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SEARCHING HUMAN BRAIN FOR MECHANISMS OF PSYCHIATRIC DISORDERS

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Abstract

In the past 25 years, research on the human brain has been providing a clear path toward understanding the pathophysiology of psychiatric illnesses. The successes that have been accrued are matched by significant difficulties identifying and controlling a large number of potential confounding variables. By systematically and effectively accounting for unwanted variance in data from imaging and postmortem human brain studies, meaningful and reliable information regarding the pathophysiology of human brain disorders can be obtained. This perspective paper focuses on postmortem investigations to discuss some of the most challenging sources of variance, including diagnosis, comorbidity, substance abuse and pharmacological treatment, which confound investigations of human brain.

Keywords

human postmortem; in vivo imaging; confounding factors; psychiatric disorders; comorbidity

INTRODUCTION

There is no controversy regarding the fundamental role of human brain studies in investigations of the pathophysiology of psychiatric disorders. These illnesses do after all involve changes in cognitive and emotional behaviors, and there is no other organ of the

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body where such functions receive their primary mediation. Because these disorders are only diagnosable in patients, the human brain becomes by necessity the primary object of investigation. A clear example of the success of this strategy is the involvement of GABAergic interneurons in the pathophysiology of disorders such as schizophrenia, bipolar disorder and autism (Akbarian and Huang, 2006; Akbarian et al., 1995; Benes, 2000; Benes and Berretta, 2001; Benes et al., 1992; Blatt and Fatemi, 2011; Costa et al., 2004; Cotter et al., 2002; Fatemi et al., 2011; Fatemi et al., 2009; Guidotti et al., 2011; Guidotti et al., 2005; Heckers et al., 2002; Lawrence et al., 2010; Lewis et al., 2005; Lewis et al., 1999; Torrey et al., 2005; Volk et al., 2000; Woo et al., 1998) (see also articles included in this Special Issue). The postulated role of the GABA system in schizophrenia, the main focus of this special issue, has originated from a variety of technological approaches to the study of human brain that include both in *vivo* brain imaging and postmortem investigations of the human brain.

While studies of this type led important breakthroughs in our knowledge, and offer great promise for the future, they also present daunting difficulties related in great part to the inherent complexity of psychiatric disorders and the many potential confounding factors. While it is inconceivable for studies on the pathophysiology of schizophrenia and other psychiatric disorders to be undertaken without investigations on the human brain, the challenges these disorders pose can only be met by a highly diverse and complementary array of methodological approaches, ones capable of integrating human studies with investigations of non-human mammalian species and in *vitro* cell systems. This integration makes it possible for studies of cognition and emotion to be understood within the context of detailed cellular and molecular mechanisms related to neural circuitry. Our understanding of psychiatric disorders critically depends upon the inherent synergy between brain imaging and postmortem studies of human brain, human genetic investigations, experimental animals and in *vitro* models, and their ability to reciprocally complement one another with their respective strengths and weaknesses. In this context, it is essential to maintain an open and constructive dialogue regarding the strengths and weaknesses of each of these respective methodologies. The intent of this perspective paper is to stimulate a dialogue that will help to highlight some of the main challenges that studies of the human brain, and postmortem in particular, in psychiatric disorders present to the field of translational neuroscience.

Methodological innovations applied to postmortem investigations on the human brain have in recent years rapidly amplified their potential and usefulness. Increasingly more sophisticated methodological approaches, such as studies of microarray-based genomic integrity, gene expression and methylation, cell level gene and microRNA expression profiling, as well as epigenetics, proteomics, and quantitative high resolution microscopy, hold important promises for progress (Benes, 2012; English et al., 2011; Horváth et al., 2011; Mitchell et al., 2014; Moreau et al., 2011; Pidsley and Mill, 2011). In parallel, attention to a growing number of potential confounding variables, and advances in our understanding of the effects on the brain of systemic physiological and pathological conditions, has contributed to elevate accepted standards. This process is leading toward increasingly more rigorous approaches with regard to diagnostic criteria, comorbidity, effects of pharmacological treatment and drugs of abuse, among many. Far from wishing to

push forth one specific approach over others, a discussion of commonly used strategies is intended to encourage an ongoing conversation about valid approaches to the study of postmortem human brain.

Although we focus on human postmortem investigations, it is important to remember that several of the aspects discussed below are also critical to in *vivo* imaging studies. Both in *vivo* and postmortem human studies bring to the fore issues related to reliable psychiatric diagnosis, comorbidity, substance abuse, current and past pharmacological treatment and compliance. Each of these issues presents a distinct challenge to both postmortem and brain imaging studies of the brain in relation to psychiatric disorders. For both, it is critical to emphasize the importance of gathering extensive, detailed, information on study subjects. While this task can be particularly challenging for postmortem studies, which rely heavily on medical records and family interviews/questionnaires, the availability of toxicological and neuropathological assessments represents a significant advantage, as discussed in more detail below. The reliance of human postmortem studies on the availability of subject information underlies the importance of modern approaches to brain banking and thoughtful screening of available information. Other important aspects related to human brain studies have been elegantly discussed by other authors (Deep-Soboslay et al., 2011; Harrison, 2011; Horváth et al., 2011; McCullumsmith and Meador-Woodruff, 2011; Tunbridge et al., 2011).

DIAGNOSIS

The debate about diagnostic criteria, highlighted during the recent release of the DSM 5, raises important issues relative to categorical versus dimensional diagnostic approaches (Barch et al., 2013; Heckers et al., 2013). These issues are equally important to clinicians and researchers, as they impact on the conceptual framework and design of group comparisons. Clinical presentations do not often fit into categories and may change over time, raising important questions with regard to the nature of a group, such as schizophrenia, and the testing of hypotheses related to this disorder. For instance, should studies on psychosis include all subjects with psychosis independent of a diagnosis of schizophrenia, schizoaffective, psychotic bipolar disorder and other psychotic disorders? Some patients with an initial diagnosis of one psychotic disorder will be reclassified if they are followed for several years (Bromet et al., 2011; Salvatore et al., 2011). Which diagnosis should be considered the best estimate diagnosis at various time points throughout the illness? While the main goal of a specific study will dictate its design in this regard, we expect that evolving diagnostic criteria in psychiatry will continue to shape the conceptual framework of research studies on these disorders. Conversely, pathophysiological findings contribute to our understanding of the relationships between disorders. For instance, abnormalities affecting the GABAergic system have been consistently reported in schizophrenia and bipolar disorder (Benes and Berretta, 2001; Blum and Mann, 2002; Costa et al., 2007; Gonzalez-Burgos and Lewis, 2008; Guidotti et al., 2011; Heckers et al., 2002; Lewis et al., 1999). However, evidence from the hippocampus indicates that the molecular mechanisms underlying these abnormalities may be different in the two disorders (Benes, 2010). Thus, a disruption of intrinsic inhibitory circuits may represent a shared pathological feature, perhaps underlying overlapping clinical domains, and yet resulting from distinct pathophysiological mechanisms.

