

Psychiatric Brain Banking: Three Perspectives on Current Trends and Future Directions

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Postmortem human brain tissue is critical for advancing neurobiological studies of psychiatric illness, particularly for identifying brain-specific transcripts and isoforms. State-of-the-art methods and recommendations for maintaining psychiatric brain banks are discussed in three disparate collections, the National Institute of Mental Health Brain Tissue Collection, the Harvard Brain Tissue Resource Center, and the Mount Sinai School of Medicine Alzheimer's Disease and Schizophrenia Brain Bank. While the National Institute of Mental Health Brain Tissue Collection obtains donations from medical examiners and focuses on clinical diagnosis, toxicology, and building life span control cohorts, the Harvard Brain Tissue Resource Center is designed as a repository to collect large-volume, high-quality brain tissue from community-based donors across a nationwide network, placing emphasis on the accessibility of tissue and related data to research groups worldwide. The Mount Sinai School of Medicine Alzheimer's Disease and Schizophrenia Brain Bank has shown that prospective recruitment is a successful approach to tissue donation, placing particular emphasis on clinical diagnosis through antemortem contact with donors, as well as stereological tissue sampling methods for neuroanatomical studies and frozen tissue sampling approaches that enable multiple assessments (e.g., RNA, DNA, protein, enzyme activity, binding) of the same tissue block. Promising scientific approaches for elucidating the molecular and cellular pathways in brain that may contribute to schizophrenia are briefly discussed. Despite different perspectives from three established brain collections, there is consensus that varied networking strategies, rigorous tissue and clinical characterization, sample and data accessibility, and overall adaptability are integral to the success of psychiatric brain banking.

Key Words: Brain banks, brain collections, postmortem human brain, psychiatric, psychosis, schizophrenia

Postmortem investigation of psychiatric and neurological illnesses using human brain tissue is a well-established approach for identifying the molecular pathways that may contribute to disease and offers a singular avenue for exploring brain-specific transcripts and isoforms not permitted by *in vivo* studies. Postmortem studies in schizophrenia and mood disorders have led to improved understanding of the structural and molecular neuropathology of these complex psychiatric disorders (1), paving the way toward elucidating mechanisms by which candidate susceptibility genes and pathways may contribute to pathogenesis (2,3). Insofar as molecules and pathways may be brain-specific, there may be no substitute for postmortem brain studies in improving our ultimate understanding of the etiology of neuropsychiatric disorders.

Postmortem neuropsychiatric brain research has expanded from case-control comparisons to increasingly complex uses, such as transcript characterization and other neurobiological phenomena associated with allelic variations in genes implicated in psychiatric illness. With the application of genome-wide association studies, copy number variation measurements, and other high throughput molecular genetics techniques to the study of psychiatric disease (4), the field of postmortem molecular genetics has evolved considerably in recent years. As a

result, there has been increased emphasis on higher standards for tissue characterization, as well as for employing larger sample sizes.

Developing a steady source of well-characterized brain tissue donations is a major challenge for postmortem brain studies of schizophrenia. While living donor or prospective recruitment has been effective in some tissue banks and has gained momentum recently in some countries (5), recruitment through autopsy centers remains one of the most common sources of tissue donation. Yet, with the worldwide autopsy rates declining (6), an increasing demand for samples as seen by some brain banks (7), and an apparent shortage of healthy control tissue for case-control studies (8), alternate approaches to collecting tissue need to be explored to expand this important resource. Furthermore, the establishment of a brain tissue collection requires a long-term investment, not only financial (with published cost estimates between \$10,000 and \$30,000 per case [7,9,10]) but also a considerable time investment to refine methods, build up a supply of well-characterized specimens, optimize long-term tissue storage to take advantage of evolving analytic methods, evaluate tissue requests, disseminate tissue, and archive experimental data.

The relative successes and longevity of established brain banks throughout the world at securing larger, nonaged sample sizes for postmortem study of schizophrenia and bipolar disorder have led to a shift whereby larger sample sizes are becoming more common. While previous studies with sample sizes of 20 or less per cell in case-control studies were acceptable just a decade ago (e.g., [11,12]), studies in the past few years have reported more than double those numbers for control samples, with the recent publication of two papers reporting on over 100 control samples (13,14), with similar increases in psychiatric samples (15,16). Thus, the demand for well-characterized postmortem human brain tissue already exceeds the supply, and this imbalance is bound to worsen without a renewed investment in tissue acquisition.

