Lifetime major depression and grey-matter volume

Head motion: the dirty little secret of neuroimaging in psychiatry
I am the only physician responsible for looking, as I left the man’s bed and was part of an elite Nazi "challenge from the ordinary. For two and a half months, I volunteered to become a tail-gunner and never come back, starting with every step, his eyes and having no idea how any of it would be his last case, he swore it to me to examine his chest and abdomen. We have to check her breath this morning. I also gave her a shot of what. She should probably see the dentist today. I also gave her a shot of dispirum too, which I didn’t know people numbered days. There’s a history of vansilla extract. Black pepper, whole Kathmandu, for which he traded a bottle guably Molly Mayfair.”

My father struggled as well. He vos when she became Jolly Mayfair. "Theories crowded my mind. My first thought as I left the man’s bed was that the wrong team.” I will tell you now that I am the only physician responsible for looking, as I left the man’s bed and was part of an elite Nazi “challenge from the ordinary. For two and a half months, I volunteered to become a tail-gunner and never come back, starting with every step, his eyes and having no idea how any of it would be his last case, he swore it to me to examine his chest and abdomen. We have to check her breath this morning. I also gave her a shot of dispirum too, which I didn’t know people numbered days. There’s a history of vansilla extract. Black pepper, whole Kathmandu, for which he traded a bottle guably Molly Mayfair.”

Many of the residents cry out from algebra every day. For two and a half months, I volunteered to become a tail-gunner and never come back, starting with every step, his eyes and having no idea how any of it would be his last case, he swore it to me to examine his chest and abdomen. We have to check her breath this morning. I also gave her a shot of dispirum too, which I didn’t know people numbered days. There’s a history of vansilla extract. Black pepper, whole Kathmandu, for which he traded a bottle guably Molly Mayfair.”

Now as I watch from shore, I real-

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Unreadable

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From the neo-Kraepelinian framework to the new mechanical philosophy of psychiatry: regaining common sense

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Head motion: the dirty little secret of neuroimaging in psychiatry

C. Makowski, M. Lepage, A.C. Evans
From the neo-Kraepelinian framework to the new mechanical philosophy of psychiatry: regaining common sense

Ridha Joober, MD, PhD; Karim Tabbane, MD

"While the other senses put us in contact with things, the common sense presides over our relations with persons."

Henri Bergson

The neo-Kraepelinian framework (NKF) dominated psychiatric practice and research for almost 50 years. In this editorial, we briefly review the historical context in which the NKF emerged and reflect on its current implications for clinical practice and research. We conclude with some reflections on how this framework is adapting to the mechanical philosophy of biology that emerged at the turn of the 21st century.

The practice of psychiatry in the 18th and 19th centuries produced a rich literature in the form of detailed clinical descriptions. These efforts were made with the premise that systematic observations are the basic method for advancing sciences, including the nascent science of behavioural medicine. Philippe Pinel (1745–1826), one of the founding fathers of psychiatry, wrote in his Treatise On Insanity that the path to success required him “to notice successively every fact, without any other object than that of collecting materials for future use; and to endeavour, as far as possible, to divest myself of the influence, both of my own prepossessions and the authority of others.” 1 Given the wide range of human behaviours, emotions and cognition, as well as their quasi-infinite variations and combination from one subject to the other and the limitless ways an observer could interact with and interpret the behaviour of their patients, there were no boundaries to what could be reported and discussed.

The aspiration to divest influence from oneself and that of authority is laudable; however, it has been strongly criticized and is now considered epistemologically flawed, particularly in human sciences. Indeed, all observations are read/decoded according to various theoretical frameworks, or disciplinary matrices,2 that are required for all levels of analyses, starting from the simplest descriptions to the highest levels of causal or other organizing principles. Thus, beyond fact collection, the European masters of psychiatry contributed to the invention of various theoretical frameworks, in which masterfully described cases were taken as exemplars to support specific models of psychopathology and were used to understand the life histories of patients, the pathways leading from normal to pathological behaviours, the grouping of patients into diagnostic categories and the treatment offered according to the models at hand.

Nineteenth-century frameworks of psychiatric disorders

Although it is difficult to do justice to the large number of authors who have marked the history of psychiatry, 2 major frameworks, 1 proposed by Emile Kraepelin and 1 by Sigmund Freud, were highly influential and shaped modern psychiatry significantly. A brief presentation of these 2 frameworks here will serve to situate them historically and underline their effects on the emergence of the NKF.

Emile Kraepelin (1856–1926) is probably the most consequential researcher for modern psychiatry.3 Marked by the European tradition of correlating signs and symptoms and tracking their evolution over time to define diseases, as typified by general paresis and its association with a specific pathogen, his main legacy to psychiatry has been the distinction between dementia praecox and manic-depressive illness as 2 different illnesses with putatively different pathological processes, genetics and biochemistry.4 His approach is the closest to the medical model. The Kraepelinian system was quickly adopted by the American Psychiatric Association, and it replaced previous classification systems based mainly on symptoms.5

Sigmund Freud (1856–1939), the founder of psychoanalysis, arguably the most influential and popular theoretical framework of the mind and its workings in the 20th century, did not seek to construct a psychiatric nosology, but rather
elaborated a theory of the psychic apparatus. He postulated 3 fundamental agencies — the id, ego and superego — and emphasized a major role of the id (unconscious) in human behaviours. These agencies are subject to conflicts between them and the outside world, leading to the emergence of symptoms, which are conceived as maladaptive defence mechanisms. Over time, psychoanalytical theories have burgeoned, seeking to explain all kinds of signs and symptoms using the initial psychic topology of Freud and all their avatars. Although its impact on human sciences in general and psychiatry in particular was major and universal, psychoanalysis was severely criticized by some philosophers of science as being pseudoscientific.

**The neo-Kraepelinian framework**

While the Kraepelinian and other 19th-century frameworks of psychiatric disorders continued to thrive along with psychoanalysis in Europe, American psychiatry was almost completely engulfed by the theories of Freud and his disciples in the ‘60s and ‘70s. This resulted in a major rift between American psychiatry and mainstream European psychiatry. The climax of this rift was epitomized by the realization that clinicians and researchers in Britain and the United States were using the same label to designate highly disparate disorders. For example, it was found that the designation of schizophrenia in the United States included depressive, manic and personality disorders. In response to this appalling state of affairs, major efforts were deployed to improve the reliability of the designation of psychiatric disorders, mainly in the form of creating operationalized criteria to diagnose mental disorders, which were included in the *Diagnostic and Statistical Manual of Mental Disorders*, third edition (DSM-III), published by the American Psychiatric Association in 1980. This work was led by a group of American psychiatrists called the neo-Kraepelians, and it received immediate, wide acceptance and usage all over the world. The major premise of the work was that a homogenization of the language used by clinicians and researchers was required for any serious attempt to develop a scientific understanding of psychiatric disorders. An important aspect of this effort to homogenize language was the reliance on criteria that had high interrater reliability. Another major theoretical foundation of the NKF was the repudiation of any top–down theoretical framework of mental illnesses. It was assumed that using reliable criteria to define disorders could, through an iterative process of validation, lead to bottom–up theories of mental illnesses.

Despite widespread acceptance of the NKF, it is important to review some of its consequences and reflect on its standing in view of the major developments in biological psychiatry, notably genetics and, to a lesser extent, brain imaging.

In the pursuit of reliability, major efforts were made to exclude any signs and symptoms with poor interrater reliability. Consequently, many of these signs and symptoms that were important — even foundational for the definition of some psychiatric disorders — were disregarded. One well-known example, that of psychotic ambivalence, was considered a cardinal sign of schizophrenia, as conceived by Bleuler, but was not included in the DSM-III and its subsequent versions because it did not fulfill the criterion of satisfactory interrater reliability. However, ambivalence is certainly a clinical reality in some patients that can be clearly perceived by phenomenologically tuned clinicians and can possibly be very helpful to better understand the patient and relate to their difficulties.

Another symptom, delusional mood, which had a pre-eminent place in the understanding of the early phases of psychotic illness, has almost disappeared in the modern psychiatric literature. This experience of delusional mood was one of the fundamental insights into the phenomenological experience of individuals facing the early dissolution of the structure of consciousness as a consequence of the psychotic process. Other symptoms, such as delusions, although pre-eminent in the DSM system, are restricted to a bare definition of false beliefs with some modifiers, which contrasts with the sophisticated phenomenological analyses offered by some masters of psychopathology who consider delusion a “particular type of existence in the world, which should be analyzed on a case-by-case basis and not defined in advance by its nonreality.”

Here, we have presented a few examples of how list-oriented psychiatry has resulted in the loss of a rich clinical and semeiotic knowledge painstakingly identified by psychiatrists and researchers over the last 2 centuries. The purpose at this point is not to blame the successive versions of the DSM for this impoverishment of clinical psychiatry. Indeed, the NKF never purported the DSM to be a clinical textbook to teach psychiatry and improve empathy. Nonetheless, an unintended consequence of the NKF, unfortunately, is the “death of phenomenology” in America, as Nancy Andreasen argued a few years ago.

Notwithstanding the unintended negative consequences of criteria-oriented psychiatry, we believe that this approach made a major contribution to psychiatric research, because patients could be grouped under relatively reliable labels and studied with modern and tremendously sophisticated technology that transformed all aspects of medicine (e.g., molecular biology, genetics, high-resolution brain imaging, high-throughput computational power). Had we continued to operate with the confused language that predated the NKF, our field would have developed into a deafening cacophony. This approach enabled researchers in psychiatry to apply cutting-edge technologies developed in the last 40 years in an attempt to validate these criteria-defined disorders (this is what most papers published in *JPN* attempt to do). These efforts represent the second and major part of the NKF agenda. Robins and Guze, who were major figures among the neo-Kraepelians, published the “manifesto” of this validation program in 1970. They proposed 5 criteria — namely, clinical description, laboratory study, exclusion of other disorders, follow-up study and family study — to validate psychiatric disorders. They gave the example of how family study led them to conclude, “good prognosis schizophrenia” is not mild schizophrenia, but a different illness.” We believe that given the tremendous developments in genetic and brain imaging research, it is legitimate to reflect on whether we have gained any added validity, as proposed by Rubins and...
Guze, of any specific major psychiatric disorder compared with our basic clinical knowledge. Most of the examples that follow pertain to schizophrenia, but the conclusions can be extended, without loss of generality, to all mental disorders.

The publication of major gene effects associated with schizophrenia and bipolar disorder in the late 1980s was met initially with enthusiasm, only to be refuted extensively later on. It can now be affirmed without any doubt that there are no major mutations that are causative of any mental disorder, although a handful of cases may be strongly influenced by mutations in a single gene (e.g., complement C4, RNA-motif binding 12, and SETD1A in schizophrenia). Furthermore, the picture that emerged from the very large and statistically powerful international collaborative effort (Psychiatric Genetic Consortium; PGC) that assembled tens of thousands of samples of patients and controls and compared their genetic variants is quite sobering from the perspective of the NKF. The most recent genome-wide association study (GWAS) in 36 180 Chinese participants, along with a concomitant analysis of the PGC data, identified 113 single nucleotide polymorphisms (SNPs) that were significantly associated with schizophrenia, and each of these variants had a tiny effect. Genome-wide association studies have also developed and validated the concept of a polygenic risk score, which reflects the aggregate effect of thousands of loci that might be implicated in a disorder under a polygenic model. This work has clearly demonstrated a highly significant genetic overlap among most of the major psychiatric disorders that were included in the analysis. Most remarkably, a recent, large study (265 218 patients and 784 643 controls) investigated the genetic commonalities among 25 brain disorders. The study found that psychiatric disorders and neurologic disorders generally do not share genetic variants, establishing a clear boundary between psychiatric and other disorders of the brain. In addition, the study found that, contrary to neurologic disorders, which show clear boundaries among themselves, psychiatric disorders do not. For example, schizophrenia has significant genetic correlation with attention-deficit/hyperactivity disorder (ADHD), anorexia nervosa, bipolar disorder, autism and major depressive disorder. These nonspecific genetic influences of SNPs on a large number of psychiatric disorders have also been shown in relation to genomic copy number variants (CNVs) that have shown stronger genetic effects, but also poor specificity. Indeed, it is now believed that any of these given pathogenic CNVs increase the risk for neurodevelopmental abnormalities and a host of psychiatric outcomes (e.g., ADHD, autism, intellectual disability, learning disabilities, epilepsy).

Genetic molecular studies have also highlighted a few important facts that have clear implications for our understanding of the architecture of psychiatric disorders. It has been reported that more than 71% of 1 MB windows in the human genome contain 1 or more genetic variants, which increase the risk for schizophrenia, and the majority of these variants are noncoding variants with weak enrichment in functional gene categories. These observations, which are shared by complex human disorders, led to the formulation of the “omnigenic” model, postulating that the disease risk is mainly due to genetic variants with no specificity to any particular disorder; however, they convey their effects through widely distributed pleiotropic effects on transcription. But how a specific disorder emerges from these largely distributed effects is far from being understood.

Thus, the genetic architecture of the major psychiatric disorders did not support the well-delimited entities reified as diseases through highly reliable criteria. Instead, molecular genetic studies support that the major mental disorders cluster in a large group, with very fuzzy delimitation among the different disorders. Consequently, under the highly polygenic nature and the complex genetic architecture of psychiatric disorders, it can be safely asserted that even affected members in the same family will differ from each other with regard to thousands of genetic variants implicated in the disorder. Conversely, even in the case of monozygotic twins who share 100% of their genetic makeup, the concordance for psychiatric disorders rarely exceeds 50%. This, of course, brings the whole issue of nongenetic contribution to the discussion, which, in most psychiatric disorders, was demonstrated to be nonshared in nature (as opposed to shared environmental risk factors within families). Analyzing these nonshared factors might be many orders of magnitude more complex than the genetic factors, given that our access to environmental factors is retrospective and tainted by various biases and circular causality. It is also quite possible that the nonshared environmental factors include all the “decisions” that an individual makes across every juncture in their life, and not only the physical environment to which a person is subjected. In that sense, the nonshared environment could also be of our own making. Eric Turkheimer took this idea to its extreme and made a bold and provocative proposition that the nonshared environment is free will.

Not the kind of metaphysical free will that no one believes in anymore, according to which human souls float free above the mechanistic constraints of the physical world, but an embodied free will, tethered to biology, that encompasses our ability to respond to complex circumstances in complex and unpredictable ways and in the process to build a self.

It can be argued that these conclusions may be specific to genetics, and other lines of biological investigations (laboratory study in the Robins and Guze proposal) may lead us to different, better delineations of specific psychiatric disorders. Notwithstanding the fact that most research in other biological fields of psychiatry remains impeded by small sample sizes and lack of robustness, some evidence suggests that the level of heterogeneity in psychiatric disorders is such that validation of specific mental disorders on the basis of brain imaging markers might be very difficult. For example, in a recent large study comparing brain structures in patients with schizophrenia (n = 163), bipolar disorder (n = 190) and controls (n = 256), it was concluded that the group-level differences disguised a high level of heterogeneity within disorders. Indeed, overlap of more than 2% among patients was reported only in a few loci, suggesting that sharing a major DSM diagnosis does not guarantee a meaningful commonality at the brain structure level.
A new mechanical philosophy of science

From the philosophy of enlightenment to the logical empiricism that dominated the epistemology of science until the late 20th century, important efforts have been made to understand phenomena on the basis of empirical evidence aided by logic and mathematics, with the ultimate goal of identifying the laws of nature on which we can base our understanding of phenomena. Much of the work that has been done by the classical writers in psychiatry had that same aspiration. At the turn of the 21st century, a new mechanical philosophy of science emerged mainly from philosophical reflections on the foundations of biological sciences, particularly neuroscience and psychology. The main impetus for the NKF was the absence of general laws in biological sciences compared with physics and chemistry. The concept of mechanism is central to this NKF and was first articulated clearly in a paper by Machamer and colleagues published in 2000.

In this seminal paper, it was proposed that “mechanisms are sought to explain how a phenomenon comes about or how some significant process works. Specifically: mechanisms are entities and activities organized such that they are productive of regular changes from start or set-up to finish or termination conditions.” In this NKF, mechanisms are defined in a very large sense, and they can span multiple levels of analysis. They are not necessarily determinant, reductionist, sequential/linear or localizable; nor are they limited to “cogwheel” kinds of Cartesian machines. In addition, mechanisms are not laws of nature.

In psychiatry, Kenneth Kendler and many others have promoted this new mechanistic approach to our field. In a paper titled “Explanatory models for psychiatric disorders,” Kendler wrote:

A more appropriate scientific model for psychiatry emphasizes the understanding of mechanisms, an approach that fits naturally with a multicausal framework and provides a realistic paradigm for scientific progress; that is, understanding mechanisms through decomposition and reassembly. (...) Biology will implement but not replace psychology within our explanatory systems.

Whether this new mechanistic approach will fill the gap between explanation (based on pure biological underpinnings) and understanding (based on psychological/phenomenological relatedness to patients) of mental phenomena, as formulated by Jaspers, the founder of phenomenological psychiatry, and later philosophers of science, remains an open question, although in a more recent paper Kendler and Campbell proposed some examples on how understanding of the life experience of patients at the phenomenological level can be enhanced by mechanistic explanations at the molecular and cognitive levels.

Conclusion

Importantly, we are not presenting a negative view of the NKF or trying to denigrate what it has achieved; on the contrary, we strongly believe that NKF has been extremely important for the development of psychiatric science in the last 50 years and that it definitely established that mental disorders are a reality etched in our genes and bodies, and not only social constructs. Genetic and brain imaging research have been the flagship of scientific validation of this framework, and their results clearly indicate a broad validation of psychiatric disorders as a group, but still with limited specificity and very few implications at the individual level. We believe that deeper phenomenological analyses of patients’ experiences are needed and should be taught, researched and cherished. This will be very useful from a clinical point of view (at the individual level) and will open new vistas of research on psychological/phenomenological mechanisms, fully rehabilitated within the new mechanistic philosophy of science, and not as epiphenomena that can be disposed of as soon as they are correlated to whatever molecular or brain activity phenomenon. In that, the NKF will have contributed to the return of common sense.

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Updated Dec. 10, 2018
A review of functional neurological symptom disorder etiology and the integrated etiological summary model

Aaron D. Fobian, PhD; Lindsey Elliott, MA

Functional neurological symptom disorder (FNSD) is characterized by neurological symptoms that are unexplained by other traditional neurological or medical conditions. Both physicians and patients have limited understanding of FNSD, which is often explained as a physical manifestation of psychological distress. Recently, diagnostic criteria have shifted from requiring a preceding stressor to relying on positive symptoms. Given this shift, we have provided a review of the etiology of FNSD. Predisposing factors include trauma or psychiatric symptoms, somatic symptoms, illness exposure, symptom monitoring and neurobiological factors. Neurobiological research has indicated that patients with FNSD have a decreased sense of agency and abnormal attentional focus on the affected area, both of which are modulated by beliefs and expectations about illness. Sick role and secondary gain may reinforce and maintain FNSD. The integrated etiological summary model combines research from various fields and other recent etiological models to represent the current understanding of FNSD etiology. It discusses a potential causal mechanism and informs future research and treatment.

Introduction

Functional neurological symptom disorder (FNSD) refers to neurological symptoms that are incompatible with neurological or medical conditions. The incidence is between 4 and 12 per 100 000, comparable to multiple sclerosis and amyotrophic lateral sclerosis, and it is the second most common diagnosis in neurology clinics. Examples of FNSD include psychogenic nonepileptic seizures (PNES), paralysis, functional movement disorders (FMD), blindness and non-dermatomal sensory deficits.

The prognosis for FNSD is linked to early diagnosis and symptom duration, but the average time to diagnosis of PNES is more than 7 years. Delayed diagnosis and unnecessary medications can lead to iatrogenic effects, delay appropriate treatment and negatively affect prognosis. However, both physicians and patients have a limited understanding of FNSD. One study demonstrated that physicians held several misperceptions about PNES, and that their confidence in their ability to treat PNES was low. This uncertainty likely affects patients with FNSD. Most patients with PNES do not have a good understanding of their diagnosis, and they report feeling confused, angry and “dumped” after physician consult.

Such uncertainty about FNSD suggests that its etiology may not be easily explained by physicians or comprehensible to patients. Traditionally, the etiology of FNSD has been explained in the context of psychoanalytic theory as a physical manifestation of psychological distress, and many physicians continue to use this as a simple explanation in clinical settings. However, there is little supporting empirical evidence for this hypothesis, and patients have been found to respond negatively to psychiatric explanations for physical symptoms. There is evidence that rates of trauma, stress and psychiatric comorbidities are higher in patients with FNSD, but recent research has demonstrated low incidence of physical or psychiatric diagnoses to directly explain patients’ symptoms, and trauma is present in only about one-third of patients. No single causal mechanism has been found; instead, predisposing factors vary among individual patients. As a result, the DSM-5 diagnostic criteria for FNSD have removed preceding stressors as a requirement, instead focusing on positive symptoms. Several cognitive and neurobiological etiological models have been proposed for medically unexplained illness and FNSD symptoms.

Given recent research and the shift in diagnostic criteria, we provide a review of recent research on the predisposing and reinforcing factors for FNSD. Then, integrating information from other models, we present an integrated etiological summary model of FNSD.

Predisposing factors

Research has demonstrated heterogeneity in the vulnerabilities for FNSD, and individual patients may not experience...
the same combination of predisposing factors (Table 1). Below is a review of factors that may predispose patients to FNSD.

Trauma/psychiatric symptoms

Trauma and psychiatric symptoms have long been regarded as the cause of FNSD, but research findings in this area have been inconsistent. Patients with FNSD have increased general trauma history, and a recent meta-analysis found that 33% of patients with PNES had a history of childhood sexual abuse. However, the meta-analysis concluded that there was not enough evidence to establish a causal relationship between childhood sexual abuse and PNES. Still, there is a demonstrated link between PNES and trauma, suggesting that trauma is a predisposing factor for the development of FNSD. As well, the magnitude of trauma experience is related to the severity of FNSD symptoms. This finding has been supported by a recent study demonstrating that childhood abuse burden was associated with left anterior insular volume reductions in women with FNSD.

Findings related to the association between FNSD, stressors and psychiatric conditions have also been inconsistent. About one-third of patients with FNSD have normal scores on psychological measures, similar to patients with organic movement disorders. Further, 2 recent studies found no difference in reported stressors between patients with FNSD and controls. In a group of pediatric patients, all denied history of sexual abuse or trauma, and 25% denied even mundane stressors. A study of adults found no difference in stressful events between patients with PNES, patients with epilepsy or controls, but patients with PNES self-reported greater stress and demonstrated fewer coping skills. This result was consistent with a study that found no difference in the number or impact of stressful life events between patients with FNSD and controls, but did find that both cortisol (hypothalamic–pituitary–adrenal axis) and α-amylase (adrenergic axis) levels were higher in patients with FNSD. Patients with FNSD and controls responded to a social stress test with similar increases in cortisol and α-amylase, but patients with FNSD self-reported significantly greater stress, which correlated with α-amylase levels. This finding suggests that patients with FNSD may perceive stress differently and have fewer skills to cope with stress.

Although some studies have found no significant increase in comorbidities, such as depression, anxiety or personality disorders, others have found increased prevalence of psychiatric disorders in patients with FNSD. Many patients with PNES have reported panic symptoms before PNES onset, but evidence about anxiety comorbidity is mixed. Some studies have demonstrated high anxiety in patients with PNES, but others have found no relationship. However, studies found that no anxiety could be the result of a lack of anxiety awareness: some patients with PNES have elevated physiologic arousal but deny anxiety. Additionally, some patients with FNSD have reported greater alexithymia (inability to identify and describe their emotions), as well as elevated scores on the hypochondriasis and hysteria scales and lower scores on the depression scale of the Minnesota Multiphasic Personality Inventory-2. Dissociative disorders are also common psychiatric comorbidities in this population, and the presence of a comorbid dissociative disorder is associated with more severe psychopathology in patients with FNSD.

While the evidence is not strong enough to indicate direct causality, there is an established connection between FNSD, trauma and psychiatric symptoms, suggesting that these factors, in combination with other predisposing factors, can increase the risk of developing FNSD.

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<th>Table 1: Overview of FNSD predisposing factors</th>
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<td>Factor</td>
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<td>Trauma/psychiatric symptoms</td>
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<td>Somatic symptoms</td>
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<td>Illness exposure</td>
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FNSD = functional neurological symptom disorder.
Somatic symptoms

Many patients with FNSD have experienced other medically unexplained symptoms in addition to their functional neurological symptoms. Between 57% and 82% of patients with PNES have a history of other medically unexplained symptoms and have rated their general health as worse than patients with epilepsy. Several explanations have been proposed for this increased experience of somatic symptoms. Some research suggests that increased somatization in patients with FNSD may be the result of heightened awareness of physical symptoms. Impairment in sensorimotor gating has been associated with FNSD, suggesting difficulty integrating information from internal and external environments.

However, other studies have suggested that increased somatization in patients with FNSD could be because of somatosensory amplification — the interpretation of somatic symptoms as injurious, extreme and distressing. As well, parental reinforcement of children’s illness behaviour is associated with those children’s concept of their illness, often resulting in beliefs and symptoms incongruent with their actual state of health and persisting into adulthood.

Illness exposure

Patients with FNSD frequently experience a precipitating physical event before the onset of FNSD. Peripheral injury was found in the majority of patients with functional dystonia, while 20% of patients with functional weakness had experienced physical injury to the affected limb near symptom onset. This link has been consistently reported since 1965, suggesting that physical trauma may play a significant role in FNSD onset.

Additionally, many patients with FNSD have a comorbid neurological disorder. Epilepsy prevalence in patients with PNES has been reported to be from 4% to 58%. One-third of patients with FMD were reported to have a significant neurological history, and 25% had a comorbid organic movement disorder. People with PNES and FMD are also more likely to have structural or functional brain abnormalities.

In addition to personal illness experiences, patients with FNSD have often been exposed to others with illness. Medically unexplained symptoms in adulthood have been associated with prior experience of family illness, and with professions in the medical field. One study reported that 66% of patients with PNES had witnessed an epileptic seizure before PNES onset, and more than one-third had a family history of epilepsy. News media, television and movies are other common sources of exposure to diseases, and media coverage of a disorder has been associated with increased presentation to physicians with concerns about the disorder. These personal and peripheral experiences of illness help shape beliefs about physical symptoms and health and may lead to symptom monitoring.

Symptom monitoring

Compared with patients with anxiety, patients with FNSD have demonstrated significant impairment in habituation to tones, which was interpreted as a deficit in selective attention. Another study found that patients with FMD were less likely to accurately report their heartbeat than controls, instead focusing on external body features. As well, fMRI research has shown increased self-monitoring in patients with lateralized paresis of the arm. Furthermore, when attention is distracted from the affected area, FMD symptoms decrease and sometimes subside.