For the large majority of postmortem studies, the diagnosis is based exclusively on medical records and family questionnaires. The retrospective nature of these diagnoses represents a clear limitation of these studies. However, it is important to consider that medical records obtained for each brain donor contain detailed information on clinical presentation, hospitalizations, prescription drugs and other therapeutic interventions. Importantly, this information often spans the duration of the illness, and thus represents the views of several clinicians at different times. Clinical records, together with extensive family questionnaires providing a wealth of useful information, such as previous use of drugs of abuse, premorbid symptomatology, and status of living, are reviewed by trained psychiatrists following brain donation. Standard toxicological panels (see below) are also obtained and used to evaluate whether drugs of abuse were taken by control and diseased subjects prior to death. Thus, although carried out in absence of the patient, the diagnostic process for postmortem studies takes into account the full course of the illness for each patient, coalescing diagnoses made at different stages of the disorder with information contained in the medical records and provided by the family, in their totality offering a unique diagnostic perspective, a 'bird's-eye' view of the disorder for each subject including changes of its presentation over time. In addition, prospective recruitment of tissue donors, when possible, allows rigorous clinical diagnosis through antemortem clinical assessment. Although this approach presents considerable logistic challenges, it holds great potential to alleviate some of the difficulties related to diagnosis (Deep-Soboslay et al., 2011).

COMORBIDITY

Comorbidity of psychiatric disorders with other brain disorders, as well as systemic disorders, is a potential source of variance and deserves careful consideration. Within the realm of psychiatric disorders, several clinical domains and/or categorical diagnosis often coexist, such as psychosis, anxiety and depression (Braga et al., 2013; Cerda et al., 2010; Dernovsek and Sprah, 2009; Pallanti et al., 2013; Potuzak et al., 2012; Simon, 2009). The variety of psychiatric disorders represented in the large Scottish family carrying a translocation of the Disrupted In Schizophrenia (DISC-1) gene, and substantial overlap of genetic vulnerabilities among several psychiatric disorders further weaken clinical boundaries between psychiatric conditions (Blackwood et al., 2001; Millar et al., 2001; Millar et al., 2000; Smoller, 2013).

Drug addiction, a condition with frequent comorbidity with other psychiatric disorders (Barnett et al., 2007; Chand et al., 2014; Conway et al., 2006; Katz et al., 2008; Lasser et al., 2000; National et al., 2011; Saban et al., 2014; Wisdom et al., 2011) and of particular interest in the context of investigations on the human brain, is discussed below under 'Substance Use Disorders". Several other brain disorders, such as vascular conditions, Alzheimer's disease and Parkinson's disease, may be comorbid particularly in elderly patients and may represent exclusion criteria in studies focusing on psychiatric disorders. Systemic illnesses, such as metabolic, cardiovascular, inflammatory conditions are often associated with psychiatric disorders (e.g. Casey et al., 2011; Ferentinos and Dikeos, 2012; Lang and Borgwardt, 2013; Mitchell et al., 2013). Growing evidence points at robust interactions between several of these conditions and disorders such as major depression (Lang and Borgwardt, 2013) and schizophrenia (Casey et al., 2011; Ferentinos and Dikeos,

2012; Mitchell et al., 2013). Notably, large numbers of cytokines/growth factors and hormones involved in systemic conditions also have distinct neural functions, some currently emerging as potential contributors to the pathophysiology of neurological and psychiatric disorders. For instance, interleukin 1 and 6, tumor necrosis factor α, transforming growth factor β1 and brain derived growth factor have been consistently found to be altered in psychiatric illnesses such as mood disorders and schizophrenia, and suggested to mediate interactions between these illnesses, inflammation and stress (Barbosa et al., 2014; Catena-Dell'Osso et al., 2013; Chavarria-Siles et al., 2007; Fineberg and Ellman, 2013; Hashimoto, 2010; Kim et al., 2004; Poon et al., 2013; Rosenblat et al., 2014). Several of these cytokines were shown directly affect GABAergic transmission, impacting GABA A-mediated synaptic strength and dendritic homeostasis (Beattie et al., 2002; Benes, 2010; Stellwagen et al., 2005; Sun et al., 2010; Vezzani et al., 2008; Wang et al., 2000), pointing to the need to better understand, as well as control for, the relationships between immune factors and neurotransmission in psychiatric disorders..(Benes, 2010)

Accumulating evidence for these interactions is not only adding to our understanding of these disorders, but also informing the design of our studies by identifying potential factors that may impact on outcome variables. A particular instance of comorbidity, relevant to postmortem studies, is that of disorders leading to the subject's death. In the majority of cases, particularly in elderly patients, the cause of death is disease-related (e.g. cancer, pneumonia), and may involve prolonged agonal states and intensive pharmacological treatment. Thus, several distinct aspects related to cause of death, such as potential tissue quality degradation due to hypoxia, exposure to opioids and other agents affecting the CNS, and the nature of the illness itself, need to be considered with attention, taking also into account methodologies and outcome measures specific to each study. For instance, if a main outcome measure of the study is directly or indirectly related to cytokines/growth factors, molecules that systemically are involved in inflammation and/or autoimmune disorders, these conditions need to be taken into account, whether they represent the direct cause of death or even were present during the last months of life. Even suicidality, a sequela of severe prolonged, unremitting psychiatric disorders, must be considered as a potential confounding variable. Studies have demonstrated that the brainstem serotonin systems show replicable abnormalities in suicide patients, regardless of their primary psychiatric diagnosis (Mann et al., 2001; Mann et al., 1999).

How can studies on human brain, and particularly postmortem investigations, take into account comorbid illnesses when analyzing data for group effects related to primary neurological or psychiatric diagnosis? Careful screening of medical records often provides sufficient information on diagnosis and treatment of comorbid conditions during the course of the illness and, particularly, before death, although there are obvious limits to the amount of detailed information available. Data from standard toxicological panels can be used to assess recent exposure to many of the medications administered during the last period before death. For instance, it is often the case, that subjects who have succumbed to a malignancy have been treated with opiates and benzodiazepines to manage pain. In addition to clinical records and toxicology, postmortem studies can avail themselves of a very powerful tool, a detailed gross and microscopic neuropathological assessment performed routinely on each and every brain as part of brain banking procedures. Neuropathological assessment provides

objective and often quantifiable evidence for a broad range of neurodegenerative disorders, such as Alzheimer's disease and other dementias, Huntington's chorea, Parkinson's diseases, tumors, infarctions, vasculopathies and inflammatory conditions. Importantly, histological evaluations included in the neuropathology reports can detect early signs of these conditions even when they may be too mild to generate clinical manifestations. Gross and microscopic evidence from neuropathology is invaluable in assessing comorbidity. We suggest that its inclusion in stepwise statistical models may be a particularly effective manner to estimate the potential contribution of co-morbid conditions to pathological findings.

Finally, tissue indices such as pH and RNA integrity number (RIN) may be used to assess tissue quality. In particular, RNA expression measurement may be substantially impacted by prolonged agonal period and/or hypoxia (McCullumsmith and Meador-Woodruff, 2011; Stan et al., 2006). Notably, protein expression was shown to remain stable even when RNA was degraded (Stan et al., 2006; Webster, 2006). RIN measurements are believed to be the most sensitive indicator of RNA quality (Stan et al., 2006), Although, RIN and pH measurements are typically correlated, a higher pH does not always predict intact RNA, and several diagnostic groups with significantly lower pH values do not show lower RIN values (Stan et al., 2006; Webster, 2006). For these reasons, RIN values are increasingly used to assess tissue quality, and RNA quality in particular. These measures can be used to exclude cases on the basis of tissue quality, and/or they can be included in statistical models to assess their potential effects on outcome measures.

