n = 162; schizophrenia, n = 83; bipolar disorder, n = 98; major depressive disorder, n = 122.
†Control, n = 101; schizophrenia, n = 114; bipolar disorder, n = 60; major depressive disorder, n = 56.
‡Control, n = 101; schizophrenia, n = 74; bipolar disorder, n = 45; major depressive disorder, n = 70.
§Among the healthy control, schizophrenia, bipolar disorder and major depressive disorder groups.
¶Among the schizophrenia, bipolar disorder and major depressive disorder groups.

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showed a significant negative correlation ($r = -0.187$, $p = 0.005$) between the ALFF balance ratio in slow-4 and BPRS factor 1, which consisted of the following: emotional withdrawal, blunted affect, conceptual disorganization, motor retardation and disorientation (Appendix 1, Tables S1 and S2). We found no significant correlation between the balance ratio in slow-5 and BPRS or HAMD factors (Appendix 1, Table S1). We found no significant correlation between the balance ratio in slow-5 or slow-4 and WCST scores (Appendix 1, Table S3). We found no significant effects of illness duration, medication status or first-episode status on the balance ratio in either frequency band (Appendix 1, Table S4).

Discussion

We observed common alterations in ALFF across schizophrenia, bipolar disorder and major depressive disorder in the slow-5 and slow-4 frequency bands. These alterations were located primarily in regions thought to subserve emotional perception, as well as in the visual cortices, whose importance in emotional perception in psychiatric disorders has recently been proposed. Specifically, we observed increased ALFF in the anterior cerebrum, including subcortical (striatum and putamen), limbic (amygdala and hippocampus), paralimbic (orbital prefrontal cortex, insula, temporal pole, parahippocampal gyrus) and heteromodal cortical (ventral prefrontal cortex and dorsolateral prefrontal cortex) regions. We observed decreased ALFF in the posterior visual cortices, including the bilateral cuneus, precuneus, lingual gyri and calcarine cortex. Interestingly, significant proportions of the observed alterations were shared across the schizophrenia, bipolar disorder and major depressive disorder groups in slow-5 and slow-4. The differences appeared to be graded such that the most significant changes were found in schizophrenia, followed by bipolar disorder and then major depressive disorder. We found ALFF alterations specific to individual disorders, with more prominent similarities between schizophrenia and bipolar disorder than with major depressive disorder, and more extensive alterations in schizophrenia. We found significant differences in the ALFF balance ratio across schizophrenia, bipolar disorder and major depressive disorder, suggesting impaired activity balance between the conventional regions of emotional perception and the visual cortices in these disorders. The ALFF ratio in slow-4 appeared to be negatively correlated with measures of negative and disorganized symptoms across schizophrenia, bipolar disorder and major depressive disorder, although the correlation was small and might have been stronger in schizophrenia than in bipolar disorder and major depressive disorder. In addition, certain ALFF alterations were specific to frequency bands. We found no significant ALFF

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Fig. 1: Significantly altered regions of ALFF values for slow-5 in participants with schizophrenia, bipolar disorder and major depressive disorder, and healthy controls (A) by ANCOVA, (B) between schizophrenia and controls, (C) between bipolar disorder and controls, and (D) between major depressive disorder and controls. Significant at $p < 0.05$, Alphasim correction. ALFF = amplitude of low-frequency fluctuation; ANCOVA = analysis of covariance.
Neuronal oscillations have been reported to bias input selection, temporally link neurons into assemblies and facilitate synaptic plasticity, mechanisms that cooperatively support temporal representation and long-term consolidation of information. While the specific mechanisms that drive resting-state spontaneous fluctuations are still unknown, more recent studies have noted that oscillations within certain classes have been linked with a variety of neural processes, including input selection, plasticity, binding and consolidation, as well as cognitive functions including salience detection, emotional regulation, attention and memory. Given the findings above and the results of our study, it is possible that ALFF alterations indicate deficits related to emotion and general network coordination, leading to psychoses.