Neurobiological factors

Three processes have been implicated in the neurobiology of FNSD: abnormal attentional focus on the affected area, beliefs and expectations about illness, and deficits in sense of control over one’s actions. Research has shown deficits in patients with FMD in movement that they had explicit, conscious control of, but no difference in performance of tasks that relied on automatic factors, suggesting that explicit movement may allow for increased attention on the production of movement in FMD.

Beliefs or expectations about health can also influence functional symptoms. Patients with FMD request less information than healthy controls before they form a decision, and they change their decision more frequently when presented with new contradictory evidence. This “jumping to conclusions” bias could be a risk factor for inappropriate updating of active inference, the theory in which the brain predicts and explains sensory input through past experiences. Additionally, patients with functional tremors self-reported tremor occurrence for 80% to 90% of their waking day, but objective measurement indicated that they had an average of only about 30 minutes of tremor per day. This overestimation was significantly greater than that in patients with organic tremor, suggesting that top–down prediction of constant tremor may prevent perception of time without tremor in patients with FMD. Research has also demonstrated the power of symptom expectation, showing that those who expected to experience analgesia in parts of their body reported analgesia in exactly those areas. This finding has been incorporated into several etiological models for general medically unexplained physical symptoms and FNSD.

Patients with FMD tend to have a decreased sense of agency or control over their actions. One study compared brain activity in mimicked tremors and functional tremors in patients with FNSD; it found hypoactivity in the right temporo-parietal junction and lower functional connectivity between the right temporo-parietal junction, sensorimotor cortices and limbic regions during functional tremors, suggesting that symptoms are perceived to be involuntary despite the use of voluntary motor pathways. These findings of decreased functional connectivity between the sensorimotor cortices and the temporo-parietal junction were later replicated with a larger sample size of patients with FMD. A computerized task has also been used to assess sense of agency in FMD by measuring patients’ action–effect binding. Compared with controls, patients with FMD showed increased perceived time between their actions and an effect, suggesting a decreased sense of control over their actions.
Several functional and structural abnormalities have consistently been present in patients with FNSD, especially in motor-processing regions and regions with dual motor- and emotion-processing functions. Compared with matched controls, patients with FNSD showed increased activity in the amygdala, supplementary motor area and periaqueductal grey matter (associated with the freeze response of fear) in response to negative emotions across several studies. Increased connectivity was demonstrated between the right amygdala and the right supplementary motor area when participants were presented with fearful and happy faces and in response to recall of stressful life events. This finding provides a potential mechanism by which certain stressors are associated with functional symptoms. Because of observed neural impairments in areas of the brain associated with emotional, perceptual and intentional awareness, Perez and colleagues suggested that patients with FNSD might experience a “neural functional unawareness,” which could also help conceptualize the brain–behaviour relationship in this disorder.

There are also some emerging functional and structural neuroimaging findings. Research has found abnormal functional connections in areas associated with cognitive control, behavioural inhibition and perceptual awareness. In patients with FNSD in response recall of stressful life events, enhanced activity has been found in the left dorsolateral prefrontal cortex, right supplementary motor area and temporo-parietal junction, and decreased activity in the left hippocampus. Evidence also suggests abnormal brain activity in areas regulating sensory integration (posterior parietal cortex and angular gyrus regions).

In terms of structural abnormalities, 1 study found no difference in insular volumes between patients with FNSD and controls. However, patients with FNSD who had self-reported severely impaired physical health had reduced left anterior insular grey matter volumes, and patients with FNSD participants who had self-reported severely impaired mental health had greater volumes of posterior-lateral cerebellar grey matter than controls. Two studies have demonstrated decreased grey-matter volumes in the thalamus and basal ganglia in patients with FNSD. Further, Labate and colleagues found abnormal cortical atrophy in the right motor and premotor areas and the right and left cerebellum in patients with PNES. Structural abnormalities have also been found in children and adolescents with FNSD, demonstrating greater volume in the left supplementary motor area, right superior temporal gyrus and dorsomedial prefrontal cortex.

It is important to note that because these findings rely on cross-sectional designs, it is unclear whether these structural and functional abnormalities are the cause of functional symptoms or a consequence of FNSD. However, some data suggest that these findings are the result of experience-dependent neuroplasticity of the brain, or the brain’s ability to change in response to the environment or learning. Labate and colleagues demonstrated that higher depression scores were associated with decreased grey matter in the premotor regions, and Aybek and colleagues found a trend for an association between greater grey matter in supplementary motor regions and duration of FNSD, and for an association between increased grey matter in the left premotor cortex and symptom severity. Additionally, Aybek and colleagues demonstrated that higher sexual abuse rates were associated with a weakened objective response to stress in patients with FNSD. The experience-dependent neuroplasticity explanation is also consistent with research in children, which found that greater supplementary motor area volumes were associated with faster emotion-identification reaction time. Unlike in adults, no differences have been displayed in the basal ganglia, thalamus or cerebellum of children and adolescents with FNSD, suggesting that decreases in grey matter in these areas could be due to the duration of FNSD symptoms. However, additional longitudinal neuroimaging data are needed to determine which effects are the result of an experience-dependent neuroplasticity reaction to FNSD symptoms; an experience-dependent neuroplasticity response to adverse life events; and/or a genetic predisposition to reacting to stress with functional neurological symptoms.

Reinforcing factors

In addition to predisposing factors, two other factors may reinforce FNSD (Table 2).

Sick role

The sick role is the acceptance of illness by the patient, and it is governed by certain social expectations, including not being responsible for one’s condition and exemption from normal social responsibilities. As noted above, expectations and beliefs about illness have been found to influence FNSD symptoms. Therefore, expectations associated with the sick role may increase FNSD symptoms.

Many studies have found evidence of the sick role in patients with FNSD. One found that only 20% of patients with PNES were employed by the time of referral for electroencephalography. Receipt of health benefits significantly increases after PNES diagnosis, and patients with PNES are more likely to receive benefits than patients with epilepsy. Research has found that patients with FNSD who worked were more than 5 times more likely to become symptom-free. Another study found that patients adopted the sick role as an important part of their identity, and patients with FNSD avoided normal social interactions. However, some studies have found contradictory

Table 2: Overview of FNSD reinforcing factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>Supporting evidence</th>
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</thead>
<tbody>
<tr>
<td>Sick role</td>
<td>Exemption from normal social responsibilities</td>
</tr>
<tr>
<td></td>
<td>Search to be healed</td>
</tr>
<tr>
<td></td>
<td>Receipt of disability benefits</td>
</tr>
<tr>
<td></td>
<td>Less likely to live independently</td>
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<tr>
<td></td>
<td>High rates of unemployment</td>
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<tr>
<td>Secondary gain</td>
<td>Receipt of disability benefits</td>
</tr>
<tr>
<td></td>
<td>Relief of stress and pressure associated with employment or school</td>
</tr>
<tr>
<td></td>
<td>Increased attention from others</td>
</tr>
</tbody>
</table>

FNSD = functional neurological symptom disorder.
evidence about the sick role in patients with FNSD. One found a decrease in general health care utilization,77 and another found that health care costs decreased 12 months after diagnosis.78 While the sick role may not be present for all patients with FNSD, it may reinforce symptoms in some.

Secondary gain

Once FNSD has developed, patients may experience secondary gain, an intrinsic or extrinsic benefit that reinforces and maintains FNSD. Traditionally, secondary gain has been described as an etiological factor for FNSD from a psychodynamic perspective, serving as an unconscious attempt to escape unwanted psychological distress.79 The concept of secondary gain as a causal mechanism is contradicted by the absence of stressors before the onset of FNSD in many patients,80,81 but there is evidence that it may reinforce symptoms or provide a disincentive for symptom resolution in some patients.82 It has been suggested that PNES are maintained by operant conditioning through both positive and negative reinforcement, such as the release from stress associated with employment or increased attention from family or friends,80,81 or the receipt of disability benefits.82

Proposed etiological models

Although traditional etiological understanding of FNSD relied simply on the psychodynamic explanation of a physical manifestation of psychological distress as the cause of the disorder, recent etiological models have acknowledged the heterogeneity of patients with FNSD. Several cognitive and neurobiological etiological models have been proposed for medically unexplained symptoms, PNES and FNSD.

Brown and Reuber recently proposed a model that provides an integrated behavioural and psychological etiological explanation.15,16 This model is based on ideas from Brown’s cognitive model of unexplained illness, in which misinterpretation of physical symptoms is affected by “rogue representations,” or information in the cognitive system about the cause of physical symptoms, which can be attained through personal experience, the observation of others’ experiences or sociocultural influence about health.14 Similarly, in their cognitive conceptual model for PNES, Brown and Reuber described the “seizure scaffold” as the central feature of PNES. The seizure scaffold is described as automatic activation of seizure behaviour from memory, occurring during autonomic arousal as a result of threat-processing.15,16

Voon and colleagues18 have proposed a neurobiological model in which FNSD occurs because of a combination of increased emotional arousal in the amygdala at symptom onset and a “previously mapped conversion motor representation,” possibly as a result of a prior physical precipitating event. They suggest that the “previously mapped conversion motor representation” is triggered and cannot be inhibited due to abnormal functional connectivity between the limbic structures and the supplementary motor area and higher activity in the right amygdala, left anterior insula and bilateral posterior cingulate.64

In another neurobiological model for FNSD, Edwards and colleagues17 have proposed a Bayesian account for FNSD. They suggest that functional symptoms are the result of actions based on inferences. These inferences are mediated by expectations about symptoms, past emotional and illness experiences and body-focused attention. Functional symptoms are the result of failures of inference occurring outside of conscious control.17

Based on their work with children and adolescents, Kozlowska and colleagues83 hypothesized a model of PNES based on Janet’s dissociation model.84 Their model suggests that a range of dissociative brain processes become triggered in response to cortical arousal, resulting in abnormalities in brain function and connectivity. Cortical arousal can be the result of illness, injury, emotional distress or trauma. This model proposes that in response to cortical arousal, the brain shifts into a defensive state in which behaviour becomes reflexive rather than voluntary. The authors suggest that FNSD is the result of this defensive state, in which the basal ganglia, midbrain and brain engage in reflexive behaviours.85

Integrated etiological summary model

The etiology of FNSD is complex and often results from a combination of factors that vary by the person. Patients and health care providers often report frustration and confusion about FNSD, which can impede accurate and timely diagnosis and hinder development of effective treatments. To integrate the recent research and current cognitive and neurobiological etiological models reviewed above, and to summarize the findings in a way that is easily comprehensible to patients, we describe the integrated etiological summary model for FNSD, which allows for the heterogeneity observed among patients and proposes a causal mechanism.

Figure 1 illustrates the sequence of events that is suggested to occur in the establishment and maintenance of FNSD.

Internal/external predisposing factors

Because of heterogeneity among patients with FNSD, it is likely that many combinations of predisposing factors yield similar functional symptoms. Some patients with FNSD may have a history of trauma or be predisposed to have anxiety and/or increased physical symptoms.30,37−39,41,50 Others may experience increased physiologic arousal without subjective reports of anxiety,79 but some may have no history of anxiety or psychiatric comorbidities. Many patients with FNSD experience functional symptoms after a physical injury or other neurological disease.12,42−46 As suggested by Kozlowska and colleagues,83 these factors may cause experience-dependent neuroplastic structural and functional changes in the brain, or epigenetic changes that may increase the risk of developing FNSD.

Model pathway

The onset of FNSD may be gradual or sudden. In patients with gradual onset, symptom presentation and duration are
progressive, worsening over time. As proposed in Clark’s cognitive model of panic, anxiety leads to physical symptoms. However, some patients with FNSD do not report anxiety about their symptoms. Instead, they may be predisposed to increased awareness or greater experiences of physical symptoms; these symptoms are then misinterpreted as a health problem. Misinterpretation of physical symptoms is influenced by a learned mental representation of physical symptoms created by a series of beliefs, expectations and motor activities formed through cultural beliefs, past injury, illness, experience and/or personal knowledge. These cognitive representations are referred to by Voon and colleagues as “previously mapped conversion motor representations,” by Brown as “rogue representations” for medically unexplained symptoms, and by Brown and Reuber as the “seizure scaffold” for PNES. As noted above, illness beliefs and expectations held by patients with FNSD may not be limited to exposure to neurological conditions or symptoms. Because this model is focused on the production of all functional neurological symptoms, it uses the broader term “health scaffold,” which encompasses all illness experiences, including personal illness, parental anxiety about the patient’s health as a child, illness of family or friends, job in a health profession, witnessed event of a stranger in public and cultural beliefs that certain symptoms are associated with a particular condition (e.g., forgetting one’s name is associated with dementia). Additionally, through news coverage of medical conditions and illnesses portrayed on television and movies, there is ample opportunity for illness exposure and health scaffold development. Intensified by the “jumping to conclusions” bias in some patients, a strong health scaffold increases sensitivity to even minor physical symptoms and reinforces beliefs that physical symptoms signify serious illness. Misinterpretation of symptoms as an illness then leads back to expectation of symptoms and/or anxiety, which produces additional physical symptoms and continues the cycle, further generating symptoms until it results in a symptom consistent with FNSD. In instances of sudden onset, the occurrence of symptoms may not cycle through the pathway as in gradual onset. Patients may have a significant trauma or injury, such as a car accident, and the associated physical symptoms are misinterpreted, resulting in the expectation and immediate onset of symptoms consistent with FNSD.

Fig. 1: The integrated biopsychosocial model for functional neurological symptom disorder. White represents predisposing factors, black represents the main model pathway and grey represents reinforcing factors.
Mechanism of action

We propose the mechanism by which FNSD is produced can be explained in the context of the placebo effect, which is the result of a combination of classical conditioning and explicit expectancies.86 Ivan Pavlov, who first discovered classical conditioning, was the first to propose it as a causal mechanism for FNSD.87 Classical conditioning occurs when an unconditioned stimulus is paired with a neutral stimulus until the neutral stimulus (then called the conditioned stimulus) elicits the reflexive unconditioned response in the absence of the unconditioned stimulus (then called the conditioned response). This is consistent with the high rate of precipitating physical events and the common co-occurrence of epilepsy and PNES. Classical conditioning may occur through repeated pairing of the unconditioned stimulus and the neutral stimulus or, if the event is sufficiently significant, through a single pairing. In a recent book chapter, Carson and colleagues suggested that the manifestation of FNSD may occur through single event of classical conditioning and be mediated by panic as the conditioned response.88 This suggestion is supported by evidence that many PNES first present as fainting in a social situation; the fainting causes anxiety, and subsequent occurrences are believed to be triggered by small fluctuations in emotion or neutral stimuli or mediated by panic.89,90 Multiple-exposure classical conditioning could explain the occurrence of FNSD in some patients, such as those with a personal or family history of neurological disorders. If the patient has repeatedly experienced seizures or been exposed to a family member’s seizures, neutral stimuli present during each of the experiences may produce a seizure-like conditioned response.

In addition to classical conditioning, negative symptom expectations have been found to directly modulate several neurochemical systems, resulting in increased symptoms.90 Expectation of a relationship between 2 stimuli can also result in classical conditioning without prior pairing of the stimuli. Dawson and Grings91 discovered that verbal information about a relationship between the unconditioned stimulus and neutral stimulus was sufficient to produce a conditioned response, consistent with high rates of personal illness experience and observed exposure to illness through family members or the media in patients with FNSD. Therefore, the health scaffold can contain classically conditioned neurological behavioural responses to neutral (conditioned) stimuli.

When a person experiences a normal physical symptom associated with their health scaffold, they misinterpret the symptom as a medical condition, and their expectation of symptoms and/or anxiety increases. If the symptom has been classically conditioned to a neurological behaviour in their health scaffold, it results in reflexive functional neurological behaviour. For example, someone with a history of epilepsy may have experienced headache (neutral stimulus) before their seizures (unconditioned response), so that headaches become a conditioned stimulus to seizure behaviour (conditioned response) and further develop their health scaffold. When the symptoms are experienced outside the context of an epileptic seizure, resulting in an expectation of symptoms, and functional seizure behaviour is automatically triggered.

While this mechanism explains the sudden onset of symptoms, many patients with FNSD experience a gradual onset. This can be explained through shaping, or the differential reinforcement of successive approximations.92 A physical symptom may trigger the health scaffold, resulting in the misinterpretation of the symptom as a health problem and leading to the expectation of symptoms and/or an increase in anxiety, but the symptom may not be conditioned to elicit a reflexive neurological response. However, expectation of symptoms has been found to increase reported experience of symptoms.93 Once the symptom recurs, the belief that the symptoms are due to a health problem is reinforced, which again results in expectation of symptoms and/or anxiety. As this cycle continues, most often on a preconscious level, symptoms are gradually shaped to become more frequent and prominent, producing additional symptoms. Once this results in a symptom that has been conditioned to a neurological response, functional neurological symptoms are triggered. This process of shaping may also be responsible for the gradual progression of functional symptoms and frequent co-occurrence of multiple functional symptoms.

Once FNSD symptoms are produced, new physical symptoms and situations occurring just before or simultaneously with the functional symptoms can be conditioned to trigger FNSD, consistent with Clark’s cognitive model of panic85 and Carson and colleagues’ suggestion that panic may mediate conditioning as the conditioned response.88 For example, heart rate increases during FNSD symptoms because of increased anxiety/panic associated with an episode can lead to FNSD symptoms (conditioned response) being triggered by an increase in heart rate (conditioned stimulus) when angry or while running.

This model is consistent with the Bayesian account of FNSD, in which it is posited that functional symptoms are the result of actions (conditioned response) based on failures of inference (misinterpretation of symptoms) from beliefs founded on prior experiences (health scaffold) and sensory evidence (physical symptoms).17

Reinforcing factors

After the onset of FNSD, the sick role and secondary gain reinforce and maintain FNSD symptoms. Reinforcers of the sick role include staying home, abstaining from responsibilities, family members acting as caregivers and repeatedly going to the emergency department.

Secondary gain reinforces symptoms through operant conditioning93 by following the symptoms with a rewarding response. This includes increased attention from family and friends (positive reinforcement) and decreased aversive responsibilities, such as attending work (negative reinforcement). The sick role and secondary gain overlap, but are reinforcing in different ways. For example, staying home from work reinforces a person’s belief that they are sick by providing cognitive support for the expectation that they are exempt from normal social responsibilities and also negatively reinforces symptoms by removing work stress.
Reinforcing factors maintain episodic functional symptoms but also contribute significantly to symptoms occurring constantly, such as anesthesia or paralysis. After the initial onset of symptoms, expectation of continued symptoms, acceptance of the sick role and secondary gain work in combination to maintain FNSD. For example, once paralysis begins, the patient develops an expectation of being paralyzed every day. Upon waking, they expect paralysis to continue, and symptoms are maintained. This is reinforced by staying home from work and receiving help from family members, which is rewarding. Figure 2 uses a real-life case example to demonstrate the clinical application of the integrated etiological summary model.

Extinction and relapse

Finally, this model also accounts for common relapse of functional neurological symptoms after remission.94 When a conditioned response is extinguished, the response is not unlearned. Instead, new learning occurs that is stored with previous learning, resulting in 2 responses for the same stimulus, and the resulting response is determined by the context of the situation, such as environment, mood or time. Therefore, the extinguished behaviour can relapse given a certain context.93

Supporting neurobiological evidence

In support of this model, the findings of several neuroimaging studies are consistent with the concept of placebo effect and classical conditioning. The automatic reflexive response produced through classical conditioning is consistent with the decreased sense of agency for symptoms. Because of abnormal functional connectivity found between the limbic structures and motor areas and higher activity in the amygdala, insula and cingulate, the “previously mapped conversion motor representation” is triggered and cannot be inhibited (conditioned response within the health scaffold).18

Fig. 2: Integrated biopsychosocial model, case example. A 23-year-old woman with history of sinus node dysfunction, syncopal spells from documented sinus arrest and a history of childhood sexual abuse, for which she has previously sought psychological treatment. She had a pacemaker implanted to treat her medical diagnosis, which resolved her syncopal episodes. However, new episodes began the day after the pacemaker implantation, with symptoms similar to those she had experienced before, such as dizziness, but without full loss of consciousness. Her episodes occurred at the same time of day and in the same location as before. She was referred for neurological assessment, which demonstrated normal sinus rhythms and no EEG changes during the events, and she was diagnosed with PNES. White represents predisposing factors, black represents the main model pathway and grey represents reinforcing factors. EEG = electroencephalography; PNES = psychogenic nonepileptic seizures.
It is also interesting to note that the same areas of the brain with increased activation in this model are associated with classical conditioning. Several other studies also demonstrate similar structural and functional patterns between classical conditioning and FNSD. For example, increased stress has been found to increase the acquisition and consolidation of classically conditioned responses in animals and humans. Specifically, noradrenaline release associated with stress facilitates the acquisition of fear conditioning, while glucocorticoids facilitate the consolidation of classical conditioning. This may explain why there are high rates of patients with FNSD and a history of stress and trauma. As well, greater cerebellar volume is associated with higher levels of classical conditioning, which could explain the association demonstrated by Perez and colleagues between greater self-reported severely impaired mental health and greater volumes of posterior-lateral cerebellar grey matter in patients with FNSD. Research assessing pain-related classical conditioning has demonstrated that an anticipation of a classically conditioned response and placebo effects due to expectations after verbal suggestion result in increased activation in areas of the brain related to attentional (posterior cingulate, anterior cingulate) and emotional (amygdala, hippocampus) processing, similar to the increased activation found in patients with FNSD. Specifically, increased amygdala activity has been consistently demonstrated in FNSD, and the amygdala is the gate for the physiologic expression of classically conditioned behaviour.

Conclusion

The etiology and maintenance of FNSD result from a variety of precipitating and reinforcing factors, and insufficient etiological understanding by physicians and patients impedes diagnosis and treatment. Although no model may be able to capture the etiological pathway for all patients with FNSD, our summary model presented here integrates current research into FNSD from various fields and recent etiological models. We have also proposed the placebo effect as the mechanism of action and emphasized how much of the research on the placebo effect and classical conditioning is consistent with the neurobiological evidence for the production and maintenance of FNSD. While others have discussed classical conditioning as a causal mechanism, this paper expands the explanation of research on the placebo effect and classical conditioning to account for additional aspects of FNSD, including its consistency with patients’ decreased sense of agency and other structural and functional neuroimaging findings; the shaping of symptoms over time; episodic and continuous symptoms; positive symptoms (tremors) and negative symptoms (paralysis); and the high rate of relapse of symptoms over time (extinction and spontaneous recovery).

This model poses multiple testable research hypotheses. The overlap between the neurobiological evidence for FNSD and classical conditioning provides ample opportunity for additional research. There are also many evidence-based interventions that address etiological factors related to classical conditioning, such as exposure and response prevention for anxiety, which will provide a foundation to inform treatment development for FNSD. Future studies can assess the overlap between brain function during classical conditioning and FNSD, measure whether symptoms are shaped to evolve over time, and evaluate patients’ acceptance of the diagnosis based on this explanation. Research can also examine whether FNSD symptoms are altered by treatment that restores a sense of agency over symptoms or by using extinction techniques such as exposure with response prevention.

This summary model explains symptoms as automatic conditioned reflexes to certain stimuli, and psychiatric factors are noted as potentially influential but unnecessary for the development and maintenance of FNSD. Classical conditioning and the placebo effect are commonly understood concepts that do not have psychiatric connotations. Since patients often reject FNSD diagnosis and prefer a medical diagnosis over a psychiatric diagnosis, this model could provide physicians with an explanation that is more acceptable to patients, potentially increasing patients’ willingness to pursue treatment.

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Competing interests: None declared.

Contributors: A. Fobian conducted a literature review, integrated previous research, and outlined and described the summary model. L. Elliott also conducted the literature review. Both authors wrote and reviewed the article, approved the final version for publication, and can certify that no other individuals not listed as authors have made substantial contributions to the paper.

References


ERβ agonist alters RNA splicing factor expression and has a longer window of antidepressant effectiveness than estradiol after long-term ovariectomy

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Introduction
Depression is a leading cause of disease-related disability. The lifetime incidence of major depressive episodes in women is almost twice that of men, and it has been suggested that the higher prevalence of major depressive episodes in women is associated with female-specific reproductive events, such as perimenstrual changes, pregnancy, the postpartum period and menopause. The menopausal transition, for example, appears to represent a period in which some women might be more vulnerable to the development of new-onset or recurrent depressive symptoms and major depressive episodes, resulting in new-onset major depression in approximately 17% of women and minor depression in another 16%. The menopausal transition, for example, appears to represent a period in which some women might be more vulnerable to the development of new-onset or recurrent depressive symptoms and major depressive episodes, resulting in new-onset major depression in approximately 17% of women and minor depression in another 16%. In late ET, only diarylpropionitrile (an ERβ-specific agonist) achieved similar results — not E2 (an ERα and ERβ agonist) or propylpyrazolotriol (an ERα-specific agonist). Limitations: Our experimental paradigm mimicked early and late ET in the clinical setting, but the contribution of age and OVX might be difficult to distinguish. Conclusion: These findings suggest that ERβ alternative splicing and altered responses in the regulatory system for serotonin may mediate the antidepressant efficacy of ET associated with the timing of therapy initiation. It is likely that ERβ-specific ligands would be effective estrogen-based antidepressants late after the onset of menopause.
For example, E2 increased expression of TPH2, the primary isoform of TPH in the brain, specifically in the dorsal raphe of OVX rats. In addition, E2 administration is associated with reduced MAO-A expression in the dorsal raphe of OVX rats, and MAO-A levels are significantly increased in the midbrain during the menopausal transition and menopause in humans.

A pivotal issue emerging in the use of estrogen therapy (ET) is the timing of administration. Although beneficial associations between ET and reduced risk of depressive disorders have been reported in both animal and clinical studies, some clinical trials in late postmenopausal women have shown no effect of ET on depression and other anxiety symptoms. Available studies and our previous work suggest that there may be a critical window of effectiveness in the use of ET for mood improvement: early initiation of ET after cessation of ovarian function sustains the normal protective role of estrogen, but later ET initiation is ineffective or even harmful.

We know that E2 acts through a number of different estrogen receptors (ERs), including ERα, ERβ, and G-protein-coupled ER (also known as GRER or GPR30). The ERβ receptor is widely distributed in the brain, with especially high expression in the dorsal raphe, hippocampus and cortex, and it plays an important role in mediating the antidepressant effect of E2.

Its main splice variants, ERβ1 and ERβ2, are derived from the same gene transcript via pre-mRNA alternative splicing, a process during the co- or post-transcriptional stage that occurs in more than 90% of multi-exon human genes. Our previous work has shown a negative association between E2 effectiveness and the level of the dominant negative ERβ2, on increasing cell proliferation and decreasing depression-like behaviour in short- and long-term OVX rats. It suggests that alternative RNA splicing may account for the differential effects of ET shortly after cessation of ovarian function (the time that has been proposed as the starting point of a critical window of effectiveness for ET) and later after cessation of ovarian function (the time after which that window has closed).