SUBSTANCE USE DISORDERS

Substance use disorders, a frequent comorbidity in psychiatric disorders, pose a significant challenge to human brain studies. Estimates of lifetime prevalence of substance use disorders in subjects with psychiatric disorders vary broadly from 15 to 50%, largely depending on the disorder, type of substance, age and length of exposure and geographical location (Barnett et al., 2007; Chand et al., 2014; Conway et al., 2006; Katz et al., 2008; Lasser et al., 2000; National et al., 2011; Saban et al., 2014; Wisdom et al., 2011). The occurrence of drug abuse also varies with socioeconomic factors. Postmortem cases acquired through medical examiner offices have a much higher incidence than those obtained through community referrals by family members and medical staff at treatment facilities. Some studies reported lifetime prevalence of substance use disorders in individuals with psychosis to be as high as 47% among people with schizophrenia and 60% among people with bipolar disorder, while it may be lower than 20% in people with anxiety disorder (Conway et al., 2006; Regier et al., 1990). Meta-analysis studies show somewhat lower lifetime prevalence estimates of substance use disorders in schizophrenia, with 40% of people having used drugs at some point in their lives, with a lifetime prevalence of 20% for alcohol abuse and 27% for cannabis (Koskinen et al., 2009, 2010). Alcohol and cannabis, together with nicotine, consistently come up as the most commonly abused substances in subjects with psychiatric illnesses, while estimates on cocaine, amphetamines and opioids are more variable (Barnett et al., 2007; Green et al., 2004; National et al., 2011; Thoma and Daum, 2013). Notably, use of these substances varies over the course of the illness.

Cannabis and class A drugs may be mostly used around the time of the first psychotic episode, while nicotine and alcohol misuse may be much more long lasting, often spanning a lifetime (Barnett et al., 2007; Lasser et al., 2000; National et al., 2011; Wisdom et al., 2011). For instance, cannabis was shown to be most commonly used by younger male patients (Koskinen et al., 2009, 2010), and reports on substance use at the time of the first psychotic episode show cannabis abuse between 28 and 50% of patients, alcohol abuse between 21 and 43%, class A drugs approximately 55% (Barnett et al., 2007; Green et al., 2004), although lower rates have also been reported (Chand et al., 2014). However, approximately 50% of patients with substance abuse became abstinent or drastically decreased drug consumption following their first psychotic episode, as shown by a meta-analysis including studies published between 1990 and 2009 (Wisdom et al., 2011). Use of alcohol and nicotine may, instead, persist. A study on smoking rates showed that people with a psychiatric condition are almost twice as likely to smoke, and are more likely to be heavier, lifetime smokers (Lasser et al., 2000). People with anxiety disorders, including phobias, post traumatic stress disorder etc., major depression, bipolar disorder and personality disorders were among those most likely to be heavy smokers (Lasser et al., 2000).

The evidence discussed above is mostly based on subjects with psychosis, while less information is available on other psychiatric disorders. Furthermore, not enough is known on the pattern of substance use disorders in psychiatric patients during the entire course of the illness and during older age, specifically, an aspect particularly relevant to human postmortem studies. Because substance use in people with psychiatric disorders typically follows the pattern observed in the rest of the population (National et al., 2011), data relative to substance use disorders in elderly people without psychiatric disorder can provide some useful hints. In this population, drug abuse was found to involve mostly prescription drugs (benzodiazepines, opioid analgesics) and legal substances, particularly alcohol (Koechl et al., 2012). These substances are highly relevant to key aspects of the pathophysiology of psychiatric disorders, among which we emphasize GABAergic abnormalities. GABA A receptors interact with a variety of pharmacological and abuse agents, such as benzodiazepines, for which they have specific binding sites, as well as barbiturates, neuroactive steroids, anaesthetics and alcohol (Laviolette et al., 2004; Rehni et al., 2013; Sieghart et al., 2012; Ting and van der Kooy, 2012; Trudell et al., 2014). Abnormalities of GABA receptors in psychiatric disorders may thus interact with exposure to these drugs, which may in turn enhance or mask disease-related changes.

Overall, current literature indicates that in approximately 40–50% of people with psychiatric disorders frequent early exposure to a variety of abused substances, including cannabis, cocaine and amphetamines, may be followed by alcohol and nicotine misuse and, in elderly people, prescription drug abuse. It is reasonable to postulate that exposure to these substances at various stages of the life cycle, i.e. in the months before death or earlier in life, may contribute to some of the abnormalities detected in the brain of people with psychiatric disorders. Estimates of drug exposure at these different stages require a combination of different approaches designed to assess recent and past use (for detailed review see Lehrmann et al., 2008). Structured next-of-kin interviews and/or questionnaires can be particularly useful in evaluating use throughout life, and may provide information on alcohol and nicotine exposure that may otherwise not be obtainable by other approaches. However,

at times family members may be unable or unwilling to provide this information. Toxicology assays of amphetamine, barbiturates, benzodiazepines, cannabinoids, cocaine, opiates and phencyclidine are crucial not only for younger brain donors, who may have been recently exposed to drugs of abuse, but also in older donors with chronic pain syndromes and terminal malignancies, often treated with to benzodiazepines and opioids. A thoughtful approach to toxicology is key to establish recent use. Toxicology of scalp hair, wherever possible, may be advantageous, as it typically reveals use within the last few months, i.e. covering longer periods with respect to urine and blood (Lehrmann et al., 2008). Even so, toxicology screening does not provide information on some commonly used substances, e.g. nicotine and alcohol, and on substance abuse earlier in life. Information on past use can be obtained from family interviews/questionnaires, as mentioned above, as well as clinical case history reviews,. Although this information typically cannot be transformed into quantitative values, it can be introduced in statistical models using ranking systems (e.g. heavy, moderate, occasional and no smoker) or simply users versus non-users (e.g. Cobb et al., 2013; Kunii et al., 2014; Pantazopoulos et al., 2010; Ray et al., 2014). In addition, inclusion of a psychiatric control group with similar substance use characteristics represents a powerful study design for assessing specific outcome measures may be affected by drug exposure (e.g. Markota et al., 2014). Finally, chronic administration of specific drugs of abuse to experimental animals may be used, particularly if there are strong reasons to suspect that a key outcome variable may be affected. Although none of these strategies alone may be sufficient, their combination, customized to each specific study, can provide an effective assessment of the potential effects of substance abuse.

PHARMACOLOGICAL THERAPY

The large majority of psychiatric patients receives, or has received in the past, pharmacological treatment extending over many years. Without a doubt, such treatment impacts on molecular, cellular and morphological neural features, and thus represents a potential confounding factor in brain imaging and postmortem brain studies of human subjects. It is important to emphasize that exposure to pharmacological agents may either induce or mask differences between diagnosis and control groups. The possibility that pharmacological treatment may conceal brain abnormalities by bringing measured parameters toward normality is perhaps the more insidious one of the two possibilities, as the effects of therapeutic drugs can plausibly be conceived of as correcting brain abnormalities intrinsic to the disorder. Evidence from postmortem studies supports this possibility. In one such report, a highly significant positive correlation between 65 kDa glutamic acid decarboxylase (GAD65) immunoreactive terminals and doses of antipsychotics was observed in the hippocampus of subjects with schizophrenia, with neuroleptic-free patients showing the lowest numbers of terminals (Todtenkopf and Benes, 1998). In another more recent study testing the expression of the dopamine transporter in the amygdala, we detected robust decreases in subjects with schizophrenia (Markota et al., 2014). Numbers of putative terminals expressing dopamine transporter were significantly, and positively, correlated with lifetime exposure to antipsychotics, which pushed numbers to these terminals toward normal values. This effect is consistent with the effects of antipsychotics, as decreases of dopamine transporter in the context of otherwise normal

densities of dopaminergic fibers implies normal dopamine release but impaired reuptake, increasing availability of dopamine at the synapse and diffusion in the extrasynaptic space (Markota et al., 2014).