Rather than reiterate previously published discussions of the pitfalls and advantages to the study of postmortem human tissue, we instead provide three different perspectives on current prac-

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Table 1. Tissue Acquisition Across Three Brain Bank Settings

	National Institute of Mental Health Brain Tissue Collection ^a	Harvard Brain Tissue Resource Center	Mount Sinai School of Medicine Alzheimer's Disease and Schizophrenia Brain Bank
Primary Donation Source(s) Founder(s)	Medical examiners' offices Dr. Joel E. Kleinman	United States–nationwide Dr. Edward D. Bird	Prospective recruitment Drs. K.L. Davis, D.P. Perl, and V. Haroutunian
Funding Source(s)	NIMH Intramural Research Program	National Institutes of Health	NIMH, NIA, VA
Date Collection Started	1977	1978	1986
Total Cases in Current Collection	1026 ^a	2000–3000	1589
Average Annual Donations	67	300	55–75
Total Cases with Schizophrenia	150	276	332
Average Schizophrenia Cases Per Year	9	10	16
Total Cases with Bipolar Disorder	50	149	52
Average Bipolar Cases Per Year	3	6	2
Total Adult Control Cases	226	618	240
Average Adult Control Cases Per Year	13	34	15
Primary Focus of Bank	Schizophrenia, normal development	Neurodegenerative disorders, psychotic disorders, nonneuropsychiatric control cases	Alzheimer's, schizophrenia, psychiatric and neurological disorders
Average Age of Cases	43.4 years	68.0 (range 1–108)	77.8
Percentage Male	65% male	54% male	49% male
Primary Manner of Death	49% natural; 22% suicide; 16% accident	90% natural; 10% suicide	99% natural; 1% suicide
Racial Data	48% Caucasian; 48% African American	95% Caucasian; 5% Asian or Hispanic	78% Caucasian; 14% African American; 5.5% Hispanic; 1% Asian
Average Tissue pH	6.4	6.6	6.4
Average PMI	34.3 hours	18.6 hours	12.1 hours

NIA, National Institute on Aging; NIMH, National Institute of Mental Health; PMI, postmortem interval; VA, Veterans Administration.

^aNational Institute of Mental Health data from 1992 to present.

tices in psychiatric brain banking from the National Institute of Mental Health Brain Tissue Collection (NIMH-BTC), the Harvard Brain Tissue Resource Center (HBTRC), and the Mount Sinai School of Medicine Alzheimer's Disease and Schizophrenia Brain Bank (MSSM-BB). We have also summarized their recommendations for the future of psychiatric brain banking.

The National Institute of Mental Health Brain Tissue Collection Perspective—Current Practices

Tissue Acquisition

The National Institute of Mental Health Brain Tissue Collection, founded in 1977, currently maintains approximately 1026 brain tissue samples (acquired from 1992 to present; with previously acquired tissue depleted or discarded). The NIMH-BTC is funded by the National Institute of Mental Health Intramural Research Program and is maintained by the Section on Neuropathology in the Clinical Brain Disorders Branch. Cases are collected from the Offices of the Chief Medical Examiner of Northern Virginia and of the District of Columbia, and consent is obtained and audiotaped with the legal next of kin at the time of autopsy. Approximately 25% to 30% of contacted families consent to tissue donation, which results in an annual accrual rate of about 70 cases.

The NIMH-BTC contains over 150 cases with a DSM-IV diagnosis of schizophrenia (17), 226 adult control cases (nonpsychiatric, nonsubstance abuse), 50 cases with bipolar disorder, and 107 cases with major depression (data from 1992 to present; Table 1). Cases donated to the National Institute of Mental Health

(NIMH) are on average 43 years old, with an average postmortem interval (PMI) of 34 hours, and are roughly 48% Caucasian and 48% African American. National Institute of Mental Health cases include suicides (22%) and death by natural causes (49%), and in general, comorbid substance abuse is high (34%). Although the NIMH-BTC is not a core facility whose primary focus is to dispense tissue, NIMH tissue is used by many other National Institutes of Health (NIH) institutes (e.g., National Institute on Aging, National Institute on Alcohol Abuse and Alcoholism, National Institute of Child Health and Human Development, National Institute of Drug Abuse, National Institute of Dental and Craniofacial Research, and National Institute of Neurological Disorders and Stroke), as well as by numerous research collaborators worldwide (e.g., Allen Institute for Brain Science, Oxford University, University of Alabama at Birmingham, Yale University). Tissue requests are carefully reviewed, with collaborators presenting their research hypotheses to the Section on Neuropathology. For the NIMH-BTC, as with any brain collection, the importance of brain pH, macroscopic and microscopic neuropathological examination, PMI, agonal state, and freezer storage methods are critical to ensuring quality postmortem tissue. These tissue characteristics have been previously well described (18–20) and tissue processing protocols have been published (21,22). Since our own work and that of others have demonstrated that RNA integrity (RNA integrity number [RIN]) may be one of the single most important indicators of tissue quality (23–25), all incoming cases are screened regionally for RIN to determine their suitability for studies.