The expression and biological function of the splicing factors are significantly changed in the homeostasis of sex hormones. We know that ERβ is involved primarily in mood and cognitive activities, and the antidepressant effects of E2 are mediated by ERβ. Our hypothesis is that the use of an ERβ-specific agonist to avoid the activation of ERα, which is involved in several cancer-related adverse effects of ET, will have a longer critical window of antidepressant effectiveness than E2, and will improve quality of life and reduce potential adverse effects in those who receive ET over the long term. In the current study, we attempted to gain evidence to support this hypothesis, exploring TPH2, MAO-A expression, immobility in behavioural tests and the expression of splicing factors as potential molecular mechanisms in response to E2 and ER-specific agonists in female rats after long-term OVX.

Methods

All studies were in compliance with University of Mississippi Medical Center institutional guidelines. Animal-use protocols were approved by the UMMC Institutional Animal Care and Use Committee and conformed with National Institutes of Health guidelines for the use of vertebrate animals.

Animals and groups

Female Sprague–Dawley rats (total n = 47; Harlan Laboratories, Inc.) were housed in pairs in a temperature- and humidity-controlled environment. They had free access to food and water and were kept on a 12 h light/dark cycle, with lights on at 6 am and lights off at 6 pm. The rats were randomly divided into 8 subgroups. We used the first 3 groups to assess the effect of early ET, the second 3 to assess the effect of late ET and the last 2 to assess the effect of ER-specific agonists in late ET: sham OVX + vehicle after 6 days (n = 6); OVX + vehicle after 6 days (n = 6); OVX + E2 after 6 days (n = 6); sham OVX + vehicle after 180 days (n = 9); OVX + vehicle after 180 days (n = 5); OVX + E2 after 180 days (n = 5); OVX + DPN after 180 days (n = 5); and OVX + PPT after 180 days (n = 5).

Bilateral ovariectomy or sham surgery was performed on rats when they were 9 months old, the age at which their estrus cycles are becoming irregular, as previously described. The rats were treated with E2 6 days post-OVX (equivalent to human early postmenopause [early ET]), or E2 or ER-specific agonists 180 days post-OVX (equivalent to 10–20 years postmenopause in humans [late ET]). This experimental design prioritized simulating a clinical setting, in which the primary interest was to compare the efficacy of ET close to the onset of menopause or later in menopause.

Treatments

We delivered E2 (30 µg/kg) or vehicle (corn oil) to OVX rats by subcutaneous injection once a day for 2 days, starting on day 7 or day 181 after surgery, to mimic the early or late initiation of ET in humans, respectively. In the last 2 groups of OVX rats, we also initiated treatments on day 181, with one group receiving the ERβ-specific agonist diarylpropionitrile (DPN; 100 µg/kg) and the other receiving the ERα-specific agonist propylpyrazoletriol (PPT; 100 µg/kg), both by subcutaneous injection. It has been reported that in behaviour tests measuring depression-like and anxiety-like behaviour, female rats perform best during proestrus, when estrogen levels are highest (about 40 pg/mL). We based the E2 dose used in the current study on our previous findings that 30 µg/kg E2 produced an antidepressant effect in OVX rats receiving early but not late ET. We have also shown that administration of the same dose produced 42 pg/g E2 in brain tissues (wet weight) and 44 pg/mL E2 in serum of OVX rats, similar to E2 levels during proestrus. We chose the doses of DPN and PPT because of their lower transcriptional activity than E2, their effectiveness at these doses has been demonstrated.

Statistical analysis

We analyzed the results from the polymerase chain reaction (PCR) array using RT2 Profiler PCR Array data analysis software, version 3.5, on the SABiosciences Web portal. We
assessed the statistical significance of the data from quantitative PCR, Western blot, immunoreactivity in immunohistochemistry, the forced swim test and the elevated plus maze using 1-way analysis of variance and a subsequent Bonferroni post hoc test to examine the effect of ovarian hormone changes in the early or late ET groups. We analyzed the normality of data distribution using a Levene test before the t test and analysis of variance. Differences were considered significant at \( p < 0.05 \).

**Results**

Estradiol showed no antidepressant effects and no effect on anxiety-related behaviours in female rats when it was initiated 180 days after OVX (late ET), but ER\( \beta \)-specific agonists did show these effects.

We tested the antidepressant and antianxiety effects of E2 and ER\( \beta \)-specific agonists using the forced swim test and the elevated plus maze, respectively, at the time points indicated in Figure 1A. In early ET, OVX significantly decreased swimming time on the forced swim test (\( p < 0.01 \)) and time in open arms (indicating anxiety reduction) in the elevated plus maze (\( p < 0.05 \)) compared with the sham groups; E2 treatment reversed these changes and significantly increased swimming time (\( p < 0.05 \)) and time in open arms (\( p < 0.02 \)) compared with OVX + vehicle (Fig. 1B, a and b). In late ET, we observed no significant difference between sham, OVX, OVX + E2, or OVX + PPT with respect to swimming time or time in open arms in the behavioural tests. Interestingly, OVX + DPN rats showed significantly increased swimming time (\( p < 0.05 \)) and time in open arms (\( p < 0.05 \)) compared to OVX + vehicle rats (Fig. 1B, c and d). Overall, E2 demonstrated a significant effect in early ET (\( F_{2,15} = 4.33, p < 0.05 \) for the forced swim test; \( F_{2,15} = 5.347, p = 0.01 \) for the elevated plus maze). The ER\( \beta \)-specific agonist DPN demonstrated a significant effect in late ET (\( F_{2,15} = 5.98, p < 0.01 \) for the forced swim test; \( F_{2,15} = 3.659, p < 0.05 \) for the elevated plus maze). Neither E2 nor PPT demonstrated effects in late ET.

**Early and late ET differentially regulated splicing factor expression profile**

Steroid hormones influence alternative splicing decisions; in turn, products from alternative splicing affect steroid hormone function. We have reported previously that OVX increased a dominant negative splicing isoform, ER\( \beta \)\( 2 \). To further understand the splicing factors involved in this process, we investigated the splicing factor expression profiles in OVX rats that received early and late ET. We used a customized RT2 Profiler PCR Array that contained the majority of genes known to regulate alternative splicing so we could analyze rat frontal cortex samples (a gene list in provided in Appendix 1, Table S1, available at jpn.ca/170199-a1).

In the resulting clustergram, most splicing factors from the heterogeneous nuclear ribonucleoprotein family, the arginine/serine-rich (SR) splicing factor family and the RNA binding motif proteins showed an expression pattern that appeared to be differentially regulated by early ET but not by late ET (Appendix 1, Fig. S1). Genes with significant fold changes were further validated using real-time qPCR. These included members of the SR protein family, SR splicing factor 7 (SRSF7), SR splicing factor 16 (SRSF16), zinc finger (CCCH type) RNA-binding motif SR2 (ZRSR2) and CTNNB1, a gene that regulates cell cycles. We observed only 1 peak in the melt curve analysis from each sample, suggesting unique PCR product and specificity of the primers for SFRS7, SFRS16, ZRSR2 and CTNNB1.

At 6 days post-OVX, expression of SFRS7 (\( p < 0.01 \)) and SFRS16 (\( p < 0.05 \)) was significantly increased compared with the sham groups, while expression of ZRSR2 (\( p < 0.05 \)) and CTNNB1 (\( p < 0.05 \)) was significantly decreased. When E2 treatment was initiated on day 7 after OVX, it reversed OVX-induced changes by significantly decreasing mRNA expression of SFRS7 (\( p < 0.05 \) v. OVX + vehicle) and SFRS16 (\( p < 0.01 \) v. OVX + vehicle; \( p < 0.05 \) v. sham + vehicle), as well as increasing expression of ZRSR2 (\( p < 0.05 \) v. OVX + vehicle) and CTNNB1 (\( p < 0.01 \) v. OVX + vehicle; \( p < 0.01 \) v. sham + vehicle; Fig. 2A, C, E and G).

At 180 days post-OVX, expression of SFRS7 (\( p < 0.05 \), ZRSR2 (\( p < 0.01 \)) and CTNNB1 (\( p < 0.05 \)) was significantly decreased compared with sham; we found no difference in SFRS16 expression. When E2 treatment was initiated on day 181 after OVX, it had no effect on OVX-induced reduction of SFRS7 or CTNNB1 mRNA (Fig. 2B and H), but it dramatically increased mRNA levels of SFRS16 (\( p < 0.01 \) v. OVX + vehicle; \( p < 0.01 \) v. sham + vehicle) and ZRSR2 (\( p < 0.01 \) v. OVX + vehicle; \( p < 0.05 \) v. sham + vehicle; Fig. 2D and F).

To examine the individual roles of ER\( \alpha \) and ER\( \beta \) in the regulation of splicing factors in late ET, we also used DPN and PPT (ER\( \beta \)- and ER\( \alpha \)-specific agonists, respectively; Fig. 2B, D, F, and H). Both DPN and PPT significantly increased SFRS7 mRNA expression (\( p < 0.01 \) v. OVX + vehicle for DPN; \( p < 0.05 \) v. OVX + vehicle for PPT), although it was still significantly lower than the sham group with PPT (\( p < 0.05 \) v. sham + vehicle); we observed no effect of DPN or PPT on SFRS16. For ZRSR2 and CTNNB1, we noted a divergence: DPN dramatically increased mRNA expression of these 2 genes (\( p < 0.01 \) v. OVX + vehicle; \( p < 0.05 \) v. OVX + vehicle), and PPT showed no effect. Gene expression values with the different treatments are summarized in Table 1. The statistical significance for SFRS7 was \( F_{2,15} = 6.79, p < 0.05 \) in early ET and \( F_{2,15} = 3.51, p < 0.05 \) in late ET; for SFRS16 was \( F_{2,15} = 14.61, p < 0.01 \) in early ET and \( F_{2,15} = 5.27, p < 0.01 \) in late ET; for ZRSR2 was \( F_{2,15} = 5.68, p < 0.05 \) in early ET and \( F_{2,15} = 12.34, p < 0.01 \) in early ET; and for CTNNB1 was \( F_{2,15} = 16.53, p < 0.01 \) in early ET and \( F_{2,15} = 3.41, p < 0.05 \) in late ET.

**E2 and ER\( \beta \)-specific agonists differentially regulated protein expression of 2 main ER\( \beta \) isoforms in leukocytes of OVX rats**

We have demonstrated that ER\( \beta \)2 expression in circulating leukocytes mirrors the expression profile in brain in OVX rats. To see the isoform expression pattern in circulating leukocytes, an easily obtainable clinical sample from humans, we examined the expression of ER\( \beta \) and ER\( \beta \)2 in rat leukocytes of the different treatment cohorts in both early and late
ET (Fig. 3). We found significant treatment effects on ERβ2 expression in both early ET ($F_{2,15} = 4.63, p < 0.05$) and late ET ($F_{3,35} = 5.6, p < 0.01$). Consistent with our previous findings, OVX significantly increased ERβ2 protein expression both 6 and 180 days after OVX ($p < 0.05$ for both) compared to sham at 6 and 180 days. Early, but not late, E2 administration

**Fig. 1:** E2 and ER-specific agonists regulate mobility and anxiety-like behaviours in OVX rats. (A) Experimental regimen. Female Sprague–Dawley rats were ovariectomized at day 0 (9 mo of age), when irregular estrous cycles usually begin in laboratory rodents. They were then separated into 2 treatment groups: early and late ET, with different durations of ovarian hormone deprivation (6 d and 180 d). This experimental paradigm mimicked a common clinical setting, in which perimenopausal women (about 50 yr of age) receive ET at different points postmenopause. In the early ET group, OVX rats were treated with either E2 or vehicle (corn oil) at day 7 (equal to 4 mo in humans). In the late ET group, OVX rats were treated with E2, DPN, PPT or vehicle at day 181 (equal to age > 11 yr in humans). After 2 days of treatment, rats were subjected to a forced swim test, and samples were collected on the following day. (B) Behavioural tests. The forced swim test (a, c) as an assessment of antidepressant activity and the elevated plus maze (b, d) as an assessment of anxiety-like behaviour were performed in rats treated with vehicle or E2 6 days post-OVX (a, b) or vehicle, E2, DPN or PPT 180 days post-OVX (c, d). Data were analyzed using 1-way ANOVA and a subsequent Bonferroni post hoc test, and are presented as mean ± SEM, $n = 6$ for early ET, $n = 5$ for late ET. #p < 0.05 v. sham + V; */p < 0.05 v. OVX + V.

ANOVA = analysis of variance; DPN = diarylpropionitrile; E2 = estradiol; ER = estrogen receptor; ET = estrogen therapy; FST = forced swim test; OVX = ovariectomy; PPT = propylpyrazoletriol; Sac = sacrifice; SEM = standard error of the mean; V = vehicle.
reversed OVX-induced elevation of ERβ2 expression ($p < 0.05$ v. OVX + vehicle; Fig. 3A and B). Activation of ERβ in late treatment with DPN decreased OVX-induced ERβ2 levels ($p < 0.01$ v. OVX + vehicle; $p < 0.05$ v. sham + vehicle) to levels that were similar to the sham group. In contrast, activa-
tion of ERα in late treatment with PPT had no effect on ERβ2 expression (Fig. 3B).

At 6 days post-OVX, ERβ protein expression was similar in the sham + vehicle, OVX + vehicle and OVX + E2 groups. At 180 days post-OVX, we observed a significant treatment

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**Fig. 2:** E2 and ER-specific agonists regulate mRNA expression of splicing factors. We measured SFRS7, SFRS16, ZRSR2 and CTNNB1 gene expression in frontal cortex using real-time qPCR with 18S rRNA expression as an internal control from rats receiving vehicle or E2 6 days post-OVX (A, C, E, G) or from rats receiving vehicle, E2, DPN or PPT 180 days post-OVX (B, D, F, H). Data were analyzed using 1-way ANOVA and a subsequent Bonferroni post hoc test, and are presented as mean ± SEM. # $p < 0.05$ v. sham + V; * $p < 0.05$ v. OVX + V.

ANOVA = analysis of variance; DPN = diarylpropionitrile; E2 = estradiol; ER = estrogen receptor; ET = estrogen therapy; OVX = ovariectomy; PPT = propylpyrazoletriol; qPCR = quantitative polymerase chain reaction; SEM = standard error of the mean; V = vehicle.
We observed treatment effects in both early and late ET. Band of TPH2 immunoreactivity at 56 kDa, consistent with late PPT treatment (Fig. 4B). We observed no effect.<ref>0.001). We observed no effect<ref> on ET increased TPH2 mRNA levels 3-fold (v. sham, <0.001). However, late DPN (0.001) and 4.1-fold (v. OVX, p<0.001) and OVX (p<0.001) further decreased it significantly (Fig. 3C and D).

By comparing ERβ2 and ERβ protein expression levels, we found a significant treatment effect for the ERβ2/ERβ ratio in early ET (F_2,15 = 5.07, p<0.05) and late ET (F_2,15 = 6.14, p<0.01). We found a persistently and significantly elevated ERβ2/ERβ ratio 6 days and 180 days post-OVX (p<0.05 and p<0.01, respectively). Early, but not late, E2 treatment reversed this elevation (p<0.05 v. OVX + vehicle). Among all ET groups, only DPN reduced the ERβ2/ERβ ratio (p<0.05 v. OVX + vehicle). In contrast, E2 and PPT failed to reduce the ERβ2/ERβ ratio: it was still significantly higher than the sham group (p<0.01 v. sham + vehicle for E2; p<0.01 v. sham + vehicle for PPT; Fig. 3E and F). These results indicate that ERβ alternative splicing is differentially modulated by OVX and ER ligands in early and late ET and that activation of ERβ successfully reduces ERβ2 expression in late ET, but E2 does not.

**E2 and ER-specific agonists regulated MAO-A protein expression in the midbrain of OVX rats**

Estradiol acts through ERβ to increase TPH2 expression, possibly by interaction with TPH2 promoter.<ref> In both early and late ET, we observed significant treatment effects on TPH2 mRNA expression (F_2,15 = 13.63, p<0.01 in early ET; F_2,15 = 15.23, p<0.01 in late ET; Fig. 4A and B). We found that TPH2 mRNA levels in the midbrain of OVX rats were significantly decreased compared with the sham group (p<0.01 in 6-day OVX; p<0.001 in 180-day OVX). Early, but not late, E2 treatment increased TPH2 mRNA levels 2.5-fold (v. sham, p<0.001) and 4.1-fold (v. OVX, p<0.001). However, late DPN treatment increased TPH2 mRNA levels 3-fold (v. sham, p<0.001) and 6-fold (v. OVX, p<0.001). We observed no effect with late PPT treatment (Fig. 4B).

In immunoprecipitation analysis, we observed a strong band of TPH2 immunoreactivity at 56 kDa, consistent with the TPH2-expressing SH-SY5Y cell line positive control<ref> (Fig. 4C). Such a band was absent in the negative control, where no antibody was used during immunoprecipitation. We observed treatment effects in both early and late ET (F_2,15 = 9.38, p<0.01 in early ET; F_2,15 = 4.87, p<0.05 in late ET; Fig. 4D and 4E). We found that OVX consistently decreased TPH2 protein expression (p<0.01 in early ET; p<0.05 in late ET); early, but not late, E2 treatment reversed this OVX-induced TPH2 reduction (p<0.01 v. OVX + vehicle). In late treatments, only activation of ERβ by DPN significantly increased TPH2 protein expression (p<0.01 v. OVX + vehicle) and restored it to levels similar to the sham group (Fig. 4D and 4E).

**Discussion**

We found that ET with an ERβ-specific agonist increased swimming time in a forced swim test, and time in open arms in an elevated plus maze task in female rats 180 days after OVX, whereas ET with E2 showed no effects. The
2 alternative RNA splicing components SFRS7 and SFRS16 were differentially expressed in the presence and absence of E2 between the mid-aged short-term and old-aged long-term OVX groups; 2 other genes, ZRSR2 and CTNNB1, showed similar responses regardless of age or length of OVX. These splicing factors may play a role in regulating the alternative splicing of ERβ that mediates the expression of TPH2 and MAO-A and ameliorates depressive and anxiety symptoms in OVX rats. Our findings suggest that E2 may regulate splicing factor expression in ovarian hormone deficiency in a time-dependent manner, possibly contributing to decreased OVX-induced ERβ2 elevation. After long-term ovarian hormone deficiency and aging, E2 treatment did not show effects on splicing factor expression similar to early ET in reducing ERβ2 levels and it was not as efficient as an antidepressant in late ET. We found that ERβ-specific agonists specifically activated ERβ to regulate the expression of components in the serotonin system and play an effective antidepressant and antianxiety role after long-term OVX in female rats, but E2 did not. Nevertheless, the precise underlying molecular mechanisms warrant further study.

**Fig. 3:** E2 and ER-specific agonists differentially regulate protein expression of 2 main ERβ isoforms in leukocytes of OVX rats. Leukocytes were extracted from whole blood, and proteins were extracted by RIPA buffer and sonication; total protein was separated by SDS–PAGE. We detected ERβ and ERβ2 protein expression using specific antibodies, with β-actin as the internal control. We compared protein levels of ERβ and ERβ2 in leukocytes from rats receiving vehicle or E2 6 days after OVX (A, C) and from rats receiving vehicle, E2, DPN or PPT 180 days after OVX (B, D). We compared ERβ2:ERβ protein expression ratio in early ET (E) and late ET (F) groups. Inserts in D and E clearly show the differences between each group. Data were analyzed using 1-way ANOVA and a subsequent Bonferroni post hoc test, and are presented as mean ± SEM of ROD; n = 5–6 for early ET, n = 4–9 for late ET. *p < 0.05 v. sham + V; **p < 0.05 v. OVX + V. ANOVA = analysis of variance; DPN = diarylpropionitrile; E2 = estradiol; ER = estrogen receptor; ET = estrogen therapy; OVX = ovariectomy; PPT = propylpyrazoletriol; RIPA = radioimmunoprecipitation assay; ROD = relative optical density; SDS–PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis; SEM = standard error of the mean; V = vehicle.
ER\(\beta\) alternative splicing regulation by splicing factors

This study showed large fold changes in the RNA profiles of SFRS7, SFRS16, ZRSR2 and CTNNB1 in response to OVX and ET. All 4 genes, highly conserved from rodent to human,\(^{46}\) are involved in many different aspects of general alternative splicing processes and play direct and indirect roles in regulating ER\(\beta\) alternative splicing. SFRS7, a classical SR protein, is constitutively expressed in cells and considered to be an activator of alternative splicing. We found 4 SFRS7 consensus binding sites (UCAACA) on intron 5, both upstream and downstream of the 54 bp nucleotide retention sequence in ER\(\beta\)2 pre-mRNA (NCBI database, Gene ID: 25149). SFRS16, also called CLK4-associating serine/arginine rich protein (CLASRP), encodes CLK4 protein. It has been reported that CLK4-induced phosphorylation of SR proteins subsequently enhances their splicing ability. ZRSR2 encodes an essential splicing factor associated with the U2 auxiliary factor heterodimer. It is required for the recognition of a functional 3SS of U2- and U12-type pre-mRNA, and plays a role in network interactions during spliceosome assembly.\(^{47}\) CTNNB1 encodes \(\beta\)-catenin protein, which regulates cell proliferation, synaptic plasticity and depression and cognitive function.\(^{48,49}\) Although \(\beta\)-catenin is not generally considered a splicing factor, it has been reported that it directly caused alternative splicing of ER\(\beta\) pre-mRNA in colon cancer cells by modulating a

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**Fig. 4:** E2 and ER-specific agonists regulate TPH2 expression in the dorsal raphe of OVX rats. We measured TPH2 mRNA expression in rat dorsal raphe using real-time qPCR with 18S rRNA expression as an internal control from rats receiving ET 6 days post-OVX (A) and 180 days post-OVX (B). We detected the expression of TPH2 protein in dorsal raphe using SDS–PAGE probed for TPH2 after immunoprecipitation (C). We used whole cell lysate from TPH2-expressing SH-SYSY neuroblastoma cells for positive controls. We used no primary antibody for the negative control in immunoprecipitation. We compared the optical density of TPH2 in rats receiving E2 6 d after OVX (D) or rats receiving E2, DPN or PPT 180 d after OVX (E). The data were analyzed using 1-way ANOVA and a subsequent Bonferroni post hoc test, and are presented as mean ± SEM; \(n = 6\) for early ET, \(n = 4–8\) for late ET. \# \(p < 0.05\) v. sham + V; * \(p < 0.05\) v. OVX + V. Ab = antibody; ANOVA = analysis of variance; DPN = diarylpropionitrile; E2 = estradiol; ER = estrogen receptor; ET = estrogen therapy; OD = optical density; OVX = ovariectomy; PC = positive control; PPT = propylpyrazoletriol; qPCR = quantitative polymerase chain reaction; SDS–PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis; SEM = standard error of the mean; TPH2 = tryptophan hydroxylase 2; V = vehicle.
ERβ in critical window of estrogen therapy

The splicing factors identified in the current study provide potential targets for future investigation to address novel mechanisms of ERβ splicing.

Regulation of TPH2 and MAO-A through ET

In brain, TPH2 is a rate-limiting enzyme for serotonin synthesis, and MAO-A is a major metabolic enzyme for serotonin degradation. Both are potential targets for estrogens to ameliorate depression. Estradiol has been reported to increase expression of TPH2, the primary isoform of TPH in the brain, specifically in the dorsal raphe of OVX rats. Furthermore, ERβ agonists specifically display dose-dependent efficacy in vivo in murine dorsal raphe assays for the induction of TPH mRNA associated with antidepressant-like effects. In ERβ knockout mice, there was a marked reduction in the expression of TPH in dorsal raphe. During major depressive episodes, MAO-A levels are elevated throughout

![Figure 5](image_url)

**Fig. 5:** E2 reduces OVX-induced oxidative stress and glial cell activation in rats (6 d post-OVX). (A) Representative immunofluorescence images of dorsal raphe of OVX female rats treated with vehicle, OVX + vehicle and OVX + E2. Nuclei were counterstained with DAPI (blue). Note the nuclear staining of NeuN (green), cytosol punctate staining of MAO-A (magenta) and glial cell–specific staining of GFAP (red). Also note the even distribution of the NeuN staining in the 3 conditions, while both MAO-A and GFAP expressions were increased in OVX rats. Scale bar = 50 µm. Arrows and arrowheads indicate the representative cells expressing MAO-A + NeuN and MAO-A + GFAP, respectively. (B) The immunoreactive intensities of MAO-A, GFAP and NeuN in the dorsal raphe of sham + vehicle, OVX + vehicle and OVX + E2 female rats, analyzed using 1-way ANOVA and a subsequent Bonferroni post hoc test. Note the significant increases of MAO-A ($F_{2,210} = 8.80, p < 0.001$) and GFAP ($F_{2,8082} = 11.71, p < 0.001$) expression in the dorsal raphe of OVX rats, but OVX did not change the expression of NeuN ($F_{2,2655} = 1.73, p = 0.18$); E2 significantly reduced OVX-induced MAO-A and GFAP expression. Although OVX did not change NeuN expression, E2 increased NeuN expression in OVX rats vs. sham + vehicle rats. ANOVA = analysis of variance; E2 = estradiol; MAO-A = monoamine oxidase A; OVX = ovariectomy; V = vehicle.
Fig. 6: E2 and ERα-specific agonist increased MAO-A and GFAP expression, and reduced NeuN expression in the dorsal raphe of OVX rats. ERβ-specific agonist ameliorated GFAP expression and maintained NeuN expression in the dorsal raphe of OVX rats (180 d post-OVX). (A) Representative immunofluorescence images of the dorsal raphe of OVX female rats treated with vehicle (sham + vehicle), OVX + vehicle, OVX + E2, OVX + DPN and OVX + PPT. Nuclei were counterstained with DAPI (blue). Note the nuclear staining of NeuN (green), cytosol punctate staining of MAO-A (magenta) and glial cell–specific staining of GFAP (red). Scale bar = 50 µm. Arrows and arrowheads indicate the representative cells expressing MAO-A + NeuN and MAO-A + GFAP, respectively. (B) We analyzed the immunoreactive intensities of MAO-A, GFAP and NeuN in the dorsal raphe of rats using 1-way ANOVA and a subsequent Bonferroni post hoc test. Note the significant increases of MAO-A (F_{4,5248} = 33.66, p < 0.001) and GFAP (F_{4,19808} = 11.88, p < 0.001) expression in OVX rats; however, OVX did not alter the expression of NeuN (F_{4,6953} = 4.95, p = 0.26). Both E2 and PPT increased MAO-A and GFAP (p < 0.001 and p < 0.05, respectively, for E2; p < 0.001 and p < 0.05, respectively, for PPT) v. OVX, but reduced NeuN expression (p < 0.001 for E2 and p < 0.06 for PPT); DPN ameliorated MAO-A and GFAP expression (p = 0.86 for MAO-A and p = 0.09 for GFAP) and maintained NeuN expression (p = 0.9) in rats 180 d after OVX. ANOVA = analysis of variance; DPN = diarylpropionitrile; E2 = estradiol; MAO-A = monoamine oxidase A; OVX = ovariectomy; PPT = propylpyrazoletriol.
grey-matter regions in the brain, including the midbrain. They are similarly elevated in high-risk states for major depressive episodes, including those associated with reduced estrogen levels, such as early postpartum, the menopausal transition and menopause. Reducing available MAO-A with antidepressants is effective, but MAO-A inhibitors have many interactions with other medications, so alternative strategies for reducing MAO-A levels need to be therapeutically strategic. Specifically related to this study, E2 administration has also been associated with reduced MAO-A expression in the dorsal raphe of OVX macaques. Collectively, these studies suggest that the antidepressant effect of E2 is mediated, at least in part, via specific activation of ERβ to increase TPH2 expression and decrease MAO-A expression in the dorsal raphe.