Shared alterations in conventional emotional perception regions across diagnostic groups

In this study, altered ALFF were prominent in the paralimbic, limbic and striatum regions across schizophrenia, bipolar disorder and major depressive disorder. The paralimbic system consists of a transitive zone between the allocortex and isocortex. It includes the orbital prefrontal cortex, insula, temporal pole, retrosplenial cingulate cortex and parahippocampal cortex. Through connections between the isocortex and limbic regions, the paralimbic system is thought to mediate the internal emotional experiences of external environmental stimuli by providing meaning and context to sensory information processed in unimodal cortical areas. Previous ALFF studies have shown significant differences in paralimbic regions in schizophrenia, bipolar disorder and major depressive disorder compared with healthy controls. Structural or functional alterations in paralimbic regions have also been demonstrated in schizophrenia, bipolar disorder and major depressive disorder.

We also found altered ALFF in limbic regions (specifically the amygdala and hippocampus, as well as the striatum) across schizophrenia, bipolar disorder and major depressive disorder. These findings were consistent with previous ALFF studies that compared the 3 disorders individually with healthy controls. Further, resting-state and task-dependent fMRI studies have implicated limbic abnormalities (including those in the amygdala and hippocampus) in schizophrenia, bipolar disorder and major depressive disorder, although the specific features of the abnormalities may vary by diagnosis. Together, previous findings suggest that limbic and striatum abnormalities are common neurobiological features across the different psychiatric disorders. The specific characteristics of these abnormalities may differ across diagnoses and serve as differentiating...
markers among different disorders. They may also influence functional integration between the limbic and cortical regions, which may, in turn, mediate the manifestation of different psychiatric syndromes from shared genetic and environmental risk factors.

Shared alterations in visual cortices across diagnostic groups

We also found altered ALFF across schizophrenia, bipolar disorder and major depressive disorder in the visual cortices,

Table 2: ALFF values for slow-5 in brain regions showing significant group differences

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Brodmann area</th>
<th>Cluster size, voxels (mm³)</th>
<th>Peak MNI coordinates</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior cerebral</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right caudate nucleus, right putamen, right thalamic, right amygdala, right hippocampus, right parahippocampal gyrus</td>
<td>47/34/25</td>
<td>378 (756)</td>
<td>9 15 −3</td>
<td>19.139</td>
</tr>
<tr>
<td>Right hippocampus, right parahippocampal gyrus</td>
<td>36/20/28</td>
<td>219 (428)</td>
<td>27 −36 9</td>
<td>13.876</td>
</tr>
<tr>
<td>Right ventral prefrontal cortex</td>
<td>47/11</td>
<td>64 (128)</td>
<td>42 33 −6</td>
<td>8.741</td>
</tr>
<tr>
<td>Right dorsal prefrontal cortex</td>
<td>10/11</td>
<td>76 (152)</td>
<td>18 60 3</td>
<td>10.996</td>
</tr>
<tr>
<td>Left caudate nucleus, left putamen, left thalamus, left amygdala, left hippocampus, left parahippocampal gyrus, left temporal pole, left insula, left anterior cingulate cortex, left orbitofrontal cortex, left ventral prefrontal cortex</td>
<td>47/34/28/36/11</td>
<td>862 (1724)</td>
<td>−9 15 −3</td>
<td>21.600</td>
</tr>
<tr>
<td>Left anterior cingulate cortex</td>
<td>10/32</td>
<td>39 (78)</td>
<td>−12 48 15</td>
<td>7.226</td>
</tr>
<tr>
<td>Left temporal pole, left inferior temporal gyrus, left middle temporal gyrus</td>
<td>21/20/38</td>
<td>88 (176)</td>
<td>−51 9 −30</td>
<td>10.692</td>
</tr>
<tr>
<td>Left inferior temporal gyrus, left middle temporal gyrus</td>
<td>21/20</td>
<td>32 (64)</td>
<td>−42 −21 −27</td>
<td>7.798</td>
</tr>
<tr>
<td>Posterior cerebral</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral cuneus gyri, bilateral precuneus gyri, bilateral lingual gyri, bilateral calcarine cortices, bilateral fusiform gyri, bilateral posterior cingulate gyri</td>
<td>18/19/17/30/31/23</td>
<td>1134 (2268)</td>
<td>−3 −93 −6</td>
<td>14.155</td>
</tr>
<tr>
<td>Left precentral gyrus</td>
<td>3/4/1/2</td>
<td>76 (152)</td>
<td>−51 −15 48</td>
<td>11.201</td>
</tr>
<tr>
<td>Bilateral paracentral lobules</td>
<td>5/4</td>
<td>30 (60)</td>
<td>0 −45 66</td>
<td>9.864</td>
</tr>
</tbody>
</table>