Clinical Characterization

The reliability of postmortem psychiatric diagnoses for subjects is a high priority. However, the diagnosis of subjects retrospectively, particularly those collected through medical examiners without antemortem study contact, remains challenging. A detailed screening questionnaire at the time of donation has proven to be a valuable resource in the initial characterization process at NIMH and often provides leads about other potential sources of clinical information. The NIMH-BTC staff believes that acquisition of clinical information from multiple sources is the best way to accurately make/confirm postmortem diagnoses. These data sources include psychological autopsy interviews with family informants, interviews with treating professionals, semistructured diagnostic assessment tools such as the Structured Clinical Interview for DSM-IV Disorders (26), medical examiner information, psychiatric records reviews, and/or semistructured tools such as the Diagnostic Instrument for Brain Science (27) or the Diagnostic Evaluation after Death (28) to review available clinical histories. Efforts must also be made to demonstrate both interrater reliability and antemortem and postmortem agreement for psychiatric diagnoses determined retrospectively (29–31). Moreover, this process must be initiated quickly after brain donation to optimize the amount of information collected.

The National Institute of Mental Health Brain Tissue Collection Perspective—Future Directions

Directed Toxicology Testing

Toxicology testing is an important component in the clinical screening and postmortem psychiatric diagnostic process. While reporting medical examiner toxicology results in postmortem studies is typical, these data are limited in both scope and sensitivity, particularly when studying cases as medication-free versus on medication at time of death. Upon review of medical examiner toxicology reports, for 130 NIMH postmortem cases with schizophrenia, we recently found that while 88% of cases had toxicology screenings as part of their autopsy (screened for basic drugs using gas chromatography/mass spectrometry), just 18 cases (13.8%) were positive for antipsychotic medication. In contrast, when additional directed toxicology testing (i.e., supplemental toxicology testing directed by extensive case history reviews of last known prescribed medications in psychiatric records, medical examiner documents, and family interviews) was conducted on postmortem blood or cerebellar tissue using gas chromatography/mass spectrometry through National Medical Services (<http://www.nmslabs.com>), 68% of cases (versus the original 13.8% reported by the medical examiner toxicology alone) were found to be positive for antipsychotic medication (Figure S1 in Supplement 1; see also preliminary data [32]).

There are a number of reasons for such a discrepancy in reported rates of acute antipsychotic use at time of death. First, the purpose of medical examiner toxicology is to determine cause of death, and in the absence of obvious overdose or known illicit drugs of abuse, screening for antipsychotic medication is irrelevant to forensic pathologists. Second, the cost is prohibitive for medical examiners to screen for every possible psychiatric medication; particularly for drugs such as risperidone, olanzapine, or aripiprazole, which are not part of routine assays. Third, even if an individual was known to be prescribed such medications, forensic toxicology laboratories may set detection limits to toxic or lethal levels, thereby leading to false-negative reports of antipsychotics at therapeutic or subtherapeutic levels.

Directed toxicology testing may be impractical for some brain banks whose funding is limited, as average costs range from \$250 to \$500 per case in blood and can be more depending on the matrix used and the number of medications tested. However, going forward, brain banks should be cautious when labeling cases as medication-free or antipsychotic-negative based on medical examiner data alone. In brain banks where no toxicology data are available, this testing may be even more crucial. Because antipsychotic treatments have long been viewed as a major confound to postmortem brain research in psychosis and gene expression studies may necessitate data on acute antipsychotic use, directed toxicology testing is recommended. Similarly, control subjects must also be screened extensively for illicit drug use when not done at autopsy, as the NIMH has found that approximately 10% of its potential control subjects are positive for acute illicit drug use such as marijuana or cocaine that were not screened at autopsy.