Manipulations that raise the release of extracellular serotonin are associated with therapeutic response in a number of illnesses, reducing symptomatic behaviours in perimenopause-related major depressive episodes, obsessive–compulsive disorder, anxiety disorders, anger and late luteal phase dysphoric disorder. Therefore, the increase of TPH2 and the reduction of MAO-A in early ET by E2 and late ET by DPN may have therapeutic potential for these conditions. Interestingly, antidepressants, including selective serotonin reuptake inhibitors and MAO-A inhibitors, have all demonstrated the generation of new neurons, as well as the differentiation of newly formed cells (normally with more GFAP) toward neuronal cells (containing NeuN) in hippocampus. The results for the mediation of NeuN and GFAP expression by early E2 and late ERβ agonist treatment in dorsal raphe may suggest a potential neuroprotective role or similar biological potential for other antidepressants in this brain region.

**ERβ-mediated therapy in long-term hormone deficiency**

Treatment with ERβ-specific agonist DPN has a longer window of antidepressant effectiveness than E2 after long-term OVX. We know that DPN has a 305-fold greater relative binding affinity to ERβ over ERα, and ERβ is highly expressed in brain regions such as raphe nuclei and substantia nigra, while ERα exhibits weak expression in those areas. Functions of ERα and ERβ are greatly overlapped in various tissues, but ERβ is primarily involved in mood and cognitive activity. In addition to mediating TPH2 expression, ERβ may underlie other antidepressant effects of E2. Indeed, ERβ agonists, but not ERα agonists, reduced depression-like behaviour in several behavioural tests when administered systemically to OVX rats. Such effects were absent in ERβ knockout mice.

The less selective agonists may not be as efficacious as the specific agonists, and their mechanism of action may be more complex. Recent studies have indicated that ethynyl estradiol (an agonist that binds to both ERα and ERβ but has an affinity that is 6 times higher for ERα than ERβ) at doses of 2.5 or 5.0 µg/rat reduced immobility 1 week after, but not 3 weeks after, OVX, even when E2 (1.25 µg/rat) was combined with citalopram (2.5 mg/kg, an antidepressant). However, other studies have reported that in combination with sertraline, E2 reduced immobility in rats 4 or 8 months post-OVX. Nevertheless, ERβ-specific agonists produced similar effects to E2 in rats after prolonged loss of ovarian hormones in early ET, potentially reducing the complexity generated by activating ERα, by increasing serotonin synthesis and reducing serotonin metabolism. Indeed, compared with ERβ, ERα is more localized in the reproductive system, such as the ovaries and uterus, so its activation may induce breast and uterine cancer. Although using progesterone replacement along with estrogens can reduce the risk of uterine cancer, progesterone diminishes the antidepressant effects of estrogens, and even exacerbates depressive symptoms. Therefore, ERβ-specific agonists may have a longer critical window of antidepressant effects than E2, with the potential for fewer complications.

**Limitations**

A limitation of our study is that the contribution of age and OVX was difficult to distinguish in the experimental paradigm. However, we accomplished our goal to investigate differential molecular and behaviour changes in clinically relevant early and late ET, and to identify the ERβ-specific agonist as an effective treatment after long-term ovariectomy.

**Conclusion**

Our study suggests potential mechanisms associated with the antidepressant efficacy of estrogen. The critical window of effectiveness for ERβ-specific therapy is longer than that for E2 in rats after long-term OVX, and ERβ-specific therapy may be a potential candidate for postmenopausal estrogen therapy with the potential for fewer complications. Further investigation is needed to understand how the splicing factors are modulated in different hormonal environments and how they alter the alternative splicing of target genes, including ERβ.

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**Competing interests:** J. Meyer reports grants from Janssen, outside the submitted work. In addition, he has patents on a brain marker and blood markers of MAO-A for predicting mood disorder and on a dietary supplement to prevent postpartum depression and sad mood during high MAO-A states. No other competing interests declared.
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Contributors: T. Mosley and J.M. Wang designed the study. X. Hou, S.O. Adeosun, X. Zhao, R. Hill, B. Zheng and R. Reddy acquired the data, while X. Hou, S.O. Adeosun, X. Zhao, R. Hill, B. Zheng, R. Reddy, X. Su, J. Meyer and J.M. Wang analyzed. X. Hou, S.O. Adeosun, X. Zhao, R. Hill and J.M. Wang 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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Psychoradiologic abnormalities of white matter in patients with bipolar disorder: diffusion tensor imaging studies using tract-based spatial statistics

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Background: An increasing number of psychoradiology studies that use tract-based spatial statistics (TBSS) of diffusion tensor imaging have reported abnormalities of white matter in patients with bipolar disorder; however, robust conclusions have proven elusive, especially considering some important clinical and demographic factors. In the present study, we performed a quantitative meta-analysis of TBSS studies to elucidate the most consistent white-matter abnormalities in patients with bipolar disorder. Methods: We conducted a systematic search up to May 2017 for all TBSS studies comparing fractional anisotropy (FA) between patients with bipolar disorder and healthy controls. We performed anisotropic effect size–signed differential mapping meta-analysis. Results: We identified a total of 22 data sets including 556 patients with bipolar disorder and 623 healthy controls. We found significant FA reductions in the genu and body of the corpus callosum in patients with bipolar disorder relative to healthy controls. No regions of increased FA were reported. In subgroup analyses, the FA reduction in the genu of the corpus callosum retained significance in patients with bipolar disorder type I, and the FA reduction in the body of the corpus callosum retained significance in euthymic patients with bipolar disorder. Meta-regression analysis revealed that the percentage of female patients was negatively correlated with reduced FA in the body of the corpus callosum. Limitations: Data acquisition, patient characteristics, and clinical variables in the included studies were heterogeneous. The small number of diffusion tensor imaging studies using TBSS in patients with bipolar disorder type II, as well as the lack of other clinical information, hindered the application of subgroup meta-analyses. Conclusion: Our study consistently identified decreased FA in the genu and body of the corpus callosum, suggesting that interhemispheric communication may be the connectivity most affected in patients with bipolar disorder.

Introduction

Bipolar disorder (BD) is a serious chronic disease characterized by the co-occurrence of manic and depressive symptoms, and it has a worldwide prevalence of approximately 1%. Two main forms are distinguished in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5): BD type I (BD I) is the classic manic-depressive disorder; a diagnosis of BD type II (BD II) requires at least 1 episode of major depression and 1 hypomanic episode. Bipolar disorder leads to severe impairments in work role function and a high lifetime suicide risk; thus, there is a pressing need for a better understanding of its neural basis. Magnetic resonance imaging has been a useful way to pursue this, and numerous cerebral morphological and functional abnormalities have been reported in patients with BD. In addition to structural and functional alterations in multiple brain regions, aberrant connectivity between regions seems to be crucial to the pathogenesis of BD. Diffusion tensor imaging (DTI), an important psychoradiology technique (https://radiopaedia.org/articles/psychoradiology), can probe the white-matter tracts that form the infrastructure of that connectivity. It maps the diffusion of water molecules, and the calculated quantity of fractional anisotropy (FA) contains information about the directionality and coherence of neuronal fibre tracts.

In studies of patients with BD, however, various regions showing both FA increases and decreases have been reported: for example in the prefrontal white matter, temporal white matter and cingulum bundle. Performing a meta-analysis is a powerful way to integrate the results from many studies in an unbiased way. Therefore, using voxel-based...
meta-analysis to detect common abnormalities in patients with BD from multiple original studies is of particular importance. To our knowledge, there have been 3 published voxel-wise whole-brain meta-analyses of pertinent DTI studies on BD.13-14 However, these reviews had some technical limitations. First, all 3 studies integrated both voxel-based analysis (VBA) and tract-based spatial statistics (TBSS) techniques to compare white-matter abnormalities between patients with BD and healthy controls. Although VBA and TBSS both explore whole-brain white-matter alterations, they have inherent methodological differences that make combined meta-analysis problematic. Voxel-based analysis is relatively straightforward and involves spatial normalization of high-resolution images from all studied participants to the same stereotactic space.15 The TBSS technique was specifically developed to analyze diffusion data, and it restricts analysis to the centre of major white-matter tracts by projecting each participant's FA data onto the mean skeleton, minimizing the misalignment problems that can arise from regular VBA16 and arguably making TBSS a more accurate technique for detecting white-matter abnormalities. Second, given the limited number of reported DTI studies, these previous meta-analyses were unable to consider the potentially different neural alterations in patients with BD I and BD II, or the influence of the mood state (manic, depressed or euthymic) during MRI scanning. Some of the inconsistencies in reported white-matter findings may be due to different selection of patient subtypes. For example, Versace and colleagues17 reported FA increases in the left uncinate fasciculus, left optic radiation and right anterohalamic radiation, and FA decreases in the right uncinate fasciculus in patients with BD I compared with healthy controls. Including patients with both BD I and BD II, Haller and colleagues18 reported FA decreases in the ventral part of the corpus callosum compared with healthy controls. Ambrosi and colleagues19 reported lower FA in the right inferior longitudinal fasciculus in patients with BD II compared with patients with BD I. These different results may reflect different neural pathophysiology in BD I and BD II, so it is important to identify their separate neurobiological markers. Now that more TBSS studies have reported results taking BD subtypes into consideration, the time is ripe to conduct an updated TBSS meta-analysis to explore how these factors influence white-matter microstructural abnormalities in patients with BD.

The first aim of this study was to conduct a meta-analysis to identify the most prominent and replicable white-matter microstructural abnormalities associated with BD. We used anisotropic effect size–signed differential mapping (AES-SDM), a recently developed meta-analytic technique with the potential to quantify the reproducibility of neuroimaging findings.20 This technique enables the results from individual studies to be weighted and controlled for multiple moderators, including demographics, clinical information and imaging factors. It has the additional advantage that all information from contributing studies is used in the same map, including positive, negative and null results. The AES-SDM technique has been successfully applied in other neuropsychiatric studies using TBSS, including major depressive disorder and attention-deficit/hyperactivity disorder.21,22 The second aim was to perform subgroup meta-analyses based on important clinical and demographic factors of BD. The third aim was to perform a multiple meta-regression analysis to explore the potential effects of demographic and clinical characteristics on white-matter microstructural differences, taking advantage of AES-SDM's ability to control for moderators.

Methods

Inclusion of studies

We conducted our meta-analysis according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.23 We searched PubMed, Web of Knowledge, MEDLINE, PsycINFO, ERIC, CINAHL, Google Scholar and Embase using the following keywords to identify relevant articles published up to May 2017: DTI < OR > diffusion tensor imaging < OR > TBSS < OR > tract-based spatial statistics < OR > fractional anisotropy; and bipolar disorder < OR > bipolar depression < OR > mania. We also manually checked the reference lists of the retrieved articles for additional relevant studies.

The inclusion criteria for the study selections were as follows: studies that compared whole-brain FA alteration between patients with BD (diagnosed according to DSM-IV criteria) and healthy controls; studies that used the TBSS approach for DTI data analysis; studies that reported Montreal Neurological Institute or Talairach coordinates of the whole brain contrast of FA; studies that were published in English in a peer-reviewed journal; and studies that used significance thresholds for data that were either corrected for multiple comparisons or uncorrected with spatial extent thresholds.

The exclusion criteria were as follows: studies that were case reports or reviews; studies that provided 3-dimensional coordinates in stereotactic space (Montreal Neurological Institute or Talairach) were unavailable; studies that reported fewer than 10 participants in either the BD or healthy control groups; studies in which the participants were explicitly recruited to have multiple combined axis I diagnoses; and the presence of sample overlap with other included studies of larger sample sizes.

Quality assessment and data extraction

Two authors (C.Y. and L.L.) independently searched the literature, assessed the quality of the retrieved articles and extracted and cross-checked the data. In cases of disagreement, another author mediated until consensus was obtained. We assessed study quality using a 12-point checklist (see Appendix 1, Table S1, available at jpn.ca/170221-a1) adapted from previous meta-analyses24 that considered not only the demographic and clinical characteristics of individual study participants (items 1 to 4), but also the specific imaging methodology (items 5 to 10) and the consistency of the conclusions and the results (items 11 and 12). Each item was given a score of 1, 0.5 or 0 to indicate whether the criteria were fully met, partially met or unfulfilled, respectively.

We extracted the following data from each selected study: the characteristics of the participants and their illness (sample
size, mean age of participants, sex, age at onset, illness duration, symptom severity, diagnosis type, drug status, mood states and comorbidities; magnetic resonance methodology (magnetic field strength, acquisition voxel size, number of diffusion directions and type of analysis); statistical methodology (statistical threshold and correction methods for multiple comparisons); and 3-dimensional coordinates (for voxel-level quantitative meta-analyses).

**Voxel-wise meta-analysis: AES-SDM**

For the voxel-wise meta-analysis of the selected studies to detect FA differences in white matter between patients with BD and healthy controls, we used AES-SDM (www.sdmproject.com). The AES-SDM technique uses effect sizes to combine reported peak coordinates that are extracted from databases with statistical parametric maps, and it recreates original maps of the effect size of FA difference in white matter between patients and controls. We performed the analysis as described in the AES-SDM tutorial and related publications. We used Mricron software (www.mricro.com/mricron/) to visualize AES-SDM maps overlaid on 3 subgroup analyses onto a high-resolution brain template generated by the International Consortium for Brain Mapping.

The AES-SDM methods are briefly summarized here but have been described in detail elsewhere. First, from each data set we extracted the peak coordinates of all white-matter differences at the whole-brain level, and the t values or z-scores of the regions. We converted z-scores for significant clusters straightforwardly to t statistics using an online converter. Studies reporting no group differences were also included. To avoid any potential bias toward liberally thresholded regions, we excluded the peaks that did not appear statistically significant at the whole brain level. Second, we recreated the peak coordinates for each study using a standard Montreal Neurological Institute map of the effect size of the group differences in FA by means of an anisotropic Gaussian kernel. We used a relatively wide full width at half maximum (20 mm) and DTI fractional anisotropy (TBSS) templates to control false-positive results. Findings from studies that reported no group difference were also recreated with effect-size maps, the difference being that all voxels in the effect-size group were estimated to have a null effect size. Third, we conducted standard meta-analysis to create a mean map via voxel-wise calculation of the random-effects mean of the study maps, taking into account sample size, intrastudy variance and between-study heterogeneity. The analytical parameters of the AES-SDM were as follows: voxel threshold \( p = 0.005 \), peak height threshold \( Z = 1.00 \) and cluster size threshold = 100 voxels.

**Jackknife sensitivity analysis and subgroup analysis**

To assess the robustness of the findings, we conducted a systematic whole-brain voxel-based jackknife analysis, in which we iteratively repeated the analysis, excluding 1 data set at a time to establish the extent to which the results could be replicated. If a brain region remained significant in all or most of the combinations of studies, we considered the finding to be highly replicable.

When the sample size was sufficient, we conducted sensitivity subgroup analyses to test the robustness of the statistically significant findings by excluding studies with potential confounds on a one-off basis. We performed subgroup analyses of patients with BD I or BD II; with psychotic or nonpsychotic symptoms; during euthymic, depressed or manic status; and of adult or pediatric patients compared with healthy controls separately if sufficient data sets were available. We also conducted a subgroup meta-analysis of studies with corrected results. We conducted jackknife sensitivity analysis for each subgroup result.

**Meta-regression analysis**

We performed meta-regression analyses using age, percentage of female patients, symptom severity (Hamilton Depression Rating Scale), age at onset and illness duration in each study as the independent variables. We could not study non-drug therapy status because of a lack of reported data. We could not explore symptom severity as rated by the Young Mania Rating Scale by meta-regression because there were only 6 data sets. The results were weighted by the square root of the sample size. To minimize the reporting of spurious relationships, we selected a more conservative threshold of \( p = 0.005 \) as used in previous studies, requiring abnormalities to be detected both in the slope and in one of the extremes of the regressor, and discarding findings in regions other than those detected in the main analyses. We displayed the main output for each variable in a map of the regression slope. Finally, we visually inspected regression plots to discard fittings that were obviously driven by too few studies.

**Analysis of heterogeneity and publication bias**

Heterogeneity refers to between-study variations. We conducted a between-study heterogeneity analysis of individual clusters using a random-effects model with Q statistics, with thresholds of \( p = 0.005 \), peak \( Z = 1.00 \) and a cluster extent of 10 voxels. Areas showing significant heterogeneity that also overlapped with the main findings were explored using meta-regression analyses to understand the sources of between-study variability. We also assessed publication bias by testing funnel plots using the Egger test via STATA (www.stata.cn), in which any result showing \( p < 0.05 \) was regarded as having significant publication bias.

**Fibre tracking**

We used DTIquery software (http://graphics.stanford.edu/projects/dti/) and an atlas of human white-matter anatomy to help identify the most probable white-matter tracts passing through the clusters of voxels that showed significant FA group difference. We used the sample data of a healthy 35-year-old male provided by the DTIquery software. We mapped the white-matter tracts using streamline tracking.
techniques and filtered them by tract length and a box-shaped region of interest centred on the coordinates of significant clusters.

Results

Included studies and sample characteristics

Systematic search of the databases yielded 883 English research papers. Of these, 18 whole-brain TBSS studies with 22 data sets met our inclusion criteria. Figure 1 shows the flow diagram of selection of studies. The included studies compared FA differences in white matter between 556 patients with BD (mean age 38.2 yr) and 623 healthy controls (mean age 39.3 yr). Four studies reported subgroup comparisons: 1 study compared drug-free patients with BD and patients treated with lithium alone with the same healthy control group; another study compared patients with BD (with and without a history of suicide attempts) with the same healthy control group; and 2 studies compared subgroups of patients with BD (BD I and BD II) with the same healthy control group. For these 4 studies, we treated each subgroup comparison as an independent data set.

The demographic and clinical characteristics of the samples are reported in Table 1. Of the 556 patients, 57 (10.3%) were in a manic state at the time of scanning, 162 (29.1%) were in a depressive state, and 232 (41.7%) were euthymic; the mood states of 105 (18.9%) were not reported. The patients included 411 with BD I, 87 with BD II and 58 whose BD subtype was not mentioned. A total of 165 patients (29.7%) were reported to have comorbidities, which included substance abuse, panic disorder, anxiety disorder, posttraumatic stress disorder, obsessive–compulsive disorder and attention-deficit/hyperactivity disorder, among others. Of the 18 DTI studies, 2 studies involved 36 adolescents with BD.

Pooled meta-analysis of all included studies

The pooled meta-analysis of the 22 data sets with 71 clusters revealed decreased FA in the genu of the corpus callosum and 2 clusters in the body of the corpus callosum in all patients with BD compared with healthy controls (Fig. 2 and

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**Fig. 1:** Flow diagram for the identification and exclusion of studies. ROI = region of interest; TBSS = tract-based spatial statistics.
Table 1: Demographic and clinical characteristics of the participants in the 18 studies (22 data sets) included in the meta-analysis (part 1 of 2)

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants, n (females)</th>
<th>Age, yr</th>
<th>Age at onset, yr</th>
<th>Illness duration, yr</th>
<th>Severity (scale type)</th>
<th>Diagnosis</th>
<th>Statistical threshold</th>
<th>Drug states</th>
<th>Mood states</th>
<th>Comorbidity*</th>
<th>Quality scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambrosi et al., BD I</td>
<td>25 (12) 50 (24)</td>
<td>48.6</td>
<td>48.3</td>
<td>NA</td>
<td>20.3</td>
<td>9.2 (HAMD)</td>
<td>BD I</td>
<td>p &lt; 0.008 (multiple comparison)</td>
<td>10 AD, 16 AP, 11 AE, 16 Li, 6 BDZ</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ambrosi et al., BD II</td>
<td>25 (12) 50 (24)</td>
<td>48.3</td>
<td>48.3</td>
<td>NA</td>
<td>23.9</td>
<td>14.5 (HAMD)</td>
<td>BD II</td>
<td>p &lt; 0.008 (multiple comparison)</td>
<td>6 AD, 12 AP, 14 AE, 10 Li, 13 BDZ</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Versace et al.</td>
<td>31 (20) 25 (14)</td>
<td>35.9</td>
<td>29.5</td>
<td>25.4</td>
<td>12.3</td>
<td>RBD 2.1, DBD 15.2 (HAMD)</td>
<td>BD I</td>
<td>p &lt; 0.05 (multiple comparison)</td>
<td>5 Li, 12 MS, 8 AP, 7 AD, 7 BDZ</td>
<td>17 euthymic, 14 depressed</td>
<td>NA</td>
</tr>
<tr>
<td>Kumar et al.</td>
<td>22 (7) 41 (11)</td>
<td>34.7</td>
<td>33.2</td>
<td>NA</td>
<td>10.7</td>
<td>8.57 (SSPI)</td>
<td>BD</td>
<td>$p_{	ext{FWE}} &lt; 0.05$</td>
<td>55 on medications, including AP and MS</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Ambrosi et al., BD III</td>
<td>15 (NA)</td>
<td>67.9</td>
<td>68.3</td>
<td>34.1</td>
<td>33.8</td>
<td>1.00 (YMRS)</td>
<td>BD</td>
<td>$p_{	ext{FWE}} &lt; 0.05$</td>
<td>10 MS, 3 AD, 6 BDZ, 4 NL</td>
<td>Euthymic</td>
<td>NA</td>
</tr>
<tr>
<td>Haller et al.</td>
<td>19 (14) 47 (22)</td>
<td>68.5</td>
<td>69.8</td>
<td>39.4</td>
<td>29.2</td>
<td>0.95 (YMRS)</td>
<td>BD I</td>
<td>$p_{	ext{FWE}} &lt; 0.05$</td>
<td>15 MS, 5 AP, 7 BDZ, 5 NL, 3 UM</td>
<td>Euthymic</td>
<td>NA</td>
</tr>
<tr>
<td>Benedetti et al., FREE</td>
<td>26 (17) 21 (10)</td>
<td>45.1</td>
<td>39.9</td>
<td>31.6</td>
<td>13.5</td>
<td>20.1 (HAMD)</td>
<td>BD I</td>
<td>$p_{	ext{FWE}} &lt; 0.05$</td>
<td>26 UM</td>
<td>Depressed</td>
<td>NA</td>
</tr>
<tr>
<td>Benedetti et al., TREAT</td>
<td>14 (11) 21 (10)</td>
<td>47.8</td>
<td>39.9</td>
<td>31.1</td>
<td>17.6</td>
<td>19.1 (HAMD)</td>
<td>BD I</td>
<td>$p_{	ext{FWE}} &lt; 0.05$</td>
<td>14 Li</td>
<td>Depressed</td>
<td>NA</td>
</tr>
<tr>
<td>Chan et al.</td>
<td>16 (4) 16 (4)</td>
<td>36.9</td>
<td>37.3</td>
<td>NA</td>
<td>0.2</td>
<td>3.8 (YMRS)</td>
<td>BD I</td>
<td>$p &lt; 0.001$ (uncorrected)</td>
<td>6 Li, 7 MS (sodium valproate), 12 AP</td>
<td>Euthymic</td>
<td>NA</td>
</tr>
<tr>
<td>Lagopoulos et al.</td>
<td>58 (41) 40 (24)</td>
<td>23.0</td>
<td>24.1</td>
<td>15.4</td>
<td>7.5</td>
<td>13.4 (HAMD)</td>
<td>BD I, BD II, spectrum</td>
<td>$p_{	ext{FWE}} &lt; 0.05$</td>
<td>25 AD, 23 MS, 37 AP, 8 UM</td>
<td>Depressed</td>
<td>NA</td>
</tr>
<tr>
<td>Mahon et al., SU</td>
<td>14 (5) 15 (7)</td>
<td>33.3</td>
<td>33.7</td>
<td>20.8</td>
<td>12.5</td>
<td>6.8 (HAMD)</td>
<td>BD I</td>
<td>$p_{	ext{FWE}} &lt; 0.05$</td>
<td>10 MS, 6 AP, 3 UM</td>
<td>NA</td>
<td>Yes</td>
</tr>
<tr>
<td>Mahon et al., NSU</td>
<td>15 (6) 15 (7)</td>
<td>36.5</td>
<td>33.7</td>
<td>24.8</td>
<td>11.7</td>
<td>5.4 (HAMD)</td>
<td>BD I</td>
<td>$p_{	ext{FWE}} &lt; 0.05$</td>
<td>14 AP, 14 MS</td>
<td>NA</td>
<td>Yes</td>
</tr>
<tr>
<td>Sprooten et al.</td>
<td>64 (46) 46 (31)</td>
<td>31.7</td>
<td>30.1</td>
<td>18.0</td>
<td>10.0</td>
<td>2.0 (HAMD)</td>
<td>BD I</td>
<td>$p_{	ext{FWE}} &lt; 0.05$</td>
<td>34 MS, 28 AD, 23 AP, 12 Li, 11 UM</td>
<td>Euthymic</td>
<td>Yes</td>
</tr>
<tr>
<td>Versace et al.</td>
<td>15 (14) 24 (15)</td>
<td>36.3</td>
<td>27.7</td>
<td>21.6</td>
<td>14.7</td>
<td>14.9 (HAMD)</td>
<td>BD I</td>
<td>$p_{	ext{FWE}} &lt; 0.05$</td>
<td>10 MS, 10 AP, 8 AP, 6 BDZ</td>
<td>Depressed</td>
<td>NA</td>
</tr>
<tr>
<td>Wessa et al.</td>
<td>22 (11) 21 (9)</td>
<td>45.4</td>
<td>43.0</td>
<td>23.0</td>
<td>22.0</td>
<td>0.1 (HAMD)</td>
<td>BD I, BD II</td>
<td>$p_{	ext{FWE}} &lt; 0.05$</td>
<td>10 Li, 11 AC, 6 AD, 5 AP, 4 UM</td>
<td>Euthymic</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Table 1: Demographic and clinical characteristics of the participants in the 18 studies (22 data sets) included in the meta-analysis (part 2 of 2)