The need to control for exposure to pharmacological treatment is well recognized in this field. Several different strategies have been applied to address it, including various approaches to estimate exposure to distinct drugs, or families of drugs, inclusion in the study of a psychiatric control group that received similar treatment, and administration to experimental animals of the same drugs used to treat the disorder under study. Although each of these strategies presents its own challenges, their combined, rigorous implementation is critical to result interpretation and the validity of the study.

Estimates of exposure to drugs or families of drugs (e.g. antipsychotics, lithium, benzodiazepines etc.) for each subject are probably the most common, challenging and direct approach. In the majority of cases, medical records contain sufficient, at times extensive, information on pharmacological treatment, and often provide important information on the patient's responsivity and adherence to pharmacological treatment (Pantazopoulos et al., 2010; Roberts et al., 2009). Estimates of drug exposure have to take into consideration that, in the majority of cases, different drugs and combination of drugs may have been tried at different points. The majority of subjects included in postmortem cohorts is typically elderly and has been exposed to long-term treatments with a variety of therapeutic drugs. Parallel to what discussed in relationship to substance abuse, it is important to make a distinction between exposure to pharmacological drugs during the last period before death and chronic, long-term exposure. The two aspects do not always correlate with each other; for instance, treatment with antipsychotic drugs or lithium continuously over several decades may be discontinued during the last few months of life. Arguably, both chronic and recent pharmacological treatment are likely to induce brain changes, although potentially distinct in nature. Thus, it is essential to assess both modalities. Typically, doses of pharmacological drugs prescribed during the last 3 to 6 months of life, or exposure at the time of death, are used to assess recent effects of drug exposure, in some cases in combination with data from toxicology (e.g. Benes et al., 2001; Cobb et al., 2013; Kunii et al., 2014; McNamara et al., 2008; Pantazopoulos et al., 2010; Shan et al., 2014). An advantage of this approach is that doses of drugs aimed at treating a psychiatric disorder are often lower during the last few months before death. In addition, directed toxicology testing on postmortem tissue has been successfully used to test for acute antipsychotic use at time of death; in a large cohort of patients with schizophrenia 68% of the patients were found to be positive for antipsychotic medication (Deep-Soboslay et al., 2011; Halim et al., 2008).

Estimates of lifetime exposure to various classes of psychotropic and neurotropic drugs, can be derived from meticulous screening of medical records and converted to estimated daily mg doses of antipsychotic drugs to the equivalent of chlorpromazine as a standard comparator (Baldessarini, 2013; Baldessarini and Tarazi, 1995), and reported as lifetime grams per patient (Markota et al., 2014; Pantazopoulos et al., 2010). Values for some other therapeutic drugs, such as lithium carbonate and valproic acid, can be reported in a similar manner (Markota et al., 2014; Pantazopoulos et al., 2010). This approach allows addressing

the effects of chronic, long-term exposure over periods often spanning several decades. In this regard non-compliance, particularly among patients with schizophrenia, represents a particularly insidious problem. For instance, in the course of the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) study, 74% of patients discontinued treatment before 18 months (Lieberman et al., 2005). Therefore, assessments of drug exposure calculated on the basis of medical records may overestimate the actual drug intake. Albeit far from exact, levels of treatment-adherence can be derived from ante mortem clinical records, and used to adjust estimates of drug exposure (Berretta et al., 2007; Markota et al., 2014; Pantazopoulos et al., 2007; Pantazopoulos et al., 2010).,

Approaches to estimating lifetime drug exposure, such as those described above and similar ones adopted by other groups (e.g. Ray et al., 2014), may be particularly valuable in assessing long term effects of chronic treatments which often last for decades. Significant effects of lifetime drug exposure have been detected even when the effects of exposure during the last months of life were not significant (Markota et al., 2014). Similar approaches have also been effectively used to assess treatment response versus treatment resistance, a particularly critical aspect in light of efforts to better understand and address drug resistance (Roberts et al., 2009). However, estimates of drug exposure, particularly in postmortem studies, are based on approximations. It is thus important to combine them with other approaches to rigorously assess the potential contribution of therapeutic drugs to a study's main findings. When possible, it is useful to include in a study a psychiatric control group with overlapping pharmacological treatment. A common example is the inclusion of subjects with schizophrenia and bipolar disorder, which are both treated with antipsychotics (e.g. Benes et al., 2001; Benes et al., 2003; Heckers et al., 2002; Konradi et al., 2011; Markota et al., 2014). Although in the past doses of antipsychotics were typically lower in subjects with bipolar disorder, the medication histories from schizophrenic and bipolar disorder cases acquired in more recent years have been remarkably similar, with both groups showing overlapping patterns of polypharmacy. Comparisons between these two groups help assessing not only the contribution of antipsychotic exposure, but also exposure to other classes of drugs commonly prescribed for schizophrenia and bipolar disorder, including mood stabilizers and antidepressants, as well as a specific factors, such as the potential stressful effects of suffering from a chronic psychiatric disorder.

Administration of pharmacological drugs to experimental animals is also effectively used to test whether a specific agent may induce, contribute, or be neutral to abnormalities detected in psychiatric patients (e.g. Konradi et al., 2011; Kunii et al., 2014; Volk et al., 2012). In one such study, chronic administration of an antipsychotic medication to rodents was found to significantly increase GABA terminals (Vincent et al., 1994). This latter study supports the view that decreases of GABA function in schizophrenia may be reversed by antipsychotic drugs and reflect their potential for therapeutic efficacy. Investigations combining postmortem studies on human and non-human primates, the latter chronically-treated with typical and atypical antipsychotics, have been particularly useful in assessing the contribution of antipsychotics to GABAergic abnormalities in subjects with schizophrenia (Hashimoto et al., 2008; Morris et al., 2008; Volk et al., 2000; Volk et al., 2012). For instance, decreased levels of mRNA for GAD67, parvalbumin, somatostatin and Lhx6 were detected in the prefrontal cortex of subjects with schizophrenia, but not in monkeys

chronically treated with antipsychotics (Hashimoto et al., 2008; Morris et al., 2008; Volk et al., 2000; Volk et al., 2012).

Several important considerations contribute to the design of experimental animal studies, including specific drugs to be tested (e.g. which typical or atypical antipsychotic), the route, dose, schedule and length of administration, and experimental animal species tested. These studies may provide compelling evidence on the potential of therapeutic drugs to induce a variety of brain changes. Species differences, and the possibility that the pharmacological effects of a specific drug on a normal brain may not reflect those occurring in a diseased brain, presenting altered neurochemical and cellular substrates, represent limitations of this approach.