Life Span Cohort

Looking to the future of psychiatric brain banking, one of the primary goals set forth by the NIMH-BTC will be to increase the number of nonpsychiatric control samples, with particular emphasis on child/adolescent control cases. Genetic variation can be studied in normal subjects free from treatment and substance abuse confounds. Recently, Myers *et al.* (13) carried out whole-genome genotyping and expression analysis by pooling tissue of 193 neuropathologically normal human brain samples (≥ 65 years old) gathered from several Alzheimer's brain banks, demonstrating that understanding normal gene expression will become an increasingly important avenue for understanding the cellular mechanisms of psychiatric illness. At NIMH, a study of a large healthy control life span cohort, comprised of 39 fetal samples and 207 control samples from birth to age 80, is currently underway in an effort to assess normal gene expression across the prenatal and postnatal life span, as well as to examine differences in age, sex, race, and single nucleotide polymorphisms (SNPs) (14,33).

SNP Database

Another integral advancement in psychiatric brain banking is the use of interactive web-based databases for exploring and sharing data, as has already been implemented with banks such as the HBTRC and the Stanley Foundation (34). The NIMH-BTC has internally launched its Genome Web Browser, a user-interactive SNP database used by investigators for exploratory data analysis of microarray gene expression and genotyping in its life span cohort. This user-interactive database will soon hold data on psychiatric cases and eventually will be accessible to outside collaborators and the public. As public genomic databases become more commonplace, a centralized data repository derived from the specimens in each brain collection will be necessary.

Cell Culture

While numerous scientific approaches and techniques have been implemented at the NIMH-BTC over the last several decades, study of postmortem tissue necessitates continual application of novel techniques. One interesting approach currently underway is the application of cell tissue culture to postmortem human scalp samples collected at autopsy. Even though cell cultures from postmortem tissue were discovered more than 40 years ago, there has only been an accelerated interest in culturing cells from autopsy materials in the last two decades (35–37). Cultured fibroblasts from human postmortem tissue have been

used to study complex neurological diseases such as Alzheimer's disease (38) and schizophrenia (39,40).

At NIMH, fresh scalp tissue from hair samples collected at autopsy for segmental hair toxicological analysis has been used to culture postmortem fibroblasts in the last year, and cells have successfully grown in 43 cases with a PMI of under 48 hours. While challenges exist in creating viable postmortem cell culture libraries, such as confounds of infection and cell senescence, cell culturing offers a promising avenue for studying the underlying genetic architecture of psychiatric disorders. One of the most exciting new techniques is the ability to create induced pluripotent stem cells from cultured fibroblasts (41). Although not identical to embryonic stem cells, induced pluripotent stem cells can also be re-differentiated into other cell types, such as neurons (42,43), which then can be used to study the epigenetics and gene expression patterns in schizophrenia.

The Harvard Brain Tissue Resource Center Perspective—Current Practices

Tissue Acquisition

The Harvard Brain Tissue Resource Center was founded in 1978 and to date, has collected postmortem brain tissue from over 8000 US donors. The HBTRC is uniquely designed, not as a research organization, but as a NIH-supported national brain tissue resource that solicits donations nationwide via both pre-registered (i.e., prospective recruitment) and previously unregistered interested donors. While the HBTRC has both types of donation, preregistration generally results in a low yield for psychiatric cases and is more successful in neurological disorders with a high and somewhat predictable mortality rate. All psychiatric donations originate from telephone calls initiated by the family when death is imminent or immediately after the donor has been pronounced dead.

The HBTRC averages about 300 donations annually, a total that includes neurodegenerative disorders (Alzheimer's disease, Parkinson's disease, Huntington's disease, frontotemporal dementias), psychotic disorders, and nonneuropsychiatric control donations (40 to 50 per year to accommodate age matching for a variety of disorders, e.g., the neurodegenerative disorders have an average age at death of approximately 73 years, while for schizophrenia and bipolar disorder, it is 60 years). The HBTRC also serves as a repository for autism and Tourette's cases, although these belong to private foundations with scientific and/or tissue advisory boards.

As a result of its method of tissue acquisition, the incidence of illicit substance abuse in HBTRC donations is low, even in the psychiatric cases (10%). About 70% of donations come from New England and the Midwest. The vast majority of HBTRC cases (approximately 95%) are Caucasian. The majority (90%) of normal control specimens are obtained through the New England Organ Bank (NEOB), a major organ procurement organization. The NEOB receives referrals from families and hospitals throughout that region but is only able to refer cases that are not on a respirator, as these are considered optimal for organ transplantation purposes. The NEOB screens all cases by conducting a telephone interview with the legal next of kin, which includes information regarding medical history, medications, substance abuse, and the presence of mental illness.