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants, n (females)</th>
<th>Age, yr</th>
<th>Age at onset, yr</th>
<th>Illness duration, yr</th>
<th>Severity (scale type)</th>
<th>Diagnosis</th>
<th>Statistical threshold</th>
<th>Drug states</th>
<th>Mood states</th>
<th>Comorbidity*</th>
<th>Quality scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liu et al., BD I</td>
<td>14 (7)</td>
<td>21 (13)</td>
<td>35.6</td>
<td>7.3</td>
<td>6.7 (HAMD)</td>
<td>BD I</td>
<td>$p &lt; 0.001$ (uncorrected)</td>
<td>2 Li, 12 AC, 12 AD, 1 AP</td>
<td>7 manic, 7 depressed</td>
<td>NA</td>
<td>11</td>
</tr>
<tr>
<td>Liu et al., BD II</td>
<td>13 (11)</td>
<td>21 (13)</td>
<td>35.1</td>
<td>9.4</td>
<td>9.5 (HAMD)</td>
<td>BD II</td>
<td>$p &lt; 0.001$ (uncorrected)</td>
<td>10 AC, 10 AD, 1 AP</td>
<td>5 manic, 8 depressed</td>
<td>NA</td>
<td>11</td>
</tr>
<tr>
<td>Magioncalda et al.</td>
<td>61 (43)</td>
<td>42 (27)</td>
<td>44.6</td>
<td>25.1</td>
<td>19.6</td>
<td>BD I</td>
<td>$p_{TFCE} &lt; 0.01$</td>
<td>52 MS, 22 AD, 35 AP, 21 BDZ, 2 UM</td>
<td>21 manic, 20 depressed, 20 euthymic</td>
<td>NA</td>
<td>11.6</td>
</tr>
<tr>
<td>Oertel-Knochel et al.</td>
<td>30 (14)</td>
<td>32 (16)</td>
<td>39.2</td>
<td>10.2</td>
<td>NA</td>
<td>BD I</td>
<td>$p_{TFCE} &lt; 0.01$</td>
<td>24 MS, 12 AD, 13 NL, 5 AL</td>
<td>Euthymic</td>
<td>NA</td>
<td>10.5</td>
</tr>
<tr>
<td>Gao et al.</td>
<td>18 (12)</td>
<td>18 (12)</td>
<td>15.1</td>
<td>13.8</td>
<td>1.3</td>
<td>BD I, BD II</td>
<td>$p_{TFCE} &lt; 0.05$</td>
<td>2 UM, 8 VP, 7 Li, 13 AP, 3 AD</td>
<td>Manic</td>
<td>Yes</td>
<td>11</td>
</tr>
<tr>
<td>Teixeira et al.</td>
<td>18 (6)</td>
<td>20 (6)</td>
<td>12.3</td>
<td>9.5</td>
<td>2.8</td>
<td>BD I, BD II</td>
<td>$p_{TFCE} &lt; 0.05$</td>
<td>All UM</td>
<td>6 manic, 8 euthymic</td>
<td>Yes</td>
<td>11</td>
</tr>
<tr>
<td>Haarmann et al.</td>
<td>21 (12)</td>
<td>22 (11)</td>
<td>44.7</td>
<td>19.5</td>
<td>25.2</td>
<td>BD I</td>
<td>$p_{TFCE} &lt; 0.05$</td>
<td>Medicated</td>
<td>Euthymic</td>
<td>No</td>
<td>11.5</td>
</tr>
</tbody>
</table>

AC = anticonvulsant; AD = antidepressant medication; AE = antiepileptic medication; AL = anxiolytics; AP = antipsychotic medication; BD = bipolar disorder; BDZ = benzodiazepine; DBP = depressed with bipolar disorder; FDR = false discovery rate; FWE = family-wise error corrected; HAMD = Hamilton Depression Rating Scale; HC = healthy controls; Li = lithium; MS = mood-stabilizing medication; NA = not available; NL = neuroleptics; NOS = not otherwise specified; RBD = remitted with bipolar disorder; SSPI = Signs and Symptoms in Psychotic Illness scale; TFCE = threshold-free cluster enhancement; UM = unmedicated; VP = valproate; YMRS = Young Mania Rating Scale.

*Comorbidity:
- Mahon et al., SU: substance abuse or dependence ($n = 3$), anorexia nervosa ($n = 1$), panic disorder with ($n = 1$) and without ($n = 1$) a history of agoraphobia, anxiety disorder NOS ($n = 2$), posttraumatic stress disorder ($n = 2$), generalized anxiety disorder ($n = 1$), specific phobia ($n = 1$) and bulimia nervosa ($n = 1$).
- Mahon et al., NSU: substance abuse or dependence ($n = 5$), obsessive–compulsive disorder ($n = 1$) and anorexia nervosa without a history of panic disorder ($n = 1$).
- Sprooten et al.: anxiety disorder ($n = 30$), alcohol use disorder ($n = 33$), substance use disorder ($n = 29$), nicotine dependence, current ($n = 21$).
- Gao et al.: anxiety ($n = 1$), attention-deficit hyperactivity disorder ($n = 1$), obsessive–compulsive disorder ($n = 2$).
- Teixeira et al.: attention-deficit/hyperactivity disorder ($n = 9$), oppositional defiant disorder ($n = 5$), generalized anxiety disorder ($n = 3$), conduct disorder ($n = 2$), simple phobia ($n = 2$), posttraumatic stress disorder ($n = 2$), panic disorder ($n = 1$), separation anxiety disorder ($n = 1$), obsessive–compulsive disorder ($n = 1$) and Tourette syndrome ($n = 1$).
- Wessa et al.: panic disorder ($n = 1$).
- FREE indicates that the study includes a subgroup of drug-free patients with BD. TREAT indicates that the study includes a subgroup of lithium-treated patients with BD. SU indicates that the study includes subgroup of patients with a previous suicide attempt. NSU indicates that the study includes subgroup of patients without a previous suicide attempt.
- Mahon et al., NSU: substance abuse or dependence ($n = 5$), obsessive–compulsive disorder ($n = 1$) and agoraphobia without a history of panic disorder ($n = 1$).

†FREE indicates that the study includes a subgroup of drug-free patients with BD. TREAT indicates that the study includes a subgroup of lithium-treated patients with BD. SU indicates that the study includes subgroup of patients with a previous suicide attempt. NSU indicates that the study includes subgroup of patients without a previous suicide attempt.

‡Children and adolescents were diagnosed as BD NOS if they presented with clear manic or hypomanic episodes without elation and/or grandiosity but lacked the duration needed to be classified as BD I or BD II.
Table 2). No regions with increased FA were found. The white-matter tracts running through these 3 regions provide interhemispheric connections to the prefrontal, orbitofrontal, inferior temporal and superior parietal cortices (Fig. 3). These results showed no significant between-study heterogeneity. Extraction of funnel plots revealed no publication bias. The Egger test showed no significant publication bias in the genu (Egger $p = 0.62$) or in the anterior (Egger $p = 0.99$) or posterior (Egger $p = 0.23$) parts of the body of the corpus callosum.

As shown in Table 3, the whole-brain jackknife sensitivity analysis of the pooled meta-analysis found that all 3 clusters were highly replicable. The most robust cluster was found in the left genu of the corpus callosum, which was preserved throughout all 22 combinations of the data sets. The other 2 results in the body of the corpus callosum remained significant in all but 2 and 7 combinations of the data sets, respectively.

Subgroup meta-analysis

The subgroup meta-analysis of the studies of patients with BD I included 13 data sets that compared 346 patients with BD I to 350 healthy controls. The FA in patients with BD I was decreased in the genu of the corpus callosum relative to that of healthy controls (Table 3). We were unable to conduct subgroup meta-analyses of the studies of patients with BD II compared with either healthy controls or patients with BD I because of the limited number of data sets.

The subgroup meta-analysis of euthymic patients with BD included 7 data sets that compared 187 euthymic patients with BD to 199 healthy controls. Relative to controls, patients in the euthymic state exhibited decreased FA in the body of the corpus callosum (Table 3). We were unable to conduct subgroup meta-analyses of manic or depressed patients compared with healthy controls because of the limited number of data sets.

Fig. 2: Results of pooled meta-analysis. Panel A shows the coronal and sagittal view showing decreased fractional anisotropy in patients with bipolar disorder versus healthy controls in the genu of corpus callosum (a1), the posterior of the body of corpus callosum (a2) and the anterior of the body of corpus callosum (a3). Panel B shows the most probable white-matter tracts passing through 3 clusters of voxels (−10, 28, 16; −18, −32, 32; and 16, −6, 36) in 3-dimensional images using DTIquery.
The subgroup meta-analysis of adult patients with BD (age > 18 yr) included 20 data sets that compared 520 adult patients with BD to 585 healthy controls. The adult patients had decreased FA in the genu and the body of the corpus callosum, sharing 2 clusters with the results of the pooled meta-analysis (Table 3). We were unable to conduct subgroup meta-analysis of adolescent patients because of a lack of data sets.

The subgroup meta-analysis of the studies with corrected results included 19 data sets that compared 513 patients with BD and 565 healthy controls. The results were consistent with the pooled meta-analysis (Appendix 1, Table S2), showing few effects of the uncorrected results on the overall conclusions.

Only 5 included studies \(^{34,37,38,43,44}\) reported white-matter alterations in patients with BD and psychosis. One study included only BD patients with psychosis, and the other 4 reported only the percentage of patients with psychosis and did no subgroup analysis. The limited number of studies prevented the detection of white-matter abnormalities when comparing patients with BD with or without psychosis.

The subgroup jackknife sensitivity analyses of bipolar subtypes, mood states and adult participants, as well as the corrected studies, found the meta-analysis results to be highly replicable when focusing only on the BD I, euthymic, adult and corrected subgroups.

**Meta-regression analysis**

The results of the meta-regression analysis showed that the percentage of female patients with BD driven by 21 data sets was negatively associated with FA reduction in the body of the corpus callosum (Fig. 3). However, the results should be interpreted with some caution, because they were seemingly driven by 2 outlier studies with very high site effects. \(^{37,38}\) We detected no effect of Hamilton Depression Rating Scale score, mean age, age at onset or illness duration on white-matter alterations.

<table>
<thead>
<tr>
<th>Region</th>
<th>MNI coordinates x, y, z</th>
<th>SDM Z value</th>
<th>p value</th>
<th>Number of voxels</th>
<th>Breakdown (number of voxels)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genu of corpus callosum</td>
<td>–10, 28, 16</td>
<td>–2.209</td>
<td>0</td>
<td>388</td>
<td>Corpus callosum (321) Left striatum (21)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Left anterior thalamic projections (20)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Left inferior fronto-occipital fasciculus (15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Left cingulum (11)</td>
</tr>
<tr>
<td>Body of corpus callosum</td>
<td>–18, –32, 32</td>
<td>–2.470</td>
<td>0</td>
<td>180</td>
<td>Corpus callosum (125) Left cingulum (43)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Left superior longitudinal fasciculus (12)</td>
</tr>
<tr>
<td>Body of corpus callosum</td>
<td>16, –6, 36</td>
<td>–1.303</td>
<td>&lt;0.001</td>
<td>127</td>
<td>Corpus callosum (112) Right anterior thalamic projections (15)</td>
</tr>
</tbody>
</table>

MNI = Montreal Neurological Institute; SDM = signed differential mapping.
The current study pooled the largest number of TBSS studies to date in patients with BD to conduct a quantitative meta-analysis. To the best of our knowledge, no similar work has been reported, especially regarding the clinical and demographic effects of BD subtypes, psychotic features, mood states, age and sex. Voxel-wise meta-analysis using AES-SDM revealed that patients with BD have decreased FA in the genu of the corpus callosum and in 2 clusters in the body of the corpus callosum; these results were robust under jackknife analysis. We found a significant negative association between the percentage of female patients and the FA in the body of the corpus callosum. Subgroup analyses of the BD I studies, euthymic studies and adult studies reproduced the significant findings of decreased FA in the genu and the body of the corpus callosum. These results suggest that abnormalities in white-matter tracts may be involved in the pathologic mechanisms of BD.

The corpus callosum is the largest white-matter bundle that connects the bilateral cerebral hemispheres, integrating emotional, cognitive, motor and sensory functions. We found decreased FA in the genu and body of the corpus callosum in patients with BD compared with healthy controls, a result that was consistent with those of 2 other DTI studies using a region-of-interest method focusing on the corpus callosum. This brain region has been increasingly implicated in patients with BD: for example, a multicentre structural MRI study reported a decrease in the mid-sagittal area of the body of the corpus callosum in patients with BD compared with healthy controls. It is tempting to speculate that impaired interhemispheric communication is important in the pathophysiology of BD. The recent meta-analysis by Wise and colleagues reported that patients with BD showed decreased FA in the left cingulum, the left genu of the corpus callosum and the right anterior superior longitudinal fasciculus compared with healthy controls. It is tempting to speculate that impaired interhemispheric communication is important in the pathophysiology of BD. The recent meta-analysis by Wise and colleagues reported that patients with BD showed decreased FA in the left cingulum, the left genu of the corpus callosum and the right anterior superior longitudinal fasciculus compared with healthy controls. Our findings are not completely consistent with those of previous meta-analyses, because we found no significant abnormalities in any tracts other than the corpus callosum. There are several possible reasons for this. First, previous meta-analyses included both TBSS and VBA studies, whereas we only included TBSS studies, because there is reason to believe that these can more accurately identify white-matter abnormalities. Second, we were able to include many more TBSS studies than the previous meta-analyses, increasing the precision of effect-size estimates.

Discussion

The current study pooled the largest number of TBSS studies to date in patients with BD to conduct a quantitative meta-analysis. To the best of our knowledge, no similar work has been reported, especially regarding the clinical and demographic effects of BD subtypes, psychotic features, mood states, age and sex. Voxel-wise meta-analysis using AES-SDM revealed that patients with BD have decreased FA in the genu of the corpus callosum and in 2 clusters in the body of the corpus callosum; these results were robust under jackknife analysis. We found a significant negative association between the percentage of female patients and the FA in the body of the corpus callosum. Subgroup analyses of the BD I studies, euthymic studies and adult studies reproduced the significant findings of decreased FA in the genu and the body of the corpus callosum. These results suggest that abnormalities in white-matter tracts may be involved in the pathologic mechanisms of BD.

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### Table 3: Results of jackknife analysis and subgroup analysis from 18 studies (22 data sets) included in the meta-analysis

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Region of FA reduction: genu of corpus callosum</th>
<th>Region of FA reduction: body of corpus callosum</th>
<th>Region of FA reduction: body of corpus callosum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jackknife sensitivity analysis</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Excluding Wessa et al.</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Excluding Versace et al.</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Excluding Chan et al.</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Excluding Benedetti et al., FREE</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Excluding Benedetti et al., TREAT</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Excluding Haller et al.</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Excluding Delaloye et al.</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Excluding Mahon et al., SUJ</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Excluding Mahon et al., NSU</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Excluding Gao et al.</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Excluding Sprooten et al.</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Excluding Gertel-Knochel et al.</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Excluding Lagopoulos et al.</td>
<td>Yes</td>
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<td>No</td>
</tr>
<tr>
<td>Excluding Teixeira et al.</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Excluding Ambrosi et al., BD</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Excluding Ambrosi et al., BD II</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
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<td>Excluding Magioncalda et al.</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
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<td>Excluding Versace et al.</td>
<td>Yes</td>
<td>Yes</td>
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</tr>
<tr>
<td>Excluding Kumar et al.</td>
<td>Yes</td>
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<td>Yes</td>
</tr>
<tr>
<td>Excluding Liu et al., BD</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Excluding Liu et al., BD II</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Excluding Haarman et al.</td>
<td>Yes</td>
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<td>Yes</td>
</tr>
</tbody>
</table>

Subgroup analysis

- Studies with adult BD: Yes, Yes, No
- Studies with BD I: Yes, No, No
- Studies with euthymic-state BD: No, No, No

BD = bipolar disorder; FA = fractional anisotropy.

**Yes** indicates that the specific region of FA reduction was significant in the specific jackknife analysis and subgroup analysis; **No** indicates that the specific region of FA reduction was not significant in specific analysis.
Third, the clinical samples in our included studies differed in detail from those in previous meta-analyses.

The white-matter fibres passing through the genu of the corpus callosum connect the bilateral prefrontal cortices, which are known to play a role in decision-making, attention, reward-processing and emotion regulation.52,53 Previous structural studies found decreased volume in both the ventral and dorsal prefrontal cortex in patients with BD,54,55 and several functional studies have observed decreased dorsal and ventral prefrontal activity in patients with BD during language tasks and executive-related tasks.56,57 Additionally, several studies have reported that the ventral prefrontal cortex is implicated in the “top–down” regulation of emotional processing in patients with BD.58,59 The FA reduction in the genu of the corpus callosum in the present study suggests impaired prefrontal interhemispheric connectivity, perhaps leading to neurocognitive deficits in processing speed and working memory,60 for example, and to emotional dysregulation in patients with BD. Our subgroup analysis of patients with BD I reproduced the finding of decreased FA in the genu of corpus callosum compared with healthy controls, which was consistent with the findings of some previous DTI studies.61,62 There are clinical differences between BD I and BD II. In DSM-5, BD I is the classic manic–depressive disorder, while BD II features depressive and hypomanic episodes; furthermore, the clinical manifestations and treatments of BD I and BD II are different.63,64 The FA reduction in the genu of the corpus callosum reflected a disconnection of the paralimbic system, which plays a central role in emotional regulation,65 and might lead to manic-type behaviours such as inappropriate euphoria, excessive psychomotor behaviour, hypersexuality and paranoia, consistent with the clinical characteristics of patients with BD I.65

The pathobiological interpretation of FA reduction is complex, because it can be influenced by many factors, such as regional myelination levels, intra- and extracellular volume, the degree of intra-voxel fibre crossing, axonal density and average axonal diameter.66 Previous studies have suggested that abnormalities in the corpus callosum that are detected early in pediatric patients with BD may be due to altered myelination during neurodevelopment.57 Meanwhile, several studies have reported a reduction of myelin-producing oligodendrocytes in the prefrontal cortex in patients with BD.68,69 The genu of the corpus callosum and the prefrontal cortex are both late-myelinating areas and are therefore more vulnerable to damage than the early-myelinating splenium.70–73 We speculate that the FA reduction in the genu of the corpus callosum may be related to a reduction of myelination and may result in a pathobiological process that directly slows the transfer of interhemispheric information in patients with BD.74 To help confirm this hypothesis, further studies with more DTI indices, such as axial diffusivity and radial diffusivity, would be useful, because there is evidence from animal studies that axial diffusivity is primarily an axonal marker and radial diffusivity is primarily a myelin marker.75–76

Our finding of decreased FA in the body of the corpus callosum in patients with BD compared with healthy controls was consistent with several previous studies.77–79 The body of the corpus callosum connects several areas, including the lateral primary motor cortex, supplementary motor areas (SMA), the primary sensory cortex and the parietal cortex.80 Previous functional studies have shown that the rostral part of the SMA (the pre-SMA) is involved in memory storage, learning, transition, and motor and speech control, and the pre-SMA connects to the cingulated gyrus, which is part of the limbic system related to cognitive and emotional regulation, while the caudal part of the SMA, the SMA proper and the primary motor area are responsible for motor function.81 Together, the SMA may be a transitional region of the limbic and motor system, which plays an important role in the translation of emotion into motor actions.82 Although emotional dysfunction is one of the main symptoms of BD, previous studies have shown that patients with BD also have motor impairments such as psychomotor retardation,83 agitation,84 attentional deficits and impairments in fine-motor skills.85,86 We therefore suggest that the FA decrease in the body of the corpus callosum might be related to motor and emotional dysfunction in patients with BD. Interestingly, our subgroup analyses of euthymic patients with BD and adult patients with BD replicated the FA decrease in the body of the corpus callosum. This finding was consistent with 1 previous study, which found that euthymic patients with BD had decreased FA in the body of the corpus callosum compared with unaffected siblings.73 Several studies have reported that brain structural and functional abnormalities are related to current mood states in patients with BD.87,88 Our results may indicate a persistent callosal dysconnectivity in euthymic patients with BD, which is consistent with the observation that cognitive impairment and executive dysfunction still exist in euthymic patients with BD.89,90

Meta-regression analysis found that FA in the body of the corpus callosum (decreased overall in patients with BD compared with healthy controls) was negatively associated with the percentage of female patients. This result may indicate that female patients have lower FA values in the body of the corpus callosum than male patients. Sex differences have been observed in the clinical characteristics of BD. Female patients with BD may be more likely to have comorbidities and depressive symptoms, which may result in high suicide risk and impaired occupational function. The sex differences of FA in the body of the corpus callosum may be related to differences in the clinical aspects of BD. A series of morphological studies reported a smaller corpus callosum in female patients than in male patients with BD.92–94 However, 1 study reported no significant difference in the integrity of the body of the corpus callosum when comparing male with female patients with BD.95 These inconsistencies may have resulted from different demographic characteristics, data acquisition methods and other confounding factors in studies of patients with BD.

Medication exposure is an important potential confounder, and understanding the effect of medications on white-matter abnormalities in patients with BD is critical for the interpretation of results. Because only 1 primary study enrolled unmedicated patients with BD, we could not exclude the confounding influences of medication. Several imaging studies have evaluated the effect of psychotropic
medications on white-matter changes in patients with BD. One structural MRI study reported larger bilateral temporal lobe white-matter volumes in patients with BD who were taking antipsychotic medications than in patients who were not taking them. Among patients with BD, longer duration of lithium treatment is associated with increased FA, suggesting that this medication might enhance white-matter integrity. In another study, decreased FA was found in participants treated with lithium, but not in those who were unmedicated; however, there was no significant difference when directly comparing lithium-using and non-lithium-using patients. It has been reported that lithium and other mood stabilizers can improve white-matter integrity and promote myelination by acting on neuroglial signalling pathways and increasing neurotrophic factors. Further studies designed to detect the effect of medication exposure on white-matter changes in patients with BD are needed.

**Limitations**

First, the participants varied in terms of sociodemographic and clinical characteristics. Although we performed subgroup analyses of patients with BD I, euthymic patients with BD, and adult patients with BD, the limited data sets precluded comprehensive subgroup analyses (in particular of the important contrast between BD I and BD II) and meta-regression analyses. Further research is needed investigating the contribution of other clinical characteristics, such as age of onset, comorbidities and disorder severity. Second, the heterogeneity of MRI image acquisition — including voxel size, field intensity of the magnetic resonance system, diffusion direction and slice thickness — may influence the accuracy of the results of our meta-analysis. Third, the main meta-analysis included primary studies, all but 1 of which enrolled medicated patients, so the confounding influences of medication could not be excluded. Fourth, the meta-regression finding that the percentage of female patients with BD was negatively related to the FA in the body of the corpus callosum was driven by 2 outlying studies. Fifth, it has been reported that more than 50% of patients with BD will experience psychotic symptoms in their lifetime, and it is difficult to accurately distinguish BD from other psychiatric disorders in patients with psychotic symptoms. However, data limitations precluded a subgroup analysis comparing patients with BD with or without psychosis. Because 5 of our primary studies included patients with BD and psychosis, we could not exclude the effects of psychosis features on our results. Future studies comparing patients who have BD with and without psychosis are needed to elucidate this.

**Conclusion**

We qualitatively and quantitatively reviewed a large number of published TBSS studies in patients with BD. Meta-analysis of FA findings found that the most robust and replicable white-matter differences were in the genu and body of the corpus callosum. These white-matter tracts connect the bilateral frontal, temporal and parietal cortices, indicating the importance of disrupted interhemispheric communication in the pathophysiology of BD. Subgroup analyses found white-matter structural differences in patients with BD I, and in adult and euthymic patients. Furthermore, the results of the meta-regression analysis shed light on the structural underpinnings of the sex differences in the clinical manifestations of patients with BD. In future studies, attention should be given to differences in clinical type, mood state, and demographics to better define neural mechanisms in patients with BD. Differentiating mood- and type-related white-matter abnormalities is important for elucidating the core pathophysiology of BD and will give better insight into the nature of the disease. The present study also adds to the development of psychoradiology, the branch of radiology that applies clinical imaging to psychiatry and psychology.

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**Competing interests:** None declared.

**Contributors:** C. Yang, L. Li, X. Hu and Q. Gong designed the study. C. Yang and L. Li acquired and analyzed the data, which X. Hu, Q. Luo, W. Kuang, S. Lui, X. Huang, J. Dai, M. He, G.J. Kemp and J.A. Sweeney also analyzed. C. Yang, L. Li, X. Hu, Q. Luo, W. Kuang and J. Dai 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**

Psychoradiologic abnormalities of white matter in bipolar disorder


Lifetime major depression and grey-matter volume

Marie-Laure Ancelin, PhD; Isabelle Carrière, PhD; Sylvaine Artero, PhD; Jerome Maller, PhD; Chantal Meslin, PhD; Karen Ritchie, PhD; Joanne Ryan, PhD; Isabelle Chaudieu, PhD

Introduction

The identification of sociodemographic, environmental and physiopathological factors associated with onset, recovery and relapse in major depressive disorder (MDD) has become a major public health priority, given the association of MDD with high rates of mortality and comorbidity. While considerable research has been done into the clinical characterization of MDD and its associated risk factors, the neuroanatomical substrates involved in the disorder are still unclear; meta-analyses of structural and functional imaging studies report inconsistent findings.1 These inconsistencies are likely due to heterogeneity in study design (case-control v. cohort), setting (general population, inpatient or outpatient), population (age, sex), depression characteristics (diagnosis or symptoms, comorbidity, age of onset, recurrent episodes, antidepressant treatment)6 and methodological issues (not controlling for total brain volume, potential confounding or modifying factors, and different neuroimaging techniques).

The most consistent findings suggest that MDD is associated with dysregulation in neural networks that are implicated in affective and cognitive processing, or in autonomic system activity, resulting in a heterogeneous array of emotive, cognitive and behavioural abnormalities.7 Grey-matter volume changes constitute network nodes in MDD, of which the hippocampus, amygdala and prefrontal cortex have been extensively examined.1,3–6 Conversely, structural alterations in deep nuclei (notably the pallidum, thalamus and hypothalamus), as well as in the insula and occipital regions, have rarely been studied despite accumulating evidence for their role in emotion and the neuropathology of stress-related affective disorder.7–10

The nature and course of volumetric changes may also vary across the lifespan.11 Although grey-matter abnormalities in frontal–subcortical and limbic networks are thought to play a key role in the pathophysiology of depression, recent meta-analyses in late-life depression have shown that the most consistent evidence for brain-volume reductions is for the hippocampus, and not for other brain areas.12 These meta-analyses made no distinction between current and past (remitted) depression and rarely considered age of onset. Most studies have been limited to clinical cohorts, which may not be representative of the case heterogeneity in the general population. In addition, studies have generally been limited in the brain regions they examine and in sample size, so they lack the power to...
explore modifying factors. More particularly, the influence of sex has rarely been evaluated, despite evidence for sexually dimorphic structural and functional brain differences across the lifespan, and the potential implications of those differences in sex-biased psychiatric conditions. This may be especially important for MDD, because prevalence, age of onset, symptomatology and etiology differ between the sexes, and steroid hormones influence brain development and onset of MDD throughout life. Genetic risk factors may also influence brain volumes and depression, but are seldom considered. Serotonergic genes — notably genetic variants in the serotonin transporter-linked promoter region (5-HTTLPR) — have been reported to influence the structure and function of certain brain regions in depressed patients. Whether grey-matter volume alterations are influenced by age-related characteristics (physical and psychiatric comorbidities) also remains to be addressed.