Conclusions

Over the past 20 years, postmortem studies of human brain have benefitted from continued improvement of their design, the availability of increasingly powerful cellular and molecular technologies, and enhanced integration of complementary approaches. Often-quoted limitations in postmortem studies of human brain obviously exist, which may be qualitatively, but not quantitatively, different from those inherent to other approaches to the study of the brain. It is critical to promote an on-going constructive dialogue on these issues, and to understand that these limitations can be addressed effectively by carefully designing studies, so that their influence on the data is well understood and carefully controlled. Potential confounding effects can never be completely eliminated from empiric investigations of human disease. Postmortem and brain imaging studies of human brain will continue to evolve strategies for assessing confounding effects, so that the study of psychiatric disorders may continue to move toward the ultimate goal of understanding their underlying cellular and molecular pathophysiology.

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References

Akbarian S, Huang HS. Molecular and cellular mechanisms of altered GAD1/GAD67 expression in schizophrenia and related disorders. Brain Res Rev. 2006; 52(2):293–304. [PubMed: 16759710]

Akbarian S, Huntsman MM, Kim JJ, Tafazzoli A, Potkin SG, Bunney WE Jr, Jones EG. GABAA receptor subunit gene expression in human prefrontal cortex: comparison of schizophrenics and controls. Cereb Cortex. 1995; 5(6):550–560. [PubMed: 8590827]

Baldessarini, RJ. Chemotherapy in Psychiatry: Pharmacologic Basis of Treatments for Major Mental Illness. 3. Springer; 2013.

Baldessarini, RJ.; Tarazi, FI. Pharmacotherapy of psychosis and mania. In: Brunton, LL.; Lazo, JS.; Parker, KL., editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 11. McGraw-Hill Press; New York: 1995. p. 461-500.

Barbosa IG, Machado-Vieira R, Soares JC, Teixeira AL. The immunology of bipolar disorder. Neuroimmunomodulation. 2014; 21(2–3):117–122. [PubMed: 24557044]

Barch DM, Bustillo J, Gaebel W, Gur R, Heckers S, Malaspina D, Owen MJ, Schultz S, Tandon R, Tsuang M, Van Os J, Carpenter W. Logic and justification for dimensional assessment of symptoms and related clinical phenomena in psychosis: relevance to DSM-5. Schizophr Res. 2013; 150(1):15–20. [PubMed: 23706415]

Barnett JH, Werners U, Secher SM, Hill KE, Brazil R, Masson K, Pernet DE, Kirkbride JB, Murray GK, Bullmore ET, Jones PB. Substance use in a population-based clinic sample of people with first-episode psychosis. Br J Psychiatry. 2007; 190:515–520. [PubMed: 17541112]

Beattie EC, Stellwagen D, Morishita W, Bresnahan JC, Ha BK, Von Zastrow M, Beattie MS, Malenka RC. Control of synaptic strength by glial TNFalpha. Science. 2002; 295(5563):2282–2285. [PubMed: 11910117]

Benes FM. Emerging principles of altered neural circuitry in schizophrenia. Brain Res Brain Res Rev. 2000; 31(2–3):251–269. [PubMed: 10719152]

Benes FM. Relationship of GAD(67) regulation to cell cycle and DNA repair in GABA neurons in the adult hippocampus: bipolar disorder versus schizophrenia. Cell Cycle. 2010; 9(4):625–627. [PubMed: 20107308]

Benes FM. Nicotinic receptors and functional regulation of GABA cell microcircuitry in bipolar disorder and schizophrenia. Handbook of experimental pharmacology. 2012; (213):401–417. [PubMed: 23027422]

Benes FM, Berretta S. GABAergic interneurons: implications for understanding schizophrenia and bipolar disorder. Neuropsychopharmacology. 2001; 25(1):1–27. [PubMed: 11377916]

Benes FM, Vincent SL, Alsterberg G, Bird ED, SanGiovanni JP. Increased GABAA receptor binding in superficial layers of cingulate cortex in schizophrenics. J Neurosci. 1992; 12(3):924–929. [PubMed: 1372045]

Benes FM, Vincent SL, Todtenkopf M. The density of pyramidal and nonpyramidal neurons in anterior cingulate cortex of schizophrenic and bipolar subjects. Biol Psychiatry. 2001; 50(6):395–406. [PubMed: 11566156]

Benes FM, Walsh J, Bhattacharyya S, Sheth A, Berretta S. DNA fragmentation decreased in schizophrenia but not bipolar disorder. Arch Gen Psychiatry. 2003; 60(4):359–364. [PubMed: 12695312]

Berretta S, Pantazopoulos H, Lange N. Neuron numbers and volume of the amygdala in subjects diagnosed with bipolar disorder or schizophrenia. Biol Psychiatry. 2007; 62(8):884–893. [PubMed: 17698040]

Blackwood DHR, Fordyce A, Walker MT, St Clair DM, Porteous DJ, Muir WJ. Schizophrenia and affective disorders - Cosegregation with a translocation at chromosome 1q42 that directly disrupts brain-expressed genes: Clinical and P300 findings in a family. Am J Hum Genet. 2001; 69(2):428–433. [PubMed: 11443544]

Blatt GJ, Fatemi SH. Alterations in GABAergic biomarkers in the autism brain: research findings and clinical implications. Anatomical record (Hoboken, NJ : 2007). 2011; 294(10):1646–1652.

Blum PB, Mann JJ. The GABAergic system in schizophrenia. Int J Neuropsychopharmacol. 2002; 5(2):159–179. [PubMed: 12135541]

Braga RJ, Reynolds GP, Siris SG. Anxiety comorbidity in schizophrenia. Psychiatry Res. 2013; 210(1):1–7. [PubMed: 23932838]

Bromet EJ, Kotov R, Fochtmann LJ, Carlson GA, Tanenberg-Karant M, Ruggero C, Chang SW. Diagnostic shifts during the decade following first admission for psychosis. Am J Psychiatry. 2011; 168(11):1186–1194. [PubMed: 21676994]

Casey DA, Rodriguez M, Northcott C, Vickar G, Shihabuddin L. Schizophrenia: medical illness, mortality, and aging. International journal of psychiatry in medicine. 2011; 41(3):245–251. [PubMed: 22073763]

Catena-Dell'Osso M, Rotella F, Dell'Osso A, Fagiolini A, Marazziti D. Inflammation, serotonin and major depression. Curr Drug Targets. 2013; 14(5):571–577. [PubMed: 23531160]

Cerda M, Sagdeo A, Johnson J, Galea S. Genetic and environmental influences on psychiatric comorbidity: a systematic review. J Affect Disord. 2010; 126(1–2):14–38. [PubMed: 20004978]

Chand P, Thirthalli J, Murthy P. Substance use disorders among treatment naive first-episode psychosis patients. Compr Psychiatry. 2014; 55(1):165–169. [PubMed: 24183888]

Chavarria-Siles I, Walss-Bass C, Quezada P, Dassori A, Contreras S, Medina R, Ramirez M, Armas R, Salazar R, Leach RJ, Raventos H, Escamilla MA. TGFB-induced factor (TGIF): a candidate gene for psychosis on chromosome 18p. Mol Psychiatry. 2007; 12(11):1033–1041. [PubMed: 17440433]