Tissue Characterization

Digital images of each brain before and after coronal dissection are available on a user-interactive website that is made

available to approved investigators. A standard set of blocks are removed from the formalin-fixed hemisphere, imbedded in paraffin, sectioned at 6 μm , and stained with hematoxylin-luxol fast blue and the Bielschowsky method. Every case receives a complete neuropathological examination that includes detailed gross and microscopic information used to confirm neurodegenerative disorder diagnoses. For normal control cases and psychiatric cases, the neuropathological assessment is used to rule out the presence of abnormalities that could interfere with their use in scientific protocols. Tissue processing methods have been previously described elsewhere (21).

Accessibility

To achieve the HBTRC mission, "to assist the neuroscience community in discovering the causes of debilitating diseases of the central nervous system and in developing novel and more effective strategies for treating them," the HBTRC must maintain a large volume of specimens (2000–3000) at any given time to promote constant tissue disbursement. However, to make these specimens easily accessible, the HBTRC has adopted entirely web-based applications to manage tissue requests, demographic data, and gene expression data.

Tissue Disbursement

First, to facilitate prompt review, tissue requests are submitted through the HBTRC website (<http://www.brainbank.mclean.org>). Investigators worldwide must submit applications indicating the diagnoses, regions, and tissue preparations being requested; describing the nature of the project; and providing a biosketch. The HBTRC then evaluates the timeliness of the project, the investigator's productivity, and the availability of appropriate federal funding and other resources that will be used to undertake the project. Staff affiliated with the HBTRC must also make application for tissue through the same process. Generally, tissue requests are processed in less than 1 month, but review times may vary depending on the volume of requests.

User-Interactive Website

In addition to its use of the internet as a portal for tissue disbursement requests, the HBTRC also offers its approved investigators a user-interactive website to cover all donated cases, containing detailed information such as final distributive diagnoses, demographic data, neuropathological reports, and photomicrographs of the brains and histological sections (44). Web-based access to this information enhances tissue use from the HBTRC, as it allows investigators to factor demographic and neuropathological parameters into statistical analyses. By maintaining a web-based application, information sharing is immediate, enhancing the research of tissue recipients from the HBTRC.

Gene Expression and SNP Database

In April 2004, the HBTRC launched the National Brain Data-bank (NBD), a public repository for depositing data obtained from postmortem tissues obtained through this facility (http://national_databank.mclean.org/brainbank/ApproveUser). It currently contains microarray-based gene expression profiling (GEP) from the hippocampus, dorsolateral prefrontal cortex, and anterior cingulate cortex from cohorts of normal control subjects, schizophrenia, and bipolar disorder matched for age, PMI, hemisphere, sex, and to the extent possible, cause of death. The NBD also contains GEP results from dopamine neurons of the substantia nigra obtained using laser microdissection from a Parkinson's disease cohort. Most recently, the HBTRC has begun depositing data from a cohort consisting of approximately 850

normal control, Alzheimer's disease, and Huntington's chorea cases that were processed by the Merck Corporation and its one-time affiliate Rosetta. This dataset consists of both SNPs and GEP from three different regions (i.e., the dorsolateral prefrontal cortex, inferior parietal area, and cerebellum). The data deposited in the NBD are available to the public, with two levels of access—guest (i.e., general public) and investigator. Demographic and medical information is not available to guests using the NBD. To be granted investigator privileges, principal investigators must submit an NIH Biosketch and demonstrate experience with clinical research and an understanding of Health Insurance Portability and Accountability Act of 1996 regulations.

Maintaining the anonymity of donors and their families is a critical issue. This is achieved by a specialized coding paradigm that helps maintain anonymity. Additionally, ages are rounded off to the nearest decade, while PMI is rounded off to the nearest 10 hours. Genotyping data will require even greater effort to protect donor identity. One approach is to permanently anonymize cases. The limitation of this approach, however, is that SNP and GEP data cannot be related to other data forms obtained from the same cases. A second approach to protecting confidentiality of genotyping data is to eliminate certain variables such as hemispheric laterality, cause of death, and possibly even PMI. In short, public genomic databases derived from postmortem studies of psychiatric disorders face the same issue confronting databases from other medical disciplines—how to preserve the confidentiality of donors because individuals can be identified with modern genetic screening methods (45).