To address the limitations of previous studies, we investigated the association between lifetime MDD and various frontal–subcortical and limbic subregions in a large, community-dwelling, elderly population. We tested the hypothesis that regional brain structure abnormalities would be more extensive in participants with a lifetime MDD diagnosis and may persist after recovery. We further hypothesized that these abnormalities would differ according to sex and genetic vulnerability to 5-HTTLPR. We also considered age of onset and the effect of physical and psychiatric comorbidities. Because prospective lifetime birth cohort data were unavailable, we conducted the study retrospectively in elderly people, for whom both lifetime MDD episodes and genotype had been recorded.

Methods

Participants

We derived data from a longitudinal study of neuropsychiatric disorders in community-dwelling French elderly adults, called “Enquête de Santé Psychologique — Risques, Incidence et Traitemement” (ESPRIT). Eligible participants who were at least 65 years of age and not institutionalized were recruited by random selection from the electoral rolls between 1999 and 2001. Ethics approval for the study was provided by the national ethics committee, and written informed consent was obtained from all participants. Of the 1863 participants initially recruited to the study, only those aged 80 years or younger were invited for an MRI; 760 participants were randomly selected to take part in the imaging study, of whom 668 had complete volumetric data for analysis. Participants with dementia (n = 14), those who were left-handed (n = 16) or were missing data about lifetime MDD or other main covariates (n = 28) were excluded, leaving 610 participants. Compared with those who were excluded, included participants were younger, more frequently men, more likely to live alone and less likely to have cognitive impairment (p < 0.001 for all characteristics). They were less likely to have never smoked and to have cardiovascular ischemic pathologies (p = 0.02 for both). They did not differ with respect to other characteristics, including the prevalence of lifetime MDD (p = 0.78).

MRI protocol and image analysis

We acquired all neuroimaging scans using the same scanner at the examination centre (Groupe des Chaulic Neurology Hospital, Montpellier, France). We used a 1.5 T GE Signa Imaging system to acquire a contiguous T-weighted sequence for volumetric estimates (axial inversion recovery prepared, spoiled gradient recalled) that was aligned on the anterior–posterior commissure (repetition time 12 ms, echo time 2.8 ms, inversion time 600 ms, matrix size 256 × 256, pixel spacing 0.9375 × 0.9375 mm, number of excitations = 1, slice thickness 1.0 mm). We performed regional reconstruction and segmentation using the FreeSurfer (5.3) image analysis suite (http://surfer.nmr.mgh.harvard.edu/) as described previously. We inspected the FreeSurfer outputs of each scan for errors or misclassifications (from 2D and 3D perspectives), and we excluded 28 scans with clear errors. We defined 16 regions of interest (ROIs) using the Desikan atlas. The regions of primary interest were the hippocampus, amygdala, orbitofrontal cortex and anterior cingulate cortex (ACC), as well as several subcortical structures (thalamus, caudate nucleus, putamen, pallidum and accumbens nuclei) that might show neuroimaging abnormalities in depressed patients. We also examined ROIs that had rarely been evaluated despite accumulating evidence for abnormalities in MDD and their potential role in emotional processing and symptom characteristics, including the insula, ventral diencephalon (a region primarily comprising the hypothalamus in FreeSurfer) and occipital visual cortex. We calculated the total brain volume (grey plus white matter) for each participant using the segmentation file in SPM5 (Wellcome Department of Cognitive Neurology), which showed greater accuracy and consistency and less systematic bias evaluation than FreeSurfer for this measure.

Diagnosis of lifetime psychiatric disorder

Current and previous MDD and anxiety disorders (phobias, generalized anxiety disorder, panic disorder, obsessive compulsive disorder and posttraumatic stress disorder) were diagnosed by psychologists and psychiatric nurses according to DSM-IV criteria and using the Mini-International Neuropsychiatric Interview (MINI, French version 5.00), a standardized psychiatric examination that has been validated in the general population. Positive cases were reviewed by a panel of independent psychiatrists.

Sociodemographic and clinical variables

We used a standardized interview to obtain information on sociodemographic characteristics, physical health and medical history. We used detailed medical questionnaires (with additional information from general practitioners) to obtain participants’ history of cardiovascular ischemic pathologies (angina pectoris, myocardial infarction, stroke, cardiovascular surgery and arthritis). We recorded all drugs used by participants in the preceding month (including antidepressants) from medical prescriptions and drug packaging. We evaluated global cognitive function using the Mini-Mental State Examination; a
score below 26 indicated cognitive impairment. Dementia was diagnosed by a neurologist as part of a standardized examination and validated by a panel of independent neurologists.\textsuperscript{34}

5-HTTLPR genotyping

We collected blood samples after the baseline clinical interview, enabling DNA extraction and 5-HTTLPR genotyping as described previously.\textsuperscript{35} We performed replicate independent genotyping using buccal DNA extracts.\textsuperscript{32}

Statistical analysis

Brain-volume measurements were normally distributed. We evaluated associations between brain regions and lifetime MDD using analysis of covariance adjusted for age, sex and total brain volume (model M0). Where we observed significant associations, we used exploratory analyses to assess the specificity of these findings. We tested the sex \times diagnosis interaction by adding a lifetime MDD \times sex term to the model. Where we observed an interaction effect (\(p < 0.10\)), we stratified analyses by sex. We made further adjustments for other covariates that have been reported to modify the association between MDD and brain volume: education level, head injury, cardiovascular ischemic pathologies and antidepressant use (model M1), as well as lifetime anxiety disorder (model M2). For significant bivariate associations, we evaluated the effect of 5-HTTLPR by stratifying it into 3 genotypes (LL, SL and SS) because of a lack of consensus about the choice of a genetic model and frequent reports of heterosis for 5-HTTLPR.\textsuperscript{36,37} To account for the fact that we examined multiple brain regions, we adjusted significance levels using the false discovery rate (FDR) method.\textsuperscript{38} All tests were 2-sided, and we used SAS (version 9.4, SAS Institute, Inc.) for the statistical analyses.

Results

Participant characteristics

The baseline characteristics of the 610 participants are summarized in Table 1. The median age of participants was 70.7 years. Of the total population, 47.5\% were male, and

<table>
<thead>
<tr>
<th>Table 1: Participant characteristics ((n = 610^*))</th>
<th>Group, median (IQR) or %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>Whole sample ((n = 610))</td>
</tr>
<tr>
<td>Age, yr</td>
<td>70.7 (67.8–74.0)</td>
</tr>
<tr>
<td>Body mass index, kg/m(^2) ((n = 604))</td>
<td>24.8 (22.8–27.1)</td>
</tr>
<tr>
<td>Cortex volume, cm(^3)</td>
<td>358 (336–382)</td>
</tr>
<tr>
<td>Total brain volume, cm(^3)</td>
<td>882 (816–958)</td>
</tr>
<tr>
<td>Education ≤ 5 yr</td>
<td>25.7</td>
</tr>
<tr>
<td>Living alone ((n = 609))</td>
<td>19.7</td>
</tr>
<tr>
<td>Smoking ((n = 609))</td>
<td>53.4</td>
</tr>
<tr>
<td>Head injury</td>
<td>10.2</td>
</tr>
<tr>
<td>Lifetime number of major depressive episodes‡</td>
<td>16.4</td>
</tr>
<tr>
<td>≥ 2</td>
<td>10.2</td>
</tr>
<tr>
<td>Current major depressive disorder‡</td>
<td>2.1</td>
</tr>
<tr>
<td>Age at first episode ((n = 609))</td>
<td>73.6</td>
</tr>
<tr>
<td>&lt; 50 yr</td>
<td>14.9</td>
</tr>
<tr>
<td>≥ 50 yr</td>
<td>11.5</td>
</tr>
<tr>
<td>Antidepressant use</td>
<td>5.1</td>
</tr>
<tr>
<td>Lifetime anxiety disorder‡ ((n = 576))</td>
<td>26.4</td>
</tr>
<tr>
<td>Hypertension§</td>
<td>68.5</td>
</tr>
<tr>
<td>Cardiovascular (ischemic pathologies¶)</td>
<td>12.8</td>
</tr>
<tr>
<td>Diabetes** ((n = 606))</td>
<td>8.8</td>
</tr>
<tr>
<td>Cognitive impairment††</td>
<td>13.4</td>
</tr>
</tbody>
</table>

IQR = interquartile range.

*Unless otherwise specified.
†Kruskal–Wallis tests for continuous variables and \(\chi^2\) tests for categorical variables.
‡Diagnosis of past and current major depression or anxiety disorder (phobias, generalized anxiety disorder, posttraumatic stress disorder, panic disorder or obsessive–compulsive disorder) according to DSM-IV criteria and using the Mini-International Neuropsychiatric Interview.\textsuperscript{31}
§Hypertension \(\geq 140/90\) mm Hg or treated.
¶History of cardiovascular ischemic pathologies (angina pectoris, myocardial infarction, stroke, cardiovascular surgery, arteritis).
**Fasting blood glucose \(\geq 7.0\) mmol/L or treatment.
††Mini-Mental State Examination score \(< 26\).
26.6% had lifetime MDD, of whom 38.3% had recurrent episodes. Only 2.1% had current MDD, and 5.1% were taking an antidepressant. Men and women differed with respect to most characteristics: women were younger, more frequently living alone and more likely to report a lifetime psychiatric disorder, but they had fewer head injuries and fewer cardiovascular risk factors.

Subsegmental brain regions according to lifetime MDD

After adjustment for age, sex and total brain volume, lifetime MDD was associated with smaller grey-matter volumes in the insula, diencephalic structures and deep nuclei, and with larger volumes in the rostral ACC and 2 visual cortex subregions (Table 2). Alterations in the insula, thalamus, ventral diencephalon, nucleus accumbens, pallidum and pericalcarine region survived FDR correction. We observed similar alterations in the multivariate model that was further adjusted for education level, head injury, cardiovascular ischemic pathologies and antidepressant use (Appendix 1, Table S1, available at jpn.ca/180026-a1). These covariates accounted for a relatively small proportion of the variance in each volume: the standardized regression coefficients ranged between −0.338 and 0.257 (Appendix 1, Table S2).

In our sample, 576 participants were also evaluated for lifetime anxiety disorder, and we observed a similar pattern after controlling for anxiety disorder (data not shown). We conducted a sensitivity analysis excluding 41 participants who were currently depressed or taking antidepressants, and observed similar patterns, except for a slightly weaker association for the nucleus accumbens but a stronger association for the lingual region (Appendix 1, Table S3).

Exploratory analyses according to sex

We observed an interacting effect of sex on the amygdala ($p = 0.09$), caudate nucleus ($p = 0.018$) and rostral ACC ($p = 0.06$). Lifetime MDD was associated with smaller grey-matter volumes in the amygdala and caudate nucleus in men (−4.3%, $p = 0.026$, and −7.2%, $p = 0.010$, respectively) but not in women (−0.8%, $p = 0.57$, and −0.2%, $p = 0.89$, respectively). Conversely, only women with lifetime MDD had larger volumes in the rostral ACC (+6.3%, $p = 0.003$ v. −1.2% in men, $p = 0.68$; Fig. 1).

Exploratory analyses as a function of 5-HTTLPR genotype

Of all participants, 30% were homozygous carriers of the L allele, and 23.2% were SS homozygotes. The frequency of the 5-HTTLPR genotypes did not deviate significantly from Hardy–Weinberg equilibrium ($p = 0.17$). Lifetime MDD was associated with a smaller thalamus in LL participants only (−4.9%, $p = 0.002$, v. −1.2% in SL participants and +0.3%, $p = 0.86$, in SS participants; Table 3). We found

<table>
<thead>
<tr>
<th>Brain region</th>
<th>No lifetime MDD ($n = 448$), mean ± SD*</th>
<th>Lifetime MDD ($n = 162$), mean ± SD*</th>
<th>$p$ value†</th>
<th>$p_{FDR}$ value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial orbitofrontal cortex</td>
<td>8 962.94 ± 43.20</td>
<td>8 939.55 ± 73.61</td>
<td>0.79</td>
<td>0.79</td>
</tr>
<tr>
<td>Lateral orbitofrontal cortex</td>
<td>11 652.40 ± 46.37</td>
<td>11 718.13 ± 79.01</td>
<td>0.48</td>
<td>0.55</td>
</tr>
<tr>
<td>Rostral anterior cingulate cortex</td>
<td>3 347.51 ± 28.37</td>
<td>3 458.73 ± 48.34</td>
<td>0.05</td>
<td>0.09</td>
</tr>
<tr>
<td>Caudal anterior cingulate cortex</td>
<td>2 993.71 ± 25.67</td>
<td>3 065.27 ± 43.73</td>
<td>0.16</td>
<td>0.22</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>7 061.54 ± 33.88</td>
<td>7 033.17 ± 57.73</td>
<td>0.68</td>
<td>0.72</td>
</tr>
<tr>
<td>Amygdala</td>
<td>2 634.05 ± 14.88</td>
<td>2 580.65 ± 25.34</td>
<td>0.07</td>
<td>0.12</td>
</tr>
<tr>
<td>Insula</td>
<td>11 954.83 ± 48.80</td>
<td>11 722.33 ± 83.14</td>
<td>0.017</td>
<td>0.045</td>
</tr>
<tr>
<td>Thalamus</td>
<td>11 806.32 ± 43.44</td>
<td>11 575.64 ± 74.01</td>
<td>0.008</td>
<td>0.038</td>
</tr>
<tr>
<td>Ventral diencephalon</td>
<td>6 794.71 ± 28.62</td>
<td>6 624.44 ± 48.76</td>
<td>0.003</td>
<td>0.038</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>6 852.97 ± 52.58</td>
<td>6 669.85 ± 89.58</td>
<td>0.08</td>
<td>0.12</td>
</tr>
<tr>
<td>Putamen</td>
<td>9 453.97 ± 52.75</td>
<td>9 215.18 ± 89.87</td>
<td>0.024</td>
<td>0.06</td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td>997.39 ± 7.14</td>
<td>960.92 ± 12.17</td>
<td>0.011</td>
<td>0.038</td>
</tr>
<tr>
<td>Pallidum</td>
<td>2 983.73 ± 16.17</td>
<td>2 893.75 ± 27.55</td>
<td>0.005</td>
<td>0.038</td>
</tr>
<tr>
<td>Cuneus</td>
<td>4 765.02 ± 30.27</td>
<td>4 839.00 ± 51.58</td>
<td>0.22</td>
<td>0.27</td>
</tr>
<tr>
<td>Pericalcarine region</td>
<td>3 404.66 ± 28.46</td>
<td>3 547.02 ± 48.48</td>
<td>0.012</td>
<td>0.038</td>
</tr>
<tr>
<td>Lingual region</td>
<td>10 714.10 ± 67.51</td>
<td>10 984.78 ± 115.01</td>
<td>0.045</td>
<td>0.09</td>
</tr>
</tbody>
</table>

*FDR = false discovery rate; MDD = major depressive disorder; SD = standard deviation.

†Raw $p$ values not adjusted for multiple comparisons.

‡False-discovery rate–corrected.
significant differences in insula, ventral diencephalon, lingual and (most significantly) pericalcarine volume for SL participants with lifetime MDD (+8.2%, p = 0.003 v. +2.5%, p = 0.41 for LL participants and +2%, p = 0.55 for SS participants). We observed the same pattern after excluding participants who had current MDD or were taking antidepressants, although association with the ventral diencephalon was weakened (p = 0.060) and that with the lingual region was strengthened (p = 0.005 in M0 and p = 0.001 in M2; data not shown).

Discussion

Lifetime MDD was associated with many grey-matter volume differences. The most robust finding was smaller volumes in the deep nuclei and insula and larger volumes in the occipital visual cortex. These findings were independent of age, sex, education level, head injury, cardiovascular ischemic pathologies, antidepressant use and lifetime anxiety disorder. They were also seen in individuals who had been free of MDD for many years (median [interquartile range] = 15 [5–24] years). Lifetime MDD was also associated with smaller caudate nuclei and amygdalae in men, and a larger rostral ACC in women. Some associations varied by 5-HTTLPR genotype: most importantly, the thalamus was smaller in LL participants with lifetime MDD, whereas the pericalcarine and lingual regions were larger in SL participants only.

Previous reports of morphological brain changes relative to MDD have mostly been based on small case-control studies that focused on the hippocampus, amygdala, orbitofrontal cortex and ACC. Inconsistent findings have been reported, probably attributable to heterogeneity in study design, size, setting, population and depression characteristics. Meta-analyses reported smaller volumes in adults, but the effects were small and/or nonsignificant; effects depended on patients’ age and whether they were experiencing a first episode, and were less likely in the community-dwelling population or in patients with remitted MDD compared with those who had current or recurrent episodes. In the community-dwelling elderly population in our study, most participants reported only 1 previous depressive episode, and the median (interquartile range) age of first onset was 47 (35–57) years, which may explain the lack of significant associations. However, we did find a highly significant difference for the rostral ACC, which was larger in women with lifetime MDD. Voxel-based morphometry studies have reported larger cingulate gyri in geriatric patients with remitted depression, and a larger ACC in medication-washout young adults with MDD. In healthy adults, a negative association has been shown between grey-matter volume and stress-related brain activity in the perigenual ACC.

Basal nuclei

In our study, lifetime MDD was associated with decreased grey-matter volumes in several basal nuclei. Smaller caudate nuclei have been reported in adult patients and, in late-life depression, effect sizes increased with age and with a smaller percentage of women. Consistent with these results, we found that lifetime MDD was associated with a smaller caudate nucleus in men, and the association was strengthened after adjusting for several confounders, including antidepressant use and the presence of an anxiety disorder (–8.6%, p = 0.003, in M2).

Some meta-analyses on the putamen have reported significant grey-matter volume reduction with lifetime MDD, and others in early- but not late-onset or current MDD. Other studies have found that associations were limited to severe or persistent subtypes. We found a marginal association between lifetime MDD and a smaller putamen in a high-dimensional set of more than 11,000 traits, the pallidum volume was reported to be a main endophenotype associated with recurrent depression. We also observed a greater grey-matter volume reduction in participants who reported multiple MDD episodes compared with those who reported 1 or no previous episodes (global p value = 0.042).

We also found an association between lifetime MDD and smaller volumes in the nucleus accumbens and pallidum. These regions have been examined rarely, and meta-analyses of studies including mainly adult patients with acute or lifetime MDD have failed to report significant associations. In an analysis of a high-dimensional set of more than 11,000 traits, the pallidum volume was reported to be a main endophenotype associated with recurrent depression. We also observed a greater grey-matter volume reduction in participants who reported multiple MDD episodes compared with those who reported 1 or no previous episodes (global p = 0.013).

Lifetime MDD was also associated with a smaller thalamus and ventral diencephalon — a region primarily comprising the hypothalamus. Small meta-analyses of the thalamus have reported moderate or no significant volume reduction, but none has included the hypothalamus, despite its crucial role in emotional behaviour and stress response as part of the

Fig. 1: Association between lifetime major depressive disorder (MDD) and volumes of rostral anterior cingulate cortex, amygdala and caudate nucleus in elderly men and women. Mean ± standard deviation (SD); values expressed in cubic millimetres and adjusted for age and total brain volume. Members of the control group did not have lifetime MDD; members of the MDD group had lifetime MDD.
hypothalamic–pituitary–adrenal axis. However, smaller ventral diencephalon volume has been reported to be the top-ranked neuroimaging endophenotype associated with recurrent MDD in adults and to correlate with the number of depressive episodes in late-life depression. 25

Insula

The insula plays a role in emotional, sensorimotor and interoceptive processing, but it has rarely been examined, despite some evidence for abnormalities in MDD and related phenotypes (sadness, irritability, sleep disorders). 7,8 A small case–control study reported reduced anterior insular cortex in current and remitted young adult patients compared with healthy controls. 44 A meta-analysis of voxel-based morphometry studies also showed left insula volume reductions in young adults with first-episode depression. 8 Our study is, to our knowledge, the first ROI study to show a significant smaller insula volume in an elderly general population with lifetime and remitted MDD.

Visual cortex

Another original finding concerns the association of MDD with larger grey-matter volume in the pericalcarine and lingual ROIs, a finding that was especially marked for late-onset MDD. The visual cortex plays a central role in the fear-conditioning paradigm in humans. 45 The pericalcarine region is the initial region of visual processing, and the lingual gyrus is associated with high-level visual processing and visual memory. The associations were highly significant after excluding participants who were currently depressed or treated for depression (p = 0.006 and 0.004, respectively, in M2). The link between the visual cortex and MDD has been described only in voxel-based morphometry studies; a meta-analysis reported a significant grey-matter volume increase in the right lingual gyrus in late-life depression. 10 A larger lingual gyrus volume was also found to predict early antidepressant response in adults and was linked to better performance on visuospatial tasks in young adults. 45 Whether the pericalcarine and lingual regions could participate in a neuronal compensatory process to facilitate the processing of aversive stimuli and fear or emotional learning in response to abnormal input from other structures, or whether they reflect resilience against relapse, remains to be examined.

5-HTTLPR genotype

Most previous studies have examined the effect of 5-HTTLPR on grey-matter volumes in either depressed or healthy participant groups, but rarely as a modifying factor between MDD risk and grey-matter alterations. 17 These studies were generally size-limited and focused on the hippocampus and amygdala.

<p>| Table 3: Association of subsegmental brain region volumes with lifetime MDD according to 5-HTTLPR genotype |
|------------------------------|------------------------------|------------------------------|------------------------------|</p>
<table>
<thead>
<tr>
<th>5-HTTLPR</th>
<th>LL (n = 160)</th>
<th>SL (n = 250)</th>
<th>SS (n = 124)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No lifetime MDD (n = 112), mean ± SD*</td>
<td>Lifetime MDD (n = 48), mean ± SD*</td>
<td>Lifetime MDD (n = 66), mean ± SD*</td>
<td>Lifetime MDD (n = 93), mean ± SD*</td>
</tr>
<tr>
<td>p value†</td>
<td>p value†</td>
<td>p value†</td>
<td>p value†</td>
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<td>----------------</td>
</tr>
<tr>
<td>No lifetime MDD (n = 184), mean ± SD*</td>
<td>Lifetime MDD (n = 66), mean ± SD*</td>
<td>Lifetime MDD (n = 31), mean ± SD*</td>
<td></td>
</tr>
<tr>
<td>p value†</td>
<td>p value†</td>
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<tr>
<td>No lifetime MDD (n = 93), mean ± SD*</td>
<td>Lifetime MDD (n = 31), mean ± SD*</td>
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<td>p value†</td>
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<td>No lifetime MDD (n = 112), mean ± SD*</td>
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<td>Lifetime MDD (n = 93), mean ± SD*</td>
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<td>p value†</td>
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<tr>
<td>No lifetime MDD (n = 184), mean ± SD*</td>
<td>Lifetime MDD (n = 66), mean ± SD*</td>
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</tr>
<tr>
<td>No lifetime MDD (n = 93), mean ± SD*</td>
<td>Lifetime MDD (n = 31), mean ± SD*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p value†</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MDD = major depressive disorder; SD = standard deviation.
*Values expressed in mm³ and adjusted for age, sex and total brain volume.
†Raw p values.
rarely on the striatum or thalamus, where 5-HTT is highly expressed. The vast majority of the structural imaging genetic studies did not consider the SL genotype individually, despite a lack of consensus regarding the genetic model and frequent report of heterosis for 5-HTTLPR. Heterogeneity in age is another potential source of concern; in younger populations, the S allele is a risk factor for mental and physical distress, but the LL genotype appears to be a risk factor in elderly people, who are highly exposed to chronic disorders and severe stressors.

We found no significant volumetric differences according to 5-HTTLPR within groups (with or without lifetime MDD; data not shown) but we did find significant between-group differences. In particular, the thalamus was smaller in LL homozygotes with lifetime MDD, but only SL heterozygotes with lifetime MDD had larger pericalcarine and lingual volumes compared with their non-MDD counterparts. The thalamus is rich in serotonergic neurons, and reduced 5-HTT availability has been described in the thalamus of depressed patients, but data on the effect of 5-HTTLPR genotypes are lacking, and no studies have examined the pericalcarine or lingual regions, despite some indication for serotonergic occipital dysfunction in depression. Our findings were unchanged after excluding patients who were currently depressed or treated for depression, suggesting that they were related to serotonergic vulnerability to the disorder.

In a subsample of the ESPRIT study, we reported that past MDD and stressful events were risk factors for current depression in LL homozygotes specifically, whereas SL heterozygotes were more resilient to these factors. We also found that some adverse events during childhood (for example, sexual or physical abuse, or having a mother with mental illness) were associated with higher risk of late-life depression but lower risk of cognitive decline, notably in visual memory, suggesting a possible cognitive adaptation or resilience effect. Although these findings are speculative, they may suggest that the pericalcarine and lingual regions could participate in a persistent neuronal compensatory process in SL heterozygotes with a history of MDD.

Sex differences

We found some evidence for sexually dimorphic alterations: smaller caudate nuclei and amygdalae in men with lifetime MDD, and larger rostral ACC in women. Specific sex differences in the depression symptomatology of older adults have been described, with women showing more mood-related symptoms and appetite disturbance and men showing more motivation-related symptoms and psychomotor changes; these differences may involve distinct biological correlates. However, only a few neuroimaging studies have investigated sex effects, many including predominantly women and, likely as a consequence, meta-analyses have seldom reported sex differences.

In a healthy sample consisting mainly of older women, a larger ACC was associated with higher levels of anhedonia. Valence-dependent sex differences in emotional reactivity have been reported, with divergent activation patterns, notably in the ACC and amygdala, suggesting a difference in recognizing, expressing or responding to emotions. The rostral ACC and caudate nucleus are involved in impulse inhibition in young adults in a sex-specific manner, suggesting different processing strategies (e.g., inhibiting inappropriate response in males v. eliciting appropriate response in females). In adults with attention-deficit/hyperactivity disorder, a smaller caudate nucleus was associated with impulsive/hyperactive symptoms in men, but not in women. In addition, increased resting rostral ACC activity has been linked to adaptive cognitive aspects of rumination and could predict better antidepressant response and recovery. Whether sex influences the nature of changes in some structures or is associated with specific symptoms, processing strategies or characteristics of depression (e.g., rumination, irritability, impulsivity) remains to be examined.