Table 3: ALFF values for slow-4 in brain regions showing significant group differences

<table>
<thead>
<tr>
<th>Brain region</th>
<th>Brodmann area</th>
<th>Cluster size, voxels (mm³)</th>
<th>Peak MNI coordinates</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior cerebral</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral caudate nuclei, bilateral putamina, bilateral amygdalae, bilateral hippocampi, left temporal pole, bilateral orbitofrontal cortices, bilateral parahippocampal gyrus, left insula, left anterior cingulate cortex, bilateral ventral prefrontal cortices, left inferior temporal gyrus, right frontal pole, right dorsal prefrontal cortex</td>
<td>47/11/36/25/10/34/38</td>
<td>2085 (4170)</td>
<td>−9 15 −3</td>
<td>15.810</td>
</tr>
<tr>
<td>Right putamen</td>
<td>27 (54)</td>
<td>30 0 3</td>
<td>8.803</td>
<td></td>
</tr>
<tr>
<td>Right temporal pole</td>
<td>38</td>
<td>42 (84)</td>
<td>30 −3 −42</td>
<td>9.195</td>
</tr>
<tr>
<td>Left inferior temporal gyrus, left middle temporal gyrus</td>
<td>20/21</td>
<td>83 (166)</td>
<td>−54 −3 −30</td>
<td>12.662</td>
</tr>
<tr>
<td>Posterior cerebral</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilateral cuneus gyri, bilateral precuneus gyri, bilateral lingual gyri, bilateral calcarine cortices, bilateral fusiform gyri, bilateral posterior cingulate gyri</td>
<td>18/19/17/30/31/23</td>
<td>1328 (2656)</td>
<td>9 −87 3</td>
<td>15.229</td>
</tr>
<tr>
<td>Right middle occipital gyrus, right middle temporal gyrus, right inferior occipital gyrus, right inferior temporal gyrus</td>
<td>37/19</td>
<td>138 (276)</td>
<td>45 −66 −12</td>
<td>11.202</td>
</tr>
<tr>
<td>Left middle occipital gyrus, left middle temporal gyrus, left inferior occipital gyrus</td>
<td>37/19</td>
<td>65 (130)</td>
<td>−45 78 −6</td>
<td>10.446</td>
</tr>
<tr>
<td>Left inferior temporal gyrus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right precentral gyrus</td>
<td>6</td>
<td>28 (54)</td>
<td>33 −15 66</td>
<td>9.39</td>
</tr>
<tr>
<td>Right postcentral gyrus</td>
<td>3/2/40/4/1</td>
<td>68 (136)</td>
<td>45 −24 48</td>
<td>8.295</td>
</tr>
<tr>
<td>Left postcentral gyrus</td>
<td>3/4/1/2</td>
<td>94 (188)</td>
<td>−54 −15 48</td>
<td>10.418</td>
</tr>
<tr>
<td>Bilateral paracentral lobules</td>
<td>6/5/4</td>
<td>200 (400)</td>
<td>−3 −42 60</td>
<td>9.524</td>
</tr>
</tbody>
</table>

ALFF = amplitude of low-frequency fluctuations; MNI = Montreal Neurological Institute.
Amplitude across frequency bands in major psychiatric disorders

including the bilateral cuneus, precuneus, lingual gyri and calcarine cortex. These regions are involved in basic and higher-order visual processing, as well as in the generation of visual imagery. Our findings of decreased ALFF in the visual cortices were consistent with previous studies in schizophrenia, bipolar disorder and major depressive disorder. Visual cortical abnormalities have been reported in psychiatric disorders, particularly in schizophrenia. They have also been shown in mood disorders, with indications of an association between visual salience and depressive symptoms. Previous studies of visual cortices and emotional tasks in schizophrenia further support a link between visualcortical abnormalities and emotions. Interestingly, one study found that activation in the lingual gyrus (which is involved in generating visual imagery) during passive viewing of emotional images differentiated schizophrenia patients with and without flat affect.