Cobb JA, Simpson J, Mahajan GJ, Overholser JC, Jurjus GJ, Dieter L, Herbst N, May W, Rajkowska G, Stockmeier CA. Hippocampal volume and total cell numbers in major depressive disorder. J Psychiatr Res. 2013; 47(3):299–306. [PubMed: 23201228]

Conway KP, Compton W, Stinson FS, Grant BF. Lifetime comorbidity of DSM-IV mood and anxiety disorders and specific drug use disorders: results from the National Epidemiologic Survey on Alcohol and Related Conditions. J Clin Psychiatry. 2006; 67(2):247–257. [PubMed: 16566620]

Costa E, Davis JM, Dong E, Grayson DR, Guidotti A, Tremolizzo L, Veldic M. A GABAergic cortical deficit dominates schizophrenia pathophysiology. Crit Rev Neurobiol. 2004; 16(1–2):1–23. [PubMed: 15581395]

Costa E, Dong E, Grayson DR, Guidotti A, Ruzicka W, Veldic M. Reviewing the role of DNA (cytosine-5) methyltransferase overexpression in the cortical GABAergic dysfunction associated with psychosis vulnerability. Epigenetics. 2007; 2(1):29–36. [PubMed: 17965595]

Cotter D, Landau S, Beasley C, Stevenson R, Chana G, MacMillan L, Everall I. The density and spatial distribution of GABAergic neurons, labelled using calcium binding proteins, in the anterior cingulate cortex in major depressive disorder, bipolar disorder, and schizophrenia. Biol Psychiatry. 2002; 51(5):377–386. [PubMed: 11904132]

Deep-Soboslay A, Benes FM, Haroutunian V, Ellis JK, Kleinman JE, Hyde TM. Psychiatric Brain Banking: Three Perspectives on Current Trends and Future Directions. Biological Psychiatry. 2011; 69(2):104–112. [PubMed: 20673875]

Dernovsek MZ, Sprah L. Comorbid anxiety in patients with psychosis. Psychiatria Danubina. 2009; 21(Suppl 1):43–50. [PubMed: 19789484]

English JA, Pennington K, Dunn MJ, Cotter DR. The neuroproteomics of schizophrenia. Biol Psychiatry. 2011; 69(2):163–172. [PubMed: 20887976]

Fatemi SH, Folsom TD, Kneeland RE, Liesch SB. Metabotropic glutamate receptor 5 upregulation in children with autism is associated with underexpression of both Fragile X mental retardation protein and GABAA receptor beta 3 in adults with autism. Anatomical record (Hoboken, NJ : 2007). 2011; 294(10):1635–1645.

Fatemi SH, Reutiman TJ, Folsom TD, Thuras PD. GABA(A) receptor downregulation in brains of subjects with autism. J Autism Dev Disord. 2009; 39(2):223–230. [PubMed: 18821008]

Ferentinos P, Dikeos D. Genetic correlates of medical comorbidity associated with schizophrenia and treatment with antipsychotics. Curr Opin Psychiatry. 2012; 25(5):381–390. [PubMed: 22842659]

Fineberg AM, Ellman LM. Inflammatory cytokines and neurological and neurocognitive alterations in the course of schizophrenia. Biol Psychiatry. 2013; 73(10):951–966. [PubMed: 23414821]

Gonzalez-Burgos G, Lewis DA. GABA neurons and the mechanisms of network oscillations: implications for understanding cortical dysfunction in schizophrenia. Schizophr Bull. 2008; 34(5): 944–961. [PubMed: 18586694]

Green AI, Tohen MF, Hamer RM, Strakowski SM, Lieberman JA, Glick I, Clark WS. First episode schizophrenia-related psychosis and substance use disorders: acute response to olanzapine and haloperidol. Schizophr Res. 2004; 66(2–3):125–135. [PubMed: 15061244]

Guidotti A, Auta J, Chen Y, Davis JM, Dong E, Gavin DP, Grayson DR, Matrisciano F, Pinna G, Satta R, Sharma RP, Tremolizzo L, Tueting P. Epigenetic GABAergic targets in schizophrenia and bipolar disorder. Neuropharmacology. 2011; 60(7–8):1007–1016. [PubMed: 21074545]

Guidotti A, Auta J, Davis JM, Dong E, Grayson DR, Veldic M, Zhang X, Costa E. GABAergic dysfunction in schizophrenia: new treatment strategies on the horizon. Psychopharmacology (Berl). 2005; 180(2):191–205. [PubMed: 15864560]

Halim ND, Lipska BK, Hyde TM, Deep-Soboslay A, Saylor EM, Herman MM, Thakar J, Verma A, Kleinman JE. Increased lactate levels and reduced pH in postmortem brains of schizophrenics: medication confounds. J Neurosci Methods. 2008; 169(1):208–213. [PubMed: 18177946]

Harrison PJ. Using Our Brains: The Findings, Flaws, and Future of Postmortem Studies of Psychiatric Disorders. Biological Psychiatry. 2011; 69(2):102–103. [PubMed: 21183008]

Hashimoto K. Brain-derived neurotrophic factor as a biomarker for mood disorders: an historical overview and future directions. Psychiatry Clin Neurosci. 2010; 64(4):341–357. [PubMed: 20653908]

Hashimoto T, Arion D, Unger T, Maldonado-Aviles JG, Morris HM, Volk DW, Mirnics K, Lewis DA. Alterations in GABA-related transcriptome in the dorsolateral prefrontal cortex of subjects with schizophrenia. Mol Psychiatry. 2008; 13(2):147–161. [PubMed: 17471287]

Heckers S, Barch DM, Bustillo J, Gaebel W, Gur R, Malaspina D, Owen MJ, Schultz S, Tandon R, Tsuang M, Van Os J, Carpenter W. Structure of the psychotic disorders classification in DSM-5. Schizophr Res. 2013; 150(1):11–14. [PubMed: 23707641]

Heckers S, Stone D, Walsh J, Shick J, Koul P, Benes FM. Differential hippocampal expression of glutamic acid decarboxylase 65 and 67 messenger RNA in bipolar disorder and schizophrenia. Arch Gen Psychiatry. 2002; 59(6):521–529. [PubMed: 12044194]

Horváth S, Janka Z, Mirnics K. Analyzing Schizophrenia by DNA Microarrays. Biological Psychiatry. 2011; 69(2):157–162. [PubMed: 20801428]

Katz G, Durst R, Shufman E, Bar-Hamburger R, Grunhaus L. Substance abuse in hospitalized psychiatric patients. The Israel Medical Association journal : IMAJ. 2008; 10(10):672–675. [PubMed: 19009943]

Kim YK, Myint AM, Lee BH, Han CS, Lee HJ, Kim DJ, Leonard BE. Th1, Th2 and Th3 cytokine alteration in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 2004; 28(7):1129–1134. [PubMed: 15610925]

Koechl B, Unger A, Fischer G. Age-related aspects of addiction. Gerontology. 2012; 58(6):540–544. [PubMed: 22722821]

Konradi C, Zimmerman EI, Yang CK, Lohmann KM, Gresch P, Pantazopoulos H, Berretta S, Heckers S. Hippocampal interneurons in bipolar disorder. Arch Gen Psychiatry. 2011; 68(4):340–350. [PubMed: 21135314]

Koskinen J, Lohonen J, Koponen H, Isohanni M, Miettunen J. Prevalence of alcohol use disorders in schizophrenia--a systematic review and meta-analysis. Acta Psychiatr Scand. 2009; 120(2):85–96. [PubMed: 19374633]

Koskinen J, Lohonen J, Koponen H, Isohanni M, Miettunen J. Rate of cannabis use disorders in clinical samples of patients with schizophrenia: a meta-analysis. Schizophr Bull. 2010; 36(6): 1115–1130. [PubMed: 19386576]

Kunii Y, Hyde TM, Ye T, Li C, Kolachana B, Dickinson D, Weinberger DR, Kleinman JE, Lipska BK. Revisiting DARPP-32 in postmortem human brain: changes in schizophrenia and bipolar disorder and genetic associations with t-DARPP-32 expression. Mol Psychiatry. 2014; 19(2):192–199. [PubMed: 23295814]

Lang UE, Borgwardt S. Molecular mechanisms of depression: perspectives on new treatment strategies. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology. 2013; 31(6):761–777.