Context of the findings

We found that retrospectively determined incidence of lifetime MDD was associated with a smaller striatum, pallidum, thalamus, hypothalamus and insula, but with larger pericalcarine and lingual regions, even many years after recovery. These findings are partly consistent with a neurobiological model of current depression that posits dysfunction of the cortico–striatal–pallidal–thalamic network involved in emotion, cognition and motor control; reward and stress systems; and sensorimotor and interoceptive processing. There is also some evidence for sex differences with respect to emotion production and regulation. We did not observe the reduced volumes in the hippocampus or frontal subregions previously reported in currently depressed adults. In a meta-analysis of functional MRI studies, Graham and colleagues suggested that frontal areas could be state markers of MDD, but that striatal regions were trait vulnerability markers that might be less affected by treatment. They also stressed the potential key roles of regions that are not included in prevailing models of MDD, such as the insula and occipital subregions, the latter showing overactivity in MDD. Sustained remission from MDD has been associated with normalized reactivity of certain prefrontal and limbic regions and greater sensory reactivity in visual cortices, as well as hyperactivity and/or reduced deactivation in the rostral ACC. Our data further suggest a key role of the visual corticostriatal loop in elderly patients with remitted MDD, with volume enlargement in visual occipital regions and reduction in subcortical structures. They also suggest a link between certain sensory/visual functions (thalamus for sensory relay, and pericalcarine and lingual regions for visual processing and visual memory) and 5-HTTLPR vulnerability (to stress-induced relapse) or resilience to MDD. However, it remains to be determined whether these abnormalities represent biological long-term vulnerability (endophenotype as intermediate expression of genetic vulnerability factors).

Limitations

Limitations of our study include the cross-sectional design; we could not determine whether volume alterations preceded or followed MDD. Data related to lifetime MDD were
retrospective, which may have introduced recall bias and led to an underestimation of associations, even if we had excluded participants diagnosed with probable/possible dementia to minimize inaccuracies. The volume variations associated with lifetime MDD were 2% to 5% for deep nuclei and insula and 5% to 8% for visual cortex ROIs, suggesting a relatively small effect size. We did not examine state-like characteristics because of the low prevalence of current MDD in this relatively healthy community sample, and the lack of associations with some ROIs could have been related to normalization after sustained remission/treatment. Finally, we performed multiple analyses, potentially increasing the risk of type 1 errors, but most findings remained significant even after correction for multiple comparisons.

This study constitutes the largest structural MRI investigation targeting lifetime MDD, in terms of the number of participants and ROIs examined. We measured brain volumes using FreeSurfer automated segmentation, enabling accurate evaluation of volumetric changes of smaller deep brain structures. Lifetime MDD was assessed by trained staff using a standardized psychiatric evaluation, according to DSM-IV criteria. Further clinical validation of the cases minimized false positives. Extensive information available on participants’ clinical status and medications helped minimize exposure misclassification. In contrast to previous studies, we controlled for numerous potential confounding factors, particularly education level, head injury, and physical and mental comorbidities.

Conclusion

We observed grey-matter volume differences between those who retrospectively reported lifetime MDD and those who did not. These structural correlates may constitute useful imaging phenotypes of depression: treatment responsiveness or resilience. It remains to be determined whether grey-matter volume increases are linked to a neuronal adaptive compensatory process in response to dysfunction in other structures, or whether they represent trait-like, developmental differences that underlie a neurobiological vulnerability associated with etiological pathways. Whether sex influences the nature of changes in some brain structures (which could help account for the sex differences observed in epidemiological and clinical studies of depression) or is associated with sex-specific traits or dimensions of depression also remains to be examined. Further work is required to understand the significance of these volumetric differences, including prospective multimodal and complementary imaging studies. This will help distinguish between causal roles and neural correlates of MDD, the result of shared underlying causes (for example, genetic predisposition) or bidirectional/mutual reinforcement, opening up the potential for effective therapeutic strategies.

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Competing interests: None declared.

Contributors: M-L. Ancelin designed the study. M-L. Ancelin and K. Ritchie led the ESPRIT study and the collection of data. J. Maller and C. Meslin processed all neuroimaging data. I. Carrière performed all statistical analyses. M-L. Ancelin, S. Artero, J. Ryan and I. Chauvel were involved in the interpretation of the data. M-L. Ancelin drafted the manuscript, 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

Depression and grey-matter volume


Apathy alters emotional arousal in chronic schizophrenia

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Background: Within the heterogeneity of schizophrenia, apathy constitutes an independent cluster of negative symptoms associated with poor outcomes. Attempts to identify an emotional deficit in patients who have schizophrenia with negative symptoms have yielded mixed results, and studies that focus on the relationship between apathy and emotional disorders are lacking. Methods: We set out to remedy this shortcoming using a validated battery of film excerpts to induce positive and negative emotions in patients with chronic schizophrenia with \( n = 20 \) or without \( n = 20 \) apathy, and in controls \( n = 20 \) comparable for age, sex and socioeconomic status. We assessed emotions using an innovative but validated technique to evaluate tonic and phasic electrodermal activity and subjective feelings using a standardized visual analogue scale. Results: Using a qualitative measure of apathy, we did not find a specific decrease in tonic activity during the induction of positive emotions. However, we did observe that patients with apathy showed reduced tonic activity independent of valence (i.e., for both positive and negative emotions) compared with controls and patients without apathy. Moreover, the quantitative measure of apathy (Apathy Evaluation Scale) was the only significant factor, explaining 24% of the variance in tonic activity during induction of positive emotions after controlling for confounding factors. Limitations: Electrodermal activity was the only physiologic measure we acquired. We induced several emotions sequentially that might have overlapped with each other, but we added an emotional “washout” period and randomized the order of each film excerpt to limit this possibility. Conclusion: Taken together, these results suggest that apathy in schizophrenia could impair tonic activity during positive emotions. Treatments aimed at enhancing positive emotions may help alleviate apathy in schizophrenia.

Introduction

Schizophrenia induces emotional impairments, causing social dysfunction.\(^1\) Negative symptoms in schizophrenia are heterogeneous, but can be grouped into 2 independent clusters: apathy/avolition and diminished emotional expression.\(^2\) Apathy is defined as a lack of motivation that reduces the emotional, cognitive and behavioural components of goal-directed behaviours in daily life.\(^3,4\) It accounts for the largest proportion of poor functioning in daily life and overall outcomes in schizophrenia, independent of cognitive impairment\(^5\) and diminished emotional expression.\(^6\) People with schizophrenia and apathy show steeper reward devaluation in relation to physical effort than patients without apathy.\(^7\) However, emotional deficits in schizophrenia with apathy have been unexplored. This is unexpected, because motivation is a key component of emotional processing in contemporary models,\(^8\) and altered emotional processing has been highlighted in Parkinson disease with apathy,\(^9,10\) suggesting that apathy might impair emotional processes.

Previous investigations into subjective emotional experiences in schizophrenia have opted for laboratory-based eliciting of emotion, using a wide variety of stimuli (e.g., odours, food, emotional faces or pictures).\(^11\) Despite such methodological heterogeneity, most studies have found that patients with schizophrenia experienced positive emotions in a similar way to healthy controls.\(^1,12\) However, some results have suggested exacerbated negative emotional experiences in response to positive or neutral stimuli,\(^13,14\) or impaired neural response to positive stimuli in schizophrenia.\(^15,16\) One approach to explaining preserved hedonic capacity in schizophrenia separates goal-directed emotions into reward anticipation and intact positive emotions during reward reception.\(^1,17\) The results of previous studies support the
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Hypothesis that apathy in schizophrenia and in healthy people with psychotic-like symptoms is associated with blunted striatal response during reward anticipation, but not during reward reception.18–20

Emotional processes include electrodermal activity (EDA) as a marker of emotional arousal.21 Few studies have explored the link between subjective emotional experiences and EDA during emotional induction in schizophrenia, and results have been mixed: studies found greater, equal or reduced EDA in response to emotional pictures.22–24 Electrodermal activity reflects autonomic nervous system activity and can be divided into tonic (slow variations) and phasic (rapid variations) activity.25 More specifically, tonic EDA activity refers to skin conductance level, and phasic activity corresponds to a skin conductance reaction (SCR) evoked by a stimulus.25 A recent review found that tonic and phasic activities can be increased during emotional episodes using various methods of emotion induction (film excerpts, music or pictures) and across all emotional categories (joy, fear, anger, surprise).21 Phasic activity is a rapid and event-related feature of EDA, but tonic activity is associated with anticipation of an outcome during conditional paradigms of long duration.26 Moreover, reduced tonic EDA has been related to apathy in a population with brain injuries.27

Because apathy has been related to the decreased ability to anticipate positive emotions in schizophrenia, and because anticipation of an outcome is associated with tonic EDA, our primary objective was to test for reduced tonic activity during the induction of positive emotions in patients with schizophrenia and apathy, compared to patients with schizophrenia without apathy and healthy controls. Our secondary objective was to examine whether quantitative apathy scores were associated with tonic activity during positive emotion when controlling for confounding factors in patients with schizophrenia.

To this end, we induced discrete positive and negative emotions using a validated battery of film excerpts28 and extracted tonic activity from EDA recordings in comparable groups of patients with schizophrenia with and without apathy, and in a control group.

Methods

Participants

We recruited 20 outpatients who had stable chronic schizophrenia (fewer than 3 positive items scored 4 or above on the Positive and Negative Syndrome Scale [PANSS] and no medication changes during the previous 2 months) and apathy (Scz-A), and 20 patients who had schizophrenia without apathy (Scz-NA), all from Rennes University Hospital in France. All participants were native French speakers. We recruited healthy controls using local advertising. Eight patients refused to participate in our study. We recruited participants to build 3 groups that were comparable in terms of age, socioeconomic status, sex, handedness and disease duration. We also recruited patients so that the chlorpromazine-equivalent dose was similar between both groups. All patients were ambulatory.

Schizophrenia had been diagnosed according to the French version of the Mini International Neuropsychiatric Inventory,20 based on DSM-IV-TR criteria.31 Exclusion criteria included psychotic depression, severe extrapyramidal symptoms and low-order visual and auditory impairments. Patients were allocated to the Scz-A or Scz-NA group according to the diagnostic criteria for clinical apathy.4 Apathy was assessed quantitatively using the Apathy Evaluation Scale (AES).32 The clinical criteria and the AES have been validated in schizophrenia.4

All patients were being treated with medication at the time of testing. In the Scz-A group, 16 patients were receiving atypical antipsychotics and 4 were receiving both typical and atypical antipsychotics; 4 were receiving benzodiazepines. In the Scz-NA group, 19 patients were receiving atypical antipsychotics and 1 was receiving a typical antipsychotic; none were receiving benzodiazepines.

Twenty healthy controls received €50 for their participation and were not taking any medications. Patients did not receive monetary compensation, following French ethics recommendations.

Exclusion criteria for all participants were neurologic and systemic illness, previous head injury, severely impaired vision or auditory acuity, and mental retardation (Mattis Dementia Rating Scale score below 120). As well, any diagnosis of a mental disorder according to the Mini International Neuropsychiatric Inventory led to exclusion in the control group.

Written informed consent was obtained from each participant, and the study met the ethical standards of the Declaration of Helsinki. The study was approved by the local ethics committee (CPP Ouest II-Angers Number: 2012/16).

Psychopathological and neuropsychological assessment

We used the PANSS29 to assess the stability and severity of schizophrenia. We assessed overall negative symptoms using the Scale for the Assessment of Negative Symptoms (SANS)34 and depressive symptoms using the Calgary Depression Scale for Schizophrenia.35 We used the Abnormal Involuntary Movement Scale36 to assess extrapyramidal symptoms.

The executive function battery included phonemic and semantic fluency tests, an adapted version of the Stroop paradigm, the Trail Making Test (TMT) and the digit span and digit symbol coding subtests of the Wechsler Adult Intelligence Scale IV. See a study by our group37 for a complete description of the battery.

Emotion elicitation procedure

Film excerpts are effective, valid and powerful for eliciting intense and specific target emotions.38–40 We used a battery of 5 film excerpts, selected from a larger validated battery28 according to their likelihood to induce a specific subjective emotional experience: happiness, anger, fear, sadness or disgust. All excerpts were in French, lasted 1 to 4 minutes, and were displayed on a 22-inch colour screen. The film excerpts had been edited to produce the apex of emotional intensity as

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they ended (Philippot P., personal communication, Nov. 15, 2017). To capture the emotional peak and standardize excerpt length, we analyzed the EDA recordings for only the last minute of each excerpt. We randomized the order of presentation for each participant so that no one experienced them in the same order. Before each excerpt, participants went through a 3-minute relaxation procedure to “wash out” previous emotions. After the washout period, we captured EDA for 1 minute to obtain a baseline before eliciting the next emotion (Fig. 1).41

Assessment of subjective emotional experience

After each relaxation and presentation phase, participants reported the intensity of their emotional experiences on the Differential Emotions Scale, which consists of 10 visual analogue scales: 1) interested, concentrated, alert; 2) joyful, happy, amused; 3) sad, downhearted, blue; 4) angry, irritated, mad; 5) fearful, scared, afraid; 6) anxious, tense, nervous; 7) disgusted, turned off, repulsed; 8) disdainful, scornful, contemptuous; 9) surprised, amazed, astonished; 10) warmhearted, gleeful, elated. Each scale ranged from 0 (no subjective emotional experience at all) to 7.5 (very intense subjective emotional experience).

Assessment of EDA

We acquired EDA following the recommendations of the Society for Psychophysiological Research. Two surface bipolar finger electrodes (Model MLT 118F; ADInstruments) were placed on the pad of the left forefinger and middle finger, without isotonic paste to preserve the natural condition of the skin. We recorded EDA using an isolated amplifier (ML116 GSR Amp; ADInstruments) connected to a PowerLab/4SP system (ADInstruments) using constant-voltage excitation (22 mVrms at 75 Hz). The signal was recorded continuously using Labchart v7.4.1 software (ADInstruments) at a sampling rate of 10 Hz. The temperature of the room was constant (approximately 20°C). Before the emotion elicitation procedure, EDA was recorded during a training session with the relaxation procedure to calibrate the EDA recording and train the participant on filling out the DES. Participants were excluded if the EDA recording did not meet the criteria for data quality (many artifacts, low or high EDA, or no SCR).

We performed analyses on the final 60 seconds of each relaxation and film excerpt phase to control for differential stimulus lengths. We removed artifacts (body movements, speech, irregular breathing or disruptions of the skin electrode interface) by visual inspection. We imported data using Ledalab V3.4.3 toolbox (www.ledalab.de/) running on MATLAB 7.7 (MathWorks, Inc.). We ran continuous decomposition analysis (CDA). This method, based on deconvolution, works on the principle that an impulse is followed by a biexponential function (i.e., increased skin conductivity). The automated CDA identifies the impulse and detects the EDA shape, physiologic deviation and interindividual differences. Based on this method, phasic EDA (SCR) can be differentiated more accurately from tonic activity. Moreover, the CDA method is more suitable for long-lasting stimuli and less sensitive to artifacts than other deconvolution methods.

The minimum amplitude criterion for an SCR was 0.05 μS. We summed the amplitudes of the SCRs (i.e., the phasic

Fig. 1: Procedure for emotional induction. First, we performed a calibration of EDA measures using a period of relaxation. Then, we ran 5 cycles using 5 different film excerpts that targeted 5 emotions in random order, with continuous recording of EDA. Each cycle began with a relaxation procedure (3 min), followed by acquisition of a baseline EDA value and a first subjective emotional rating using the DES. Then, a target emotion was induced using a film excerpt. We used the final minute of the film excerpt for EDA processing, and then a second subjective emotional rating (DES) was performed. DES = Differential Emotion Scale; EDA = electrodermal activity.
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directly related to what is experienced as “unpleasant.” By doing so, positive emotions were related to what is experienced as “pleasant,” and negative emotions were related to what is experienced as “unpleasant.”

Target and nontarget subjective emotional experiences

Positive emotions (e.g., joy) were target items for happiness induction. Negative target items were those that best described the discrete emotion being induced (e.g., “disgusted, turned off, repulsed” was the target item for induction of disgust). Each film excerpt had only 1 target item from the 10 items on the DÉS; the other 9 were nontargets. For the positive film excerpt, we used the mean of the 9 nontarget items for comparison. For the negative film excerpts, we used the mean of all nontarget items across all 4 negative film excerpts (fear, anger, disgust and sadness). We used the mean of all 4 negative target and nontarget items to score negative subjective emotional experiences. We computed these scores before and after participants viewed the film excerpts.

Statistical analysis

We compared sociodemographic and neuropsychological variables using 1-way analyses of variance (ANOVA; 3 groups). We supplied post hoc comparisons (using a Dunnett test) if main or interaction effects were significant. We compared clinical variables for the Scz-A and Scz-NA groups using a Student t test.

We subjected tonic and phasic EDA measures to repeated-measures ANOVAs (RM-ANOVAs). Two within-participant factors had 2 levels (valence [positive and negative] and induction [relaxation and film viewing]), and 1 between-participant factor had 3 levels (group [controls, Scz-A and Scz-NA]). When RM-ANOVA main effects involving group differences were significant, we ran planned comparisons defined a priori for reduced emotional EDA measures in the Scz-A group compared with the other groups. To perform these planned comparisons, we first computed the differences between emotional measures (i.e., tonic and phasic) at baseline and during the induction phase: for example, emotional measurefilm excerpt – emotional meausurebaseline. We then used independent t tests to compare the mean of the variables between the Scz-A group and the control and Scz-NA groups. Planned comparisons increase statistical power by limiting the number of statistical tests that might result from complex factorial designs. As long as the planned comparisons rely on a priori hypotheses and are limited, it is not necessary to adjust for multiple comparison testing.

We performed a multiple linear regression to test for significant association between quantitative apathy measures (AES score) and tonic activity during induction of positive emotions, while accounting for confounding factors.

We used an RM-ANOVA to test for the effectiveness of the battery in individuals and groups in inducing subjective emotional experiences. We used an RM-ANOVA with 3 within-participant factors that had 2 levels (before and after the film excerpt), valence [positive and negative] and item [target and nontarget], and 1 between-subject factor with 3 levels (group [Scz-A, Scz-NA and control]). For group differences, we tested the main effects of the RM-ANOVA for induction × item × group and induction × item × group × valence. We tested the effectiveness of the battery using the main effect of induction × item. We did not perform post hoc comparisons.

Finally, we used an RM-ANOVA with 1 within-participant factor that had 5 levels (happiness, anger, fear, sadness, disgust) to test for an influence of film excerpt order on EDA measures during relaxation phases in controls.

We performed statistical analysis using SPSS version 22 (IBM Inc.).

Results

Table 1 displays the sociodemographic, clinical and neuropsychological characteristics for each group. We found no statistical differences between groups for age, sex, handedness, educational level or disease duration. We found no significant chlorpromazine-equivalent differences between the Scz-NA and Scz-A groups.

The findings for tonic and phasic activity, as well as target and nontarget item ratings for each group, are displayed in Table 2.

Executive function

Although it was not part of our working hypothesis, we found selective executive impairment in initiation (categorical fluency), mental flexibility (TMT), inhibition (Stroop) and strategy switching (Modified Wisconsin Sorting Test) in the Scz-A group compared with the control and Scz-NA groups. Attention was also impaired in the Scz-A and Scz-NA groups (Table 1).

Effectiveness of emotion elicitation procedure

We found a significant induction × item contrast (F1,25 = 118.6, p = 0.001), suggesting a satisfying effect of the battery on the target items, independent of group (i.e., across all individuals; n = 60). We found no group differences in target subjective emotional experiences for induction × item × group (F1,25 = 1, p = 0.7) or induction × item × valence × group (F1,25 = 1.9, p = 0.2). These results suggest that the targeted subjective emotional experiences did not differ between groups after emotional induction (Table 2).
Influence of film order on relaxation phase in controls

To verify that previous emotions did not alter baseline EDA recordings, we ran an RM-ANOVA with 1 within-subject factor that had 5 levels (happiness, anger, fear, sadness, disgust) for both the tonic and the phasic measures. We found no significant effect of emotion for the tonic ($F_{2,57} = 0.7$, $p = 0.6$) or phasic ($F_{2,57} = 0.3$, $p = 0.8$) measure. These nonsignificant results suggest that the order of the film excerpts had no influence on the EDA recordings.

Table 1: Participant sociodemographic, clinical and neuropsychological characteristics

<table>
<thead>
<tr>
<th>Characteristic*</th>
<th>Control (n = 20)</th>
<th>Scz-A (n = 20)</th>
<th>Scz-NA (n = 20)</th>
<th>Statistical test</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>35.5 ± 7.6</td>
<td>33.4 ± 9</td>
<td>33.8 ± 7.3</td>
<td>$F_{2,57} = 0.4$</td>
<td>0.7</td>
</tr>
<tr>
<td>Sex, male/female, n</td>
<td>14/6</td>
<td>15/5</td>
<td>14/6</td>
<td>$F_{2,57} = 0.9$</td>
<td>0.6</td>
</tr>
<tr>
<td>Educational level</td>
<td>13.3 ± 1.9</td>
<td>11.9 ± 2.1</td>
<td>13.2 ± 2.6</td>
<td>$F_{2,57} = 2.4$</td>
<td>0.1</td>
</tr>
<tr>
<td>Handedness, right/ left, n</td>
<td>17/3</td>
<td>15/5</td>
<td>17/3</td>
<td>$F_{2,57} = 0.9$</td>
<td>0.6</td>
</tr>
<tr>
<td>Chlorpromazine equivalent, mg</td>
<td>—</td>
<td>729.6 ± 451.1</td>
<td>528.3 ± 412.2</td>
<td>$t_a = 1.4$</td>
<td>0.2</td>
</tr>
<tr>
<td>Disease duration, yr</td>
<td>—</td>
<td>11.6 ± 5.9</td>
<td>10.5 ± 6.9</td>
<td>$t_a = 0.6$</td>
<td>0.6</td>
</tr>
<tr>
<td>Neuropsychological test scores</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calgary Depression Scale for Schizophrenia</td>
<td>—</td>
<td>3.15 ± 2.4</td>
<td>2.2 ± 1.7</td>
<td>$t_a = 1.4$</td>
<td>0.2</td>
</tr>
<tr>
<td>Abnormal Involuntary Movement Scale</td>
<td>—</td>
<td>0.2 ± 0.5</td>
<td>0.6 ± 0.9</td>
<td>$t_a = −1.6$</td>
<td>0.1</td>
</tr>
<tr>
<td>Apathy Evaluation Scale</td>
<td>—</td>
<td>50 ± 5.4</td>
<td>32.6 ± 4.3</td>
<td>$t_a = 11.3$</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Scale for the Assessment of Negative Symptoms</td>
<td>—</td>
<td>63.9 ± 16.7</td>
<td>28.4 ± 11.2</td>
<td>$t_a = 7.9$</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PANSS, overall</td>
<td>—</td>
<td>73.7 ± 12.2</td>
<td>54.6 ± 14.8</td>
<td>$t_a = 4.4$</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PANSS, positive subscale</td>
<td>—</td>
<td>10.1 ± 1.8</td>
<td>10.5 ± 4.1</td>
<td>$t_a = −0.4$</td>
<td>0.7</td>
</tr>
<tr>
<td>PANSS, negative subscale</td>
<td>—</td>
<td>29.1 ± 6.2</td>
<td>16.9 ± 4.6</td>
<td>$t_a = 7.1$</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PANSS, general psychopathology subscale</td>
<td>—</td>
<td>34.5 ± 6.2</td>
<td>27.2 ± 7.5</td>
<td>$t_a = 3.4$</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Digit span test, forward</td>
<td>5.75 ± 0.97</td>
<td>5.7 ± 1.3</td>
<td>6.1 ± 1</td>
<td>$F_{2,57} = 1.09$</td>
<td>0.3</td>
</tr>
<tr>
<td>Digit span test, backward</td>
<td>4.5 ± 1.4</td>
<td>4 ± 1.2</td>
<td>4.6 ± 1.2</td>
<td>$F_{2,57} = 1.2$</td>
<td>0.3</td>
</tr>
<tr>
<td>Verbal fluency test, categorical</td>
<td>31.1 ± 7.6</td>
<td>21.1 ± 8.7</td>
<td>28.2 ± 7.6</td>
<td>$F_{2,57} = 8.5$</td>
<td>&lt; 0.001†‡</td>
</tr>
<tr>
<td>Verbal fluency test, lexical</td>
<td>20.8 ± 7.1</td>
<td>17.1 ± 6.3</td>
<td>20.6 ± 6.7</td>
<td>$F_{2,57} = 1.9$</td>
<td>0.2</td>
</tr>
<tr>
<td>Stroop, interference</td>
<td>4.3 ± 7.6</td>
<td>−2.1 ± 9.1</td>
<td>1.8 ± 7.8</td>
<td>$F_{2,57} = 3.1$</td>
<td>0.05†</td>
</tr>
<tr>
<td>Trail Making Test, B–A time</td>
<td>34.1 ± 14.7</td>
<td>78.6 ± 57</td>
<td>40.1 ± 23.3</td>
<td>$F_{2,57} = 8.7$</td>
<td>&lt; 0.001†‡</td>
</tr>
<tr>
<td>Digit symbol coding test</td>
<td>72.1 ± 17.7</td>
<td>49.3 ± 12.8</td>
<td>56.7 ± 13.5</td>
<td>$F_{2,57} = 12$</td>
<td>&lt; 0.001†§</td>
</tr>
</tbody>
</table>

PANSS = Positive and Negative Syndrome Scale; Scz-A = schizophrenia with apathy; Scz-NA = schizophrenia without apathy.
*Results are shown as mean ± standard deviation unless otherwise indicated
†Significant post hoc Dunnett test between Scz-A and controls.
‡Significant post hoc Dunnett test between Scz-A and Scz-NA.
§Significant post hoc Dunnett test between Scz-NA and controls.