**ALFF balance**

We observed increased ALFF in the anterior paralimbic and limbic regions and decreased ALFF in the posterior visual cortices across schizophrenia, bipolar disorder and major depressive disorder, suggesting anterior versus posterior imbalance in brain function in these disorders. Further, we observed significant differences in the ALFF balance ratio in slow-5 and slow-4 compared with healthy controls across schizophrenia, bipolar disorder and major depressive disorder. The differences appeared to be graded across the disorders, with schizophrenia having the lowest ratio, followed by bipolar disorder and then major depressive disorder. The lower ratios reflect smaller differences in ALFF between the visual cortices and conventional regions of emotional perception compared with the total ALFF of the visual cortices and emotional-perception regions. The interplay between the visual cortices and conventional regions of emotional perception may contribute to perception and biases in stimulus-processing and higher-order cognitive processes, such as learning and decision-making, as well as mental imagery and self-perception.

Measures of a slow-frequency fMRI signal such as ALFF may relate to modulatory signalling in large cortical regions. Specifically, ALFF may reflect neural activity in the deeper cortical layers V and VI and inhibitory modulation and signalling. Taken altogether, the altered ALFF balance ratios suggest abnormal modulation of activity in the regions across diagnostic groups. It is unclear how these altered balance ratios may manifest; however, the observed correlation (although small) between the ALFF balance ratio in slow-4 and BPRS factor 1 suggest that it may relate to negative and disorganized symptoms. Nevertheless, network balance appears to be important in understanding how neural commonalities among disorders result in similarities and differences in clinical features, both between and within disorders.

**Potential differentiating findings**

The ALFF alterations were most prominent in schizophrenia, followed by bipolar disorder and then major depressive disorder, compared with healthy controls. This indicates that schizophrenia has the greatest severity in ALFF disruption, major depressive disorder has the least, and bipolar disorder has an intermediate phenotype. This finding mirrors the clinical prognosis for these disorders. As well, major depressive disorder did not demonstrate significantly increased ALFF in the right anterior brain areas, which may help to differentiate major depressive disorder from other disorders. Further study is needed to determine whether gradation of alterations could be used as a biomarker to differentiate between schizophrenia, bipolar disorder and major depressive disorder, particularly early in their course or in prodromal periods.

**Limitations**

This study had several limitations. First, approximately 50% of participants with schizophrenia, bipolar disorder and major depressive disorder were taking psychotropic medications at the time of the study. However, we found no
significant effects of medication status (taking or not taking) on the regions showing significant 4-group differences in any patient group, suggesting that there were no major effects of medication on the investigated parameters. Our findings for medication effects were consistent with those of previous studies. Although the effects of medication on ALFF are still unknown, future studies in medication-naive patients or with a focus on specific psychotic medications are needed to clarify these issues. Second, our sample had a relatively wide age range (13–30 years). The broad age range and cross-sectional design may limit interpretation of our findings. Third, although most of the patients we recruited had been followed for nearly 2 years, there may have been other factors that contributed to their diagnostic categorization. Finally, the exact origins and mechanisms underlying ALFF are unknown. The fMRI signals hinge on the assumption that neuronal metabolism and cerebral blood flow are proportionately related to electrophysiological activity of neurons, but these relationships are far more complex than generally considered, and need further exploration and clarification.

Conclusion

Our major findings suggest that the extent of the commonalities we observed further supports the concept of core neurobiological disruptions shared among schizophrenia, bipolar disorder and major depressive disorder. The balance of ALFF signals in the neural system for emotional perception might be an important neuroimaging marker for the diagnosis and treatment of these major psychiatric disorders.

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Competing interests: None declared.

Contributors: F. Wang designed the study. X. Jiang, Y. Tang, S. Wei, F. Womer, K. Xu, Q. Zhou and Y. Zhou acquired the data, which H. Huang, Y. Ye and X. Zuo analyzed. M. Chang and E. Edmiston wrote the article, which all authors reviewed. All authors approved the final version to be published and can certify that no other individuals not listed as authors have made substantial contributions to the paper.