Lasser K, Boyd JW, Woolhandler S, Himmelstein DU, McCormick D, Bor DH. Smoking and mental illness: A population-based prevalence study. JAMA. 2000; 284(20):2606–2610. [PubMed: 11086367]

Laviolette SR, Gallegos RA, Henriksen SJ, van der Kooy D. Opiate state controls bi-directional reward signaling via GABAA receptors in the ventral tegmental area. Nat Neurosci. 2004; 7(2):160–169. [PubMed: 14730310]

Lawrence YA, Kemper TL, Bauman ML, Blatt GJ. Parvalbumin-, calbindin-, and calretinin-immunoreactive hippocampal interneuron density in autism. Acta Neurol Scand. 2010; 121(2):99–108. [PubMed: 19719810]

Lehrmann E, Afanador ZR, Deep-Soboslay A, Gallegos G, Darwin WD, Lowe RH, Barnes AJ, Huestis MA, Cadet JL, Herman MM, Hyde TM, Kleinman JE, Freed WJ. Postmortem diagnosis and toxicological validation of illicit substance use. Addiction biology. 2008; 13(1):105–117. [PubMed: 18201295]

Lewis DA, Hashimoto T, Volk DW. Cortical inhibitory neurons and schizophrenia. Nat Rev Neurosci. 2005; 6(4):312–324. [PubMed: 15803162]

Lewis DA, Pierri JN, Volk DW, Melchitzky DS, Woo TU. Altered GABA neurotransmission and prefrontal cortical dysfunction in schizophrenia. Biol Psychiatry. 1999; 46(5):616–626. [PubMed: 10472415]

Lieberman JA, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO, Keefe RS, Davis SM, Davis CE, Lebowitz BD, Severe J, Hsiao JK. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. N Engl J Med. 2005; 353(12):1209–1223. [PubMed: 16172203]

Mann JJ, Brent DA, Arango V. The neurobiology and genetics of suicide and attempted suicide: a focus on the serotonergic system. Neuropsychopharmacology. 2001; 24(5):467–477. [PubMed: 11282247]

Mann JJ, Oquendo M, Underwood MD, Arango V. The neurobiology of suicide risk: a review for the clinician. J Clin Psychiatry. 1999; 60(Suppl 2):7–11. discussion 18–20, 113–116. [PubMed: 10073382]

Markota M, Sin J, Pantazopoulos H, Jonilionis R, Berretta S. Reduced Dopamine Transporter Expression in the Amygdala of Subjects Diagnosed With Schizophrenia. Schizophr Bull. 2014

McCullumsmith RE, Meador-Woodruff JH. Novel Approaches to the Study of Postmortem Brain in Psychiatric Illness: Old Limitations and New Challenges. Biological Psychiatry. 2011; 69(2):127–133. [PubMed: 21094488]

McNamara RK, Jandacek R, Rider T, Tso P, Stanford KE, Hahn CG, Richtand NM. Deficits in docosahexaenoic acid and associated elevations in the metabolism of arachidonic acid and saturated fatty acids in the postmortem orbitofrontal cortex of patients with bipolar disorder. Psychiatry Res. 2008; 160(3):285–299. [PubMed: 18715653]

Millar JK, Christie S, Anderson S, Lawson D, Loh DH, Devon RS, Arveiler B, Muir WJ, Blackwood DH, Porteous DJ. Genomic structure and localisation within a linkage hotspot of Disrupted In Schizophrenia 1, a gene disrupted by a translocation segregating with schizophrenia. Mol Psychiatry. 2001; 6(2):173–178. [PubMed: 11317219]

Millar JK, Christie S, Semple CA, Porteous DJ. Chromosomal location and genomic structure of the human translin-associated factor X gene (TRAX; TSNAX) revealed by intergenic splicing to DISC1, a gene disrupted by a translocation segregating with schizophrenia. Genomics. 2000; 67(1):69–77. [PubMed: 10945471]

Mitchell AC, Bharadwaj R, Whittle C, Krueger W, Mirnics K, Hurd Y, Rasmussen T, Akbarian S. The Genome in Three Dimensions: A New Frontier in Human Brain Research. Biol Psychiatry. 2014; 75(12):961–969. [PubMed: 23958183]

Mitchell AJ, Vancampfort D, Sweers K, van Winkel R, Yu W, De Hert M. Prevalence of metabolic syndrome and metabolic abnormalities in schizophrenia and related disorders--a systematic review and meta-analysis. Schizophr Bull. 2013; 39(2):306–318. [PubMed: 22207632]

Moreau MP, Bruse SE, David-Rus R, Buyske S, Brzustowicz LM. Altered MicroRNA Expression Profiles in Postmortem Brain Samples from Individuals with Schizophrenia and Bipolar Disorder. Biological Psychiatry. 2011; 69(2):188–193. [PubMed: 21183010]

Morris HM, Hashimoto T, Lewis DA. Alterations in somatostatin mRNA expression in the dorsolateral prefrontal cortex of subjects with schizophrenia or schizoaffective disorder. Cereb Cortex. 2008; 18(7):1575–1587. [PubMed: 18203698]

National, Collaborating, Centre. Psychosis with Coexisting Substance Misuse: Assessment and Management in Adults and Young People. The British Psychological Society & The Royal College of Psychiatrists; Leicester UK: 2011.

Pallanti S, Cantisani A, Grassi G. Anxiety as a core aspect of schizophrenia. Curr Psychiatry Rep. 2013; 15(5):354. [PubMed: 23532444]

Pantazopoulos H, Lange N, Baldessarini RJ, Berretta S. Parvalbumin neurons in the entorhinal cortex of subjects diagnosed with bipolar disorder or schizophrenia. Biol Psychiatry. 2007; 61(5):640–652. [PubMed: 16950219]

Pantazopoulos H, Woo TUW, Lim MP, Lange N, Berretta S. Extracellular Matrix-Glial Abnormalities in the Amygdala and Entorhinal Cortex of Subjects Diagnosed With Schizophrenia. Arch Gen Psychiatry. 2010; 67(2):155–166. [PubMed: 20124115]

Pidsley R, Mill J. Epigenetic Studies of Psychosis: Current Findings, Methodological Approaches, and Implications for Postmortem Research. Biological Psychiatry. 2011; 69(2):146–156. [PubMed: 20510393]

Poon VY, Choi S, Park M. Growth factors in synaptic function. Frontiers in synaptic neuroscience. 2013; 5:6. [PubMed: 24065916]