Table 2: Tonic and phasic EDA and emotion ratings on target and nontarget scales for positive and negative films*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 20)</th>
<th>Scz-A (n = 20)</th>
<th>Scz-NA (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tonic activity, positive emotion, µS</td>
<td>0.086 ± 0.155</td>
<td>0.016 ± 0.056</td>
<td>0.123 ± 0.133</td>
</tr>
<tr>
<td>Tonic activity, negative emotion, µS</td>
<td>0.033 ± 0.128</td>
<td>−0.019 ± 0.076</td>
<td>0.006 ± 0.093</td>
</tr>
<tr>
<td>Phasic activity, positive emotion, µS</td>
<td>0.344 ± 0.347</td>
<td>0.087 ± 0.333</td>
<td>0.377 ± 0.433</td>
</tr>
<tr>
<td>Phasic activity, negative emotion, µS</td>
<td>0.034 ± 0.316</td>
<td>0.016 ± 0.245</td>
<td>0.089 ± 0.286</td>
</tr>
<tr>
<td>Target scale ratings, positive film</td>
<td>2.798 ± 2.039</td>
<td>2.230 ± 2.398</td>
<td>3.138 ± 2.345</td>
</tr>
<tr>
<td>Nontarget scale ratings, positive film</td>
<td>0.018 ± 0.078</td>
<td>−0.174 ± 0.55</td>
<td>−0.136 ± 0.628</td>
</tr>
<tr>
<td>Target scale ratings, negative film</td>
<td>1.678 ± 1.074</td>
<td>1.969 ± 1.310</td>
<td>1.512 ± 1.364</td>
</tr>
<tr>
<td>Nontarget scale ratings, negative film</td>
<td>0.350 ± 0.433</td>
<td>0.300 ± 0.612</td>
<td>0.358 ± 0.702</td>
</tr>
</tbody>
</table>

EDA = electrodermal activity; Scz-A = schizophrenia with apathy; Scz-NA = schizophrenia without apathy.
*Mean ± standard deviation
†We have displayed findings for only the emotional induction procedure, such as elicitation = emotional variable, t = emotional variable, _t = emotional variable.
calculated the mean tonic activity between the positive and negative emotions for each participant, and then used planned comparisons to compare tonic activities across emotions between groups. We found decreased tonic activity across emotions (i.e., both positive and negative emotions) in the Scz-A group (mean ± standard deviation [SD] −0.002 ± 0.053) compared with the Scz-NA group (mean ± SD 0.065 ± 0.083, t = 3, p = 0.004) and the control group (mean ± SD 0.06 ± 0.12, t = 2.04, p = 0.048). This revealed significant reduced tonic activity in the Scz-A group across the emotions (i.e., both positive and negative) compared with the Scz-NA and control groups (Fig. 2).

Phasic activity

We found significant effects of induction (F₁,57 = 20.7, p < 0.001) and induction × valence (F₂,57 = 19.8, p < 0.001). We found no effect of group × valence (F₀,57 = 2.4, p = 0.1). We also found no effect of group × induction (F₁,57 = 2.6, p = 0.08), group × induction × valence (F₀,57 = 2.5, p = 0.09) or induction × valence × group (F₂,57 = 2.3, p = 0.1).

Quantitative apathy as a predictor of tonic EDA activity during induction of positive emotion

Disease severity, medications and executive dysfunction can confound the relationship between apathy and EDA in schizophrenia. The AES score was highly correlated with the PANSS total score (r = 0.8, p < 0.001), and with the SANS score (r = 0.9, p < 0.001). We did not control for these variables in the linear regression, because it would have violated the assumption of noncollinearity. Instead, we used the PANSS positive subscore to control for the severity of positive symptoms. We conducted a multiple linear regression; the dependent variable was tonic activity during induction of positive emotions, and the independent variables were AES, chlorpromazine equivalent, Wechsler Adult Intelligence Scale digit symbol coding, TMT B–A, categorical fluency (the 3 tests with significant differences between the 3 groups; Table 1) and PANSS positive subscore in the total sample of patients with schizophrenia. Apathy was significantly associated with tonic activity during positive emotions (t = −3.5, p = 0.001), accounting for 24% of the variance (Fig. 3).

Discussion

Using an innovative method to extract tonic and phasic activity from EDA recordings, we tested primarily for decreased tonic activity during induction of positive emotion Scz-A patients compared with Scz-NA patients and controls. We did not find specific decreased tonic activity during induction of positive emotion in the Scz-A group compared with the Scz-NA group and controls. However, RM-ANOVA revealed a significant effect of group × induction, with decreased tonic activity in Scz-A compared with Scz-NA and controls, independent of valence (i.e., across both positive and negative emotions). Although it was not part of our a priori hypothesis, this result suggested decreased emotion-induced arousal in the Scz-A group versus the Scz-NA group and controls. Using a quantitative measure of apathy in the total sample of schizophrenia patients, we also found that apathy was significantly associated with tonic-related emotional EDA during induction of positive emotion, accounting for 24% of the variance. Our results were consistent with the suggestion that apathy in schizophrenia is associated with reduced emotional experience, especially positive emotion.

We also found impaired executive function in the Scz-A group, in line with previous reports of apathy-related executive impairment in both chronic schizophrenia and first episode of psychosis. These results support the assessment of apathy in our sample.

Limitations

The first limitation of this study was the lack of a priori estimation of sample size. Indeed, the negative results (i.e., the valence × induction × group interaction) from our primary
hypothesis may have stemmed from lack of power, because the quantitative measure of apathy was significantly associated with tonic activity during induction of positive emotion. We are not aware of previous work trying to identify deficits in subjective emotional experiences in schizophrenia relative to apathy, so we were unable to estimate a specific sample size. We based our hypothesis on previous studies that induced emotion in people with schizophrenia using film excerpts and used EDA activities to measure subjective emotional experiences.\(^4^6\)

We used only EDA as a physiologic measure of arousal, limiting our conclusions. Future confirmation studies should combine different modalities to quantify arousal.

We induced several emotions sequentially that might have overlapped with each other. This limitation is inherent in all emotion-elicitation paradigms. We added an emotional "washout" period and randomized the order of the film excerpts to limit this possibility. Moreover, post hoc analyses showed no influence of film excerpt order on tonic and phasic measures during relaxation phases in controls. Despite our efforts to reduce the possible overlap of emotion induction, the possibility of overlap should be kept in mind when interpreting our results.

We deliberately avoided assessing apathy in healthy young adults because, from our experience, the AES shows only small variability in this population (data not published).

Participants with schizophrenia were taking antipsychotic and antidepressant medications that might have affected the EDA recordings.\(^4^9\) Antipsychotics can cause hypohidrosis with an antimuscarinic effect and induce lower EDA activity. Antidepressants can cause hyperhidrosis, leading to a noisy signal during EDA recordings. Our ethics committee did not authorize a drug washout period, such as the one in a previous work.\(^4^8\) To limit the potential effect of this issue, we carefully inspected EDA recordings and applied a conservative method of artifact rejection. Moreover, we included the measure of chlorpromazine equivalents in the linear regression, and it was the least significant predictor of tonic activity during positive emotion, explaining only 6.5% of the variance (\(p = 0.8\)). This finding suggests that the antimuscarinic effect associated with antipsychotic medications could not explain the difference in EDA recordings during emotion induction in this sample.

We induced more discrete negative emotions (anger, fear, sadness and disgust) than positive emotions (happiness). We averaged the scores for the negative film excerpts and used single scores for the positive excerpt. As a result, the general category of positive emotion was restricted to happiness, and positive emotions were measured with a single estimator, while negative emotions were measured with 4 estimators. Using this approach, we could not form conclusions about differences between positive tonic EDA emotional components and a specific discrete negative tonic EDA emotional component in schizophrenia patients with apathy. As well, we arbitrarily assigned anger, fear, sadness and disgust as “negative” emotions and happiness as a “positive” emotion. Although this seemed intuitive, it was a limitation for the present study.

Conclusion

With the above limitations in mind, the present study yields new insights into the emotional component of apathy in schizophrenia. Alongside the specific cognitive deficits associated with apathy in chronic schizophrenia, our findings provide further arguments for an independent cluster of negative symptoms, namely apathy, associated with altered positive emotions in schizophrenia. Therapeutics that enhance the anticipation of positive emotions could offer new strategies for alleviating apathy in schizophrenia. Our results also point to the need to assess apathy in future studies using EDA (and possibly other psychophysiological recordings) during emotional induction in schizophrenia.

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Head motion: the dirty little secret of neuroimaging in psychiatry

Carolina Makowski, BSc; Martin Lepage, PhD; Alan C. Evans, PhD

Introduction

Neuroimaging studies of psychiatric disorders often face the dilemma of how to handle patient head motion — a dilemma we are reluctant to confront. On one hand, assembling a sufficiently large cohort for meaningful study is a painstaking process, and there is a natural desire to use all the data collected. On the other hand, many patient populations exhibit significantly increased motion in the scanner compared with healthy controls,1,2 suggesting that more scans must be excluded to obtain a clean enough sample for a significant result. Do common artifacts such as motion really make a difference in large samples? If so, what should be done about it?

Defining motion is deceivingly simple at the core, but it has been surprisingly difficult for researchers to reach consensus over the threshold of acceptable motion that can be tolerated within an MRI study. Moreover, it has become apparent that motion artifacts in neuroimaging research cannot be ignored.3 Both structural and functional imaging domains are affected by motion artifacts; studies have highlighted that results obtained with the original sample compared with a clean, quality-controlled subset of the data yield significantly different effect sizes and even different neuroanatomical substrates for interpretation. Recently, an editorial by Weinberger and Radulescu4 brought this issue to the attention of researchers in psychiatry, challenging the common interpretation of findings derived from case-control MRI studies as altered “neurobiology” in patients compared with controls. Instead, the authors urged the field to more critically and carefully acknowledge MRI-derived confounds, such as head motion, that may be clouding key findings in the literature.

In 2002, Blumenthal and colleagues5 were among the first to report a significant negative association between grey matter brain volumes and severity of motion artifact. This finding is of particular importance in the study of pediatric populations and neurodevelopmental disorders, given children’s tendency to be more restless and, in turn, exhibit more movement in the scanner. This realization has rebounded in the literature as researchers attempt to disentangle and reconcile the disparate structural trajectories associated with normal brain development — a critical concept that requires consolidation if we are to broach the topic of abnormal brain trajectories in psychiatric disorders. Nearly a decade ago, Shaw and colleagues6 conducted a seminal study on the cortical thickness trajectories underlying normal development in a sample ranging from 3.5 to 33 years of age and found predominantly nonlinear associations between cortical thickness and age, with notable peaks within the first decade of life. However, the age window leading up to these peaks of cortical development encompasses an age group (i.e., 5–10 yr) where children have been shown to exhibit the most movement in the MRI scanner.7 These confounds of movement provide a feasible explanation for the challenge in replicating these findings subsequently in independent samples.8–12 Ducharme and colleagues13 addressed such inconsistencies
using a sample with a similar age range as that in the study by Shaw and colleagues,6 but with the additional component of three levels of quality control: none, standard and stringent. With increasing stringency of quality control (i.e., removal of more scans with motion), the reported nonlinear cortical thickness associations with age disappeared; instead, predominantly linear associations across most of the brain remained. Characterization of neurodevelopmental trajectories of white matter have also been of great interest and are similarly likely to be significantly influenced by motion. A recent investigation by Realf and colleagues5 confirmed this sentiment, showing that significant correlations between commonly reported diffusion metrics (i.e., fractional anisotropy and mean diffusivity) and age are weakened with increased motion estimates.

These examples are convincing evidence that motion artifacts have a significant impact on structural imaging results, but why? As a simple point for comparison, motion in an MRI is akin to motion in any image taken with a camera: the higher degree of motion present, the more blurred and fuzzy the image will be. The basic physics underlying MRI data acquisition add another layer of complexity to the effects of motion or head displacement in a resulting image. When a patient moves in the scanner, it is the spatial frequencies of the MRI, or k-space, that are perturbed. The errors introduced give rise to motion artifacts that are not localized, but propagated throughout the image (e.g., ghosting, ringing, reduced surface area and cortical thickness) and age are weakened with increased motion estimates.

Attention-deficit/hyperactivity disorder (ADHD) poses a particularly interesting challenge in psychiatry, as some of the prominent clinical features characterizing this neurodevelopmental disorder include motion-related symptoms. Two recent studies assessing functional connectivity in ADHD have used rigorous quality-control procedures to more confidently draw upon biologically relevant networks underlying the disorder.24,25 Mirroring findings in structural imaging, one of these studies also reported reduced effect sizes compared with those reported in previous literature on functional connectivity patterns in ADHD, which the authors largely attribute to their cleaner dataset.25 Of note, the study by Fair and colleagues24 demonstrated that correcting for motion can have clinical utility in predicting subtypes of ADHD (i.e., combined v. inattentive subtypes). Specifically, the authors used three different models of motion correction in a machine learning algorithm to classify subgroups of patients with ADHD, finding a relatively high degree of accuracy (i.e., 71%–77%) with two of these models.

One of the largest and most comprehensive investigations examining motion bias across clinical cohorts (i.e., ADHD, ASD and schizophrenia) and different post-processing software was recently conducted by Pardoe and colleagues.7 This
study used resting-state fMRI acquisitions to inform the degree of movement present in T1-weighted images and examined this quantitative metric of motion in the context of brain morphometry, namely cortical thickness, grey/white matter contrast and grey matter/subcortical volumes. As expected, motion estimates were higher in all clinical populations than in controls and for the extreme ends of the age distribution (i.e., < 20 yr and > 40 yr). Intriguingly, cortical thickness and measures of cortical contrast were more affected by motion than volumetry, although the latter was affected to a greater degree by the choice of segmentation method. This paints a rather intricate and complex picture of the various levels by which motion may impact neuroimaging analyses of clinical cohorts and underscores the importance of, at minimum, having a clear and consistent quality-assurance protocol to exclude scans visibly affected by motion artifacts. Furthermore, given the manner by which different pipelines may handle motion, it is equally important to study thoroughly the processed outputs — successful runs may not always be linked to trustworthy results.

Many of the examples discussed thus far have come from cross-sectional study designs. However, longitudinal neuroimaging studies are integral in capturing dynamic brain changes that underlie the clinical course of psychiatric illness. Notably, it has been shown previously that head motion shows some degree of test–retest reliability. 26–30 Thus, for longitudinal studies, motion correction should be considered for all time points. It may also be the case that only a subset of longitudinal scans from a single participant are retained for analysis after completing quality control. Fortunately, statistical methods, such as multilevel modelling (i.e., mixed-effects models, hierarchical linear models) are designed to handle missing data points. For a more complete description of these methods, see Singer and Willet. 31

Guidelines to minimize and correct for head motion artifacts

In the sections that follow, we propose a quality-control workflow that can serve as a framework for both prospective and retrospective datasets. This workflow is depicted in Figure 1. In addition to psychiatric patient samples, it should be noted that the confounds of head motion in the scanner also exist in other fields of biomedical imaging, such as neurology. As such, we propose that this quality-control workflow can be readily generalized to other participant populations, such as patients with neurologic disorders characterized by motor symptoms.

Considerations for prospective datasets

Behavioural training

One of the simplest and arguably most effective techniques in minimizing head motion is to ensure the participant remains as still as possible within the MRI scanner. Sedation and general anesthesia have been successful in minimizing motion, particularly within pediatric populations, but such methods introduce additional confounds and are not always feasible or desirable in most research studies. 32–34 Further, several studies have shown that it is possible to obtain successful scans within pediatric and psychiatric patient samples without the use of more invasive procedures. 7,32,33,35–37 For instance, it is good practice to acclimatize the participant to the MRI setting using a mock scanner and to provide adequate training tailored to the population of interest. If a mock scanner is not available, videos simulating the MRI environment may be used (e.g., http://vimeo.com/32255381). 38 Acclimatizing children to the scanner environment requires more personnel and preparation time than required for adolescents or adults. Detailed pediatric protocols, such as the inclusion of visual aids or allowing the child to view a movie during the scan, have been reported with high success rates. 7,39,40 Neurofeedback paradigms have also been explored, where real-time feedback is provided to participants on their patterns of movement in the scanner, 41 although this technique has had limited returns thus far. In summary, incorporating pre-scan protocols with attention to participant compliance and behaviour during scan acquisition will enhance the likelihood of a successful scan. These points are highlighted in Figure 1, under the “behavioural training” portion of the quality-control workflow.

Technical and methodological considerations

A clear study protocol is required before any scans are collected. The mantra of “quality over quantity” is an important consideration when deciding upon a scanning protocol for a psychiatric neuroimaging study. Focusing on one or two modalities and optimizing their quality (e.g., by acquiring multiple structural imaging scans or functional imaging runs) may be a more efficient use of scan time than acquiring images using many modalities at suboptimal quality. Minimizing motion during scan acquisition may also require technical intervention. In recent years, various research groups have worked diligently toward developing and improving imaging protocols to correct for motion as scans are being acquired in real time. These new techniques hold promise in significantly reducing the proportion of scans that might otherwise need to be excluded owing to motion confounds. For instance, prospective motion correction (PROMO) has been proposed to counter the effects of inevitable participant movement in a proactive manner. 7,39,40 The PROMO framework acts in real time during the acquisition of a scan, where motion can be detected through maintenance of a fixed coordinate system in relation to the participant’s position within the scanner, and images are automatically rescanned if significant motion artifact is sensed. This procedure has been shown to drastically reduce the caveats associated with motion in children, and intuitively is expected to hold similar benefits for clinical populations. Framewise integrated real-time MRI monitoring (FIRMM) has also been developed recently to quantify degree of motion from frame to frame as the data are being acquired, allowing MRI technicians and researchers on site to actively monitor head movement accurately and proactively. 44 Similarly, measures of head position and orientation while scanning have been used to improve the quality of images acquired using positron emission tomography (PET). 45 These points can be found in the “technical/methodological” portion of the quality-control workflow in Figure 1.
Fig. 1: Proposed quality control workflow for MRI data sets, both prospective and retrospective. Note: this figure is designed as a simplified guide and is not a comprehensive workflow for all imaging modalities and clinical populations. DTI = diffusion tensor imaging; fMRI = functional MRI; QC = quality control.
Considerations for retrospective datasets

The previous section introduced practical suggestions for prospective data collection. A natural question that follows is what solutions exist to handle motion artifacts within the abundance of clinical neuroimaging data that have already been amassed? Having a quantitative measure of motion — a variable that could be used as a means to better match patient and control samples — could be beneficial. We provide some suggestions in Figure 1 that can be applied to raw imaging data to help mitigate the effects of motion artifacts on retrospective datasets.

Correcting for motion after image acquisition

Motion estimates from functional imaging can be used to inform analysis of $T_1$-weighted structural scans, although caution should be exercised when using a proxy measure of motion from another imaging modality. Motion during one acquisition does not necessitate motion during another, even if both acquisitions are collected within the same session. In the realms of diffusion imaging, a total motion index (TMI) has been defined based on several proxies for motion, including calculations of translation, rotation and signal drop-out, to obtain a collective “score” of motion. The TMI can then be used as a regressor in the comparison of group differences. Scores of modality-specific toolboxes and workflows have also been developed to help manage the effects of motion on image quality, for application to structural $T_1$-weighted images, diffusion-weighted imaging, resting-state and task-based fMRI, positron emission tomography and arterial spin labelling.

Choosing MRI scans for analysis

We have argued the idea that a “clean” dataset, with minimal motion impact, will yield a more biologically valid finding. However, a clear consensus on data cleaning standards has not yet emerged, despite worthy efforts in that direction. This is largely attributable to the fact that defining inclusion/exclusion criteria of an MRI scan rests largely upon the research question at hand and on the imaging modality. Standards in fMRI may be more explicit on this point; for instance, Power and colleagues proposed a widely cited method of “scrubbing” fMRI data to remove frames with a high degree of motion and significant amplitude changes in the blood oxygen level–dependent (BOLD) signal. Alternatives to handle different types of motion-related variance in fMRI acquisitions have been described recently by Caballero-Gaudes and Reynolds. Image-quality metrics can also be derived automatically (e.g., signal-to-noise and contrast-to-noise ratios), which provides a quantitative metric that can allow researchers to quickly pinpoint “outliers” that should be flagged (see the “raw image quality control” section of Fig. 1). This can be particularly useful when carrying out quality control of large imaging datasets. The critical point is that all quality-control procedures, either on raw images or derived measurements, must be completed prior to any statistical analysis across study participants. There is now an increased sensitivity to the perils of “data fishing,” or post hoc analysis (p-hacking), where final published samples are chosen based on the desired result. The field is responding to these issues and, for some journals, preregistration of studies and proposed methods are mandatory. It is likely that these initiatives will resonate quickly in the publication sphere. As alluded to earlier, recent initiatives to standardize and consolidate best practices for data analysis and sharing include recommendations for how to handle motion in neuroimaging (http://www.humanbrainmapping.org/COBIDASreport) and active participation in hackathons to reach a consensus on these pressing issues. Finally, many researchers with open datasets are beginning to publish quality-control procedures pertinent to their studies and are encouraging users to follow similar standards; examples include the Human Connectome Project and UK Biobank (see the “raw image quality control” section of Fig. 1). The availability of these large, open datasets will also enable researchers to replicate results and approach the “ground truth” for many questions that still plague the field regarding brain development and alterations in psychiatric disorders. A recent example can be found in the work of Mills and colleagues, where four independent datasets from three different countries were used to examine trajectories of brain development from childhood to early adulthood, finding high replicability across samples. Such replication studies are highly encouraged in the field of psychiatry, especially as more samples of psychiatric patients are being placed in the public domain (e.g., ABIDE, ADHD-200 [http://fcon_1000.projects.nitrc.org/indi/adhd200/] and the Bipolar-Schizophrenia Network on Intermediate Phenotypes [http://schizconnect.org/]).

Conclusion

The quantification of motion to be accounted for when analyzing data is certainly attractive, compared with mere exclusion of scans, in the analysis of retrospective data. However, sometimes a bad scan is just a bad scan, and it may be worthwhile to exercise the art of “letting go” in severe cases. Neuroimaging technology is developing quickly, and it is reasonable to expect that better algorithms and solutions for handling the blurred edges in our scans will be coming our way. Until then, do not shy away from data cleaning; the rewards gained in validity are worth the loss of a few scans.

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Psychopharmacology for the Clinician

The information in this column is not intended as a definitive treatment strategy but as a suggested approach for clinicians treating patients with similar histories. Individual cases may vary and should be evaluated carefully before treatment is provided. The patient described in this column is a composite with characteristics of several real patients.

Clozapine-induced obsessive–compulsive symptoms: mechanisms and treatment

David D. Kim, MS; Alasdair M. Barr, PhD; Randall F. White, MD; William G. Honer, MD; Ric M. Procynshyn, PharmD, PhD

A 35-year-old man with treatment-resistant schizophrenia was prescribed clozapine. Three weeks later, at a dose of 400 mg/d, his psychosis improved substantially, but contamination obsessions and cleaning compulsions developed, occupying a lot of his time. His family history was positive for obsessive–compulsive disorder (OCD); he had an aunt with OCD. Clozapine was thought to be the cause of his obsessive–compulsive symptoms (OCS), so the dose was reduced to 300 mg/d over a period of 1 week. Although the OCS abated, his psychosis worsened. Clozapine was reincreased to 400 mg/d and the OCS returned. Aripiprazole (15 mg/d) was added, and the OCS gradually diminished, with complete resolution after 5 weeks.

Determining the origin of OCS in individuals with schizophrenia is a clinical challenge, because OCS can be an idiopathic component of positive symptoms or an adverse effect of antipsychotic medications.1 Clozapine is the antipsychotic most well known to induce OCS.2,3 Although the prevalence of OCS among patients treated with clozapine tends to vary widely owing to methodological heterogeneity among studies, a recent study reported a mid-point prevalence of 47%.4

Mechanisms underlying clozapine-induced OCS have not been fully delineated. However, theories implicating various neurotransmitter systems, in particular serotonin and dopamine, have been put forward.

As selective serotonin reuptake inhibitors (SSRIs) are known to treat OCD and OCS,5 clozapine’s antiserotonergic effects (e.g., antagonism of 5-HT1A and 5-HT2C receptors) are potential causal factors.6,7 However, the mechanism may be more complex, given the evidence that 5-HT2C receptor antagonists have beneficial effects in animal models of OCD.8–9 Such an effect may be region-specific (e.g., orbitofrontal cortex).10–11 It has been hypothesized that hypersensitivity of 5-HT2C receptors, as a result of chronic antagonism, may play a role in clozapine-induced OCS.12–13 This is in line with evidence that SSRIs act not only by enhancing 5-HT neurotransmission, but also by normalizing 5-HT2C receptor function via its desensitization.14

In patients with SSRI-resistant OCD, antipsychotic augmentation has been particularly effective, implying dopaminergic involvement in OCD.15 Neuroimaging studies implicate hyperdopaminergic activity in the pathophysiology of OCD, where dopaminergic and serotonergic pathways have reciprocal relationships.16 In this regard, clozapine’s strong antagonism of 5-HT1A receptors is posited to disinhibit nigrostriatal dopaminergic neurons. Another hypothesis implicates dopamine supersensitivity secondary to treatment with antipsychotics with high affinities for D2 receptors.17,18 Thus, when switching patients to clozapine, increased dopaminergic activity resulting from reduced striatal dopaminergic inhibition may unmask OCS.19

Other causal factors need to be considered. For instance, genetics may play a role, according to a finding that individuals with polymorphisms in the genes that regulate glutamate transmission, such as SLC1A1 and GRIN2B, were more susceptible to clozapine-induced OCS.20 Findings of abnormal brain activation patterns and neurocognitive deficits associated with treatment with antiserotonergic antipsychotics (e.g., clozapine and olanzapine) also need to be considered in relation to clozapine-induced OCS.21,22

Clozapine-induced OCS may improve if the dose is reduced or discontinued.23 However, some case reports found OCS to improve when the dose of clozapine was increased.2 The conflicting results may be explained by the heterogeneous etiology of OCS.1,3 As clozapine dose reduction or discontinuation carries the risk of exacerbation of target symptoms (e.g., psychosis), other treatment options may be preferred.

Add-on SSRIs can be effective in treating clozapine-induced OCS.2,24 Mechanisms may involve enhancement of 5-HT neurotransmission and normalization of 5-HT2C receptor function.14 Of note, SSRIs, in particular fluvoxamine, can increase clozapine blood levels, and given that higher plasma clozapine and norclozapine concentrations may be associated with the development of clozapine-induced OCS,20 careful drug monitoring should be considered when using add-on SSRIs.

Add-on antipsychotics can also be considered. Studies have found add-on aripiprazole to be effective in treating clozapine-induced OCS.21,22 The antiobsessive effect of aripiprazole may come from its 5-HT1A partial agonism.23 Another candidate is amisulpride, a selective D2/D3 antagonist without an affinity for 5-HT; receptors.24 However, evidence for amisulpride, as an add-on or a switch, to treat clozapine-induced OCS is lacking, although a switch to amisulpride was shown to improve OCS induced by other atypical antipsychotics.24

Other pharmacological considerations include adjunct clonipramine and mood stabilizers (e.g., lamotrigine and valproic acid).3 Evidence for the effectiveness of nonpharmacological treatment, such as cognitive-behavioral therapy, on clozapine-induced OCS is not clear.

Current evidence suggests that clozapine may induce OCS via complex...
serotonin–dopamine mechanisms and could be treated with adjunct agents (e.g., SSRIs and aripiprazole) that alter these neurotransmitter systems. Clinicians need to be vigilant for clozapine-induced OCS and consider appropriate treatment strategies for these symptoms when they become a clinical concern.

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