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Treating resistant depression with 2 forms of convulsive therapy: a clinical case study

Anastasios A. Daskalakis; Zafiris J. Daskalakis, MD, PhD

Electroconvulsive therapy (ECT) is effective for treatment-resistant depression, but its use is limited owing largely to stigma and its cognitive adverse effects. Magnetic seizure therapy (MST) is a new, alternative seizure treatment for treatment-resistant depression (TRD), and early studies suggest that MST does not produce any adverse effects on memory.1

These differences between ECT and MST are best illustrated in the clinical case of a 34-year-old patient with a longstanding history of TRD. Numerous trials of antidepressant medications (selective serotonin reuptake inhibitors, tricyclic antidepressants) had failed while she continued to experience marked depressive symptoms with passive suicidal ideation. She began a course of ECT in 2012. She experienced a marked improvement in depressive symptoms following 10 right-unilateral ultrabrief ECT treatments delivered 2–3 times per week on nonconsecutive days. She also reported subjective worsening of memory; ECT was discontinued as she could no longer tolerate this memory impairment. Unfortunately, she experienced a marked worsening of depressed mood within 1 month of discontinuing ECT. At that time, her 24-item Hamilton Rating Scale for Depression (HAM-D) score was 29. Given her response to ECT and lack of response to numerous antidepressants, the only viable option to be considered was an alternative seizure treatment. She was offered a course of MST as part of a clinical trial at the Centre for Addiction and Mental Health. She received 9 MST treatments, delivered bilaterally over the frontol cortex using the Tonica Mag-Pro MST and twin coil, and she experienced complete remission of her depressive symptoms as indexed by the 24-item HAM-D. Subjectively, she reported no memory loss. This patient continues to be treated with MST on a maintenance basis, and she remains in remission of depressive symptoms.

Electroconvulsive therapy was first introduced in Italy by Ugo Cerletti and Lucio Bini in 1938.2 Within a few years, the therapeutic benefit of ECT became widely recognized, and it was quickly adopted as a treatment for severe and persistent mental illness across the globe. Evidence suggests that 50%–75% of patients with TRD experience significant improvement with ECT—more than any other treatment option for this disorder.3 By delivering a series of electrical stimuli to the cortex, ECT produces a generalized seizure. This seizure has been shown to release neurotransmitters, including serotonin, dopamine and noradrenaline.4 It has been postulated that deficiencies in these neurotransmitters are associated with depression and that the release of these neurotransmitters has been linked to the therapeutic effects of ECT.4 Additionally, ECT may also have antidepressant effects by increasing cerebral blood flow and by improving synaptic plasticity.5

Many patients, however, are reluctant to undergo ECT, and fewer than 1% of patients with TRD receive ECT.6 There are likely 2 main reasons. The first is that ECT is highly stigmatized in our society, in part because of the negative stereotype of ECT delivery—as shown in One Flew Over the Cuckoo’s Nest. A second reason limiting the use of ECT is its effect on memory. Most patients receiving ECT experience some degree of both anterograde and retrograde amnesia.

Magnetic seizure therapy was first developed in 1998 as a potential alternative treatment to ECT in patients with TRD.7 It delivers a focused magnetic field that produces a seizure; MST activates the cortex in a focal manner, as magnetic fields are not shunted by the skull and are not volume-conducted by the cerebrospinal fluid, unlike with ECT.8 As such, studies suggest that MST does not interfere with memory, as it does not affect deeper brain regions (e.g., hippocampus).9 Studies suggest that MST produces significant mood improvements without any significant cognitive impairment10,11 and show that MST can produce remission of suicidal ideation.11 However, further research is needed to determine if the clinical efficacy of MST is comparable to that of ECT.12

Although studies comparing ECT with MST report comparable clinical efficacy with superiority of MST in relation to cognition,9,10 to our knowledge there are no case reports illustrating within-patient effects of ECT and MST in relation to clinical outcomes and adverse effects on memory. The present case highlights these differences in adverse effects in a single patient and emphasizes the importance of offering advancements in seizure treatments to patients with TRD.

Affiliations: From the Temerty Centre for Therapeutic Brain Intervention, Centre for Addiction and Mental Health, University of Toronto, Toronto, Ont., Canada.

Competing interests: A. Daskalakis declares no competing interests. In the last 5 years, Z.I. Daskalakis has received research and equipment in-kind support for an investigator-initiated study through Brainsway Inc and Magventure Inc. His work was supported by the Ontario Mental Health Foundation (OMHF), the Canadian Institutes of Health Research (CIHR), the National Institutes of Mental Health (NIMH) and the Temerty Family and Grant Family and through the Centre for Addiction and Mental Health (CAMH) Foundation and the Campbell Institute.

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