Potuzak M, Ravichandran C, Lewandowski KE, Ongur D, Cohen BM. Categorical vs dimensional classifications of psychotic disorders. Compr Psychiatry. 2012; 53(8):1118–1129. [PubMed: 22682781]

Ray MT, Shannon Weickert C, Webster MJ. Decreased BDNF and TrkB mRNA expression in multiple cortical areas of patients with schizophrenia and mood disorders. Translational psychiatry. 2014; 4:e389. [PubMed: 24802307]

Regier DA, Farmer ME, Rae DS, Locke BZ, Keith SJ, Judd LL, Goodwin FK. Comorbidity of mental disorders with alcohol and other drug abuse. Results from the Epidemiologic Catchment Area (ECA) Study. JAMA. 1990; 264(19):2511–2518. [PubMed: 2232018]

Rehni AK, Jaggi AS, Singh N. Opioid withdrawal syndrome: emerging concepts and novel therapeutic targets. CNS & neurological disorders drug targets. 2013; 12(1):112–125. [PubMed: 23244430]

Roberts RC, Roche JK, Conley RR, Lahti AC. Dopaminergic synapses in the caudate of subjects with schizophrenia: relationship to treatment response. Synapse. 2009; 63(6):520–530. [PubMed: 19226604]

Rosenblat JD, Cha DS, Mansur RB, McIntyre RS. Inflamed moods: a review of the interactions between inflammation and mood disorders. Prog Neuropsychopharmacol Biol Psychiatry. 2014; 53:23–34. [PubMed: 24468642]

Saban A, Flisher A, Laubscher R, London L, Morojele N. The association between psychopathology and substance use: adolescent and young adult substance users in inpatient treatment in Cape Town, South Africa. The Pan African medical journal. 2014; 17(Suppl 1):8. [PubMed: 24643118]

Salvatore P, Baldessarini RJ, Tohen M, Khalsa HM, Sanchez-Toledo JP, Zarate CA Jr, Vieta E, Maggini C. McLean-Harvard International First-Episode Project: two-year stability of ICD-10 diagnoses in 500 first-episode psychotic disorder patients. J Clin Psychiatry. 2011; 72(2):183–193. [PubMed: 20673546]

Shan D, Mount D, Moore S, Haroutunian V, Meador-Woodruff JH, McCullumsmith RE. Abnormal partitioning of hexokinase 1 suggests disruption of a glutamate transport protein complex in schizophrenia. Schizophr Res. 2014; 154(1–3):1–13. [PubMed: 24560881]

Sieghart W, Ramerstorfer J, Sarto-Jackson I, Varagic Z, Ernst M. A novel GABA(A) receptor pharmacology: drugs interacting with the alpha(+) beta(−) interface. Br J Pharmacol. 2012; 166(2):476–485. [PubMed: 22074382]

Simon NM. Generalized anxiety disorder and psychiatric comorbidities such as depression, bipolar disorder, and substance abuse. J Clin Psychiatry. 2009; 70(Suppl 2):10–14. [PubMed: 19371501]

Smoller JW. Disorders and borders: psychiatric genetics and nosology. Am J Med Genet B Neuropsychiatr Genet. 2013; 162B(7):559–578. [PubMed: 24132891]

Stan AD, Ghose S, Gao XM, Roberts RC, Lewis-Amezcua K, Hatanpaa KJ, Tamminga CA. Human postmortem tissue: what quality markers matter? Brain Res. 2006; 1123(1):1–11. [PubMed: 17045977]

Stellwagen D, Beattie EC, Seo JY, Malenka RC. Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor-alpha. J Neurosci. 2005; 25(12):3219–3228. [PubMed: 15788779]

Sun M, Gewirtz JC, Bofenkamp L, Wickham RJ, Ge H, O'Connor MB. Canonical TGF-beta signaling is required for the balance of excitatory/inhibitory transmission within the hippocampus and prepulse inhibition of acoustic startle. J Neurosci. 2010; 30(17):6025–6035. [PubMed: 20427661]

Thoma P, Daum I. Comorbid substance use disorder in schizophrenia: a selective overview of neurobiological and cognitive underpinnings. Psychiatry Clin Neurosci. 2013; 67(6):367–383. [PubMed: 23890122]

Ting AKR, van der Kooy D. The neurobiology of opiate motivation. Cold Spring Harbor perspectives in medicine. 2012; 2(10)

Todtenkopf MS, Benes FM. Distribution of glutamate decarboxylase 65 immunoreactive puncta on pyramidal and nonpyramidal neurons in hippocampus of schizophrenic brain. Synapse. 1998; 29:323–332. [PubMed: 9661250]

Torrey EF, Barci BM, Webster MJ, Bartko JJ, Meador-Woodruff JH, Knable MB. Neurochemical markers for schizophrenia, bipolar disorder, and major depression in postmortem brains. Biol Psychiatry. 2005; 57(3):252–260. [PubMed: 15691526]

Trudell JR, Messing RO, Mayfield J, Harris RA. Alcohol dependence: molecular and behavioral evidence. Trends Pharmacol Sci. 2014; 35(7):317–323. [PubMed: 24865944]

Tunbridge EM, Eastwood SL, Harrison PJ. Changed Relative to What? Housekeeping Genes and Normalization Strategies in Human Brain Gene Expression Studies. Biological Psychiatry. 2011; 69(2):173–179. [PubMed: 20673871]

Vezzani A, Balosso S, Ravizza T. The role of cytokines in the pathophysiology of epilepsy. Brain, behavior, and immunity. 2008; 22(6):797–803.

Vincent SL, Adamec E, Sorensen I, Benes FM. The effects of chronic haloperidol administration on GABA- immunoreactive axon terminals in rat medial prefrontal cortex. Synapse. 1994; 17(1): 26–35. [PubMed: 8042144]

Volk DW, Austin MC, Pierri JN, Sampson AR, Lewis DA. Decreased glutamic acid decarboxylase67 messenger RNA expression in a subset of prefrontal cortical gamma-aminobutyric acid neurons in subjects with schizophrenia. Arch Gen Psychiatry. 2000; 57(3):237–245. [PubMed: 10711910]

Volk DW, Matsubara T, Li S, Sengupta EJ, Georgiev D, Minabe Y, Sampson A, Hashimoto T, Lewis DA. Deficits in transcriptional regulators of cortical parvalbumin neurons in schizophrenia. Am J Psychiatry. 2012; 169(10):1082–1091. [PubMed: 22983435]

Wang S, Cheng Q, Malik S, Yang J. Interleukin-1beta inhibits gamma-aminobutyric acid type A (GABA(A)) receptor current in cultured hippocampal neurons. J Pharmacol Exp Ther. 2000; 292(2):497–504. [PubMed: 10640285]

Webster MJ. Tissue preparation and banking. Prog Brain Res. 2006; 158:3–14. [PubMed: 17027689]

Wisdom JP, Manuel JI, Drake RE. Substance use disorder among people with first-episode psychosis: a systematic review of course and treatment. Psychiatr Serv. 2011; 62(9):1007–1012. [PubMed: 21885577]

Woo TU, Whitehead RE, Melchitzky DS, Lewis DA. A subclass of prefrontal gamma-aminobutyric acid axon terminals are selectively altered in schizophrenia. Proc Natl Acad Sci U S A. 1998; 95(9):5341–5346. [PubMed: 9560277]