The Harvard Brain Tissue Resource Center Perspective—Future Directions

Tissue Accessibility

Accessibility to tissue samples is a vital part of the mission of publicly funded brain banks. The HBTRC's straightforward use of the internet for a three-tiered, web-based database application facilitates its ability to disseminate tissue and associated data in a streamlined process. Most brain banks likely have some form of database already in place and could realistically develop a web-based application for tissue request reviews and reviews to simplify this process for collaborators. Granting investigators real-time web access to demographic, clinical, and neuropathological data, while not a simple task given confidentiality concerns and firewall issues, is a step that vastly improves the utility of tissue samples. Increased accessibility to SNP and GEP databases will further promote research on psychiatric and countless other disorders and should be explored by other brain banks in the future. With a world-renowned reputation and a long-standing track record in psychiatric and neurological brain banking, the HBTRC emphasizes maintaining relationships with the national community as well as continued accessibility to tissue resources as integral to the future of brain research.

The Mount Sinai School of Medicine Alzheimer's Disease and Schizophrenia Brain Bank Perspective—Current Practices

Tissue Acquisition

The Mount Sinai Department of Psychiatry received its first donation in November 1986 and to date has banked brain and other biological specimens from 1589 donors. This collection is dedicated to supporting specific studies in aging, dementia, and major mental illnesses that are associated with the Mount Sinai

Conte Center for Neuroscience of Mental Disorders on White Matter Abnormalities in Schizophrenia (MH066392), Alzheimer's Disease Research Center (AG-02219), Clinical and Biological Studies of Early Alzheimer's Disease (AG-02219), and the James J. Peters Veterans Affairs Medical Center's Mental Illness Research and Education Clinical Center. Through these projects and programs, the Mount Sinai Alzheimer's Disease and Schizophrenia Brain Bank distributes brain tissue specimens to participating laboratories within these programs and to collaborative investigators worldwide. The criteria for tissue distribution for collaborative studies are 1) general scientific merit determined by the executive committees of the supporting research grants; 2) broad conformity with the general scientific missions of the supporting research grants; and 3) nonreplication of current or proposed projects within the supporting grants.

The overarching emphasis of the MSSM-BB is on high-quality objective phenotypic and postmortem characterization of collected specimens. Thus, every effort is made to accept donations from persons who have participated in antemortem diagnostic and neuropsychological evaluation protocols, including control subjects. Specifically, when subjects are recruited for antemortem studies and at each subsequent assessment interval, they are informed that postmortem examination of the brain and clinico-pathological correlative study is among the primary research goals. Subjects are free to opt in or out of the postmortem donation program at any time. Family members, institutional representatives, or other caregivers are asked to contact the brain bank upon a subject's hospitalization or death. Research personnel are on-call through a manned hotline telephone system at all times to either accept postmortem consent for donation from the next of kin, discuss the donation procedures and related questions, or in case of medically ill and hospitalized subjects, to be alerted to their health status. Although percentages vary from year to year, over a 5-year interval, 62% of all donations had been preregistered. Brain specimens are processed for fixed and snap-frozen storage as described elsewhere (46,47).

Clinical Characterization

At MSSM-BB, emphasis is placed on donations that are free of potentially confounding factors such as drug and alcohol dependence, ambiguous or violent circumstances of death, and neurological or neuropsychiatric comorbidities. Of course, not all comorbidities are apparent at the time of donation and antemortem diagnoses are not always consistent with neuropathological findings. Therefore, all donations undergo detailed structured neuropathological characterization with quantitation of neuropathologic lesions (e.g., neuritic plaques and neurofibrillary tangles; number, size, and location of vascular lesions) so that distributions for research can match the intended study hypotheses/objectives as closely as possible and preclude the use of specimens with confounding comorbidities. Because postmortem neurobiology represents, in part, a snapshot of the biological state at the time of death, even when subjects have been directly assessed antemortem, the interval between the last assessment and death is a critical period. Potential changes in the neurological, cognitive, and psychiatric status of donors is assessed with extensive structured medical record reviews and semistructured interviews of informants who had 10 hours per week or more contact with the deceased. Compiling a detailed and accurate phenotype of donors is a major focus of the MSSM-BB. This is especially true for control cases, because unlike neurological diseases like Alzheimer's or Parkinson's disease, the absence of discernable neuropathology does not indicate the absence of

psychopathology (47). Similarly, multiple studies have shown that agonal events such as coma, hypoxia, and seizures can significantly affect the integrity of cells, RNA, and proteins. Thus, in addition to proxy measures such as tissue pH and RIN, medical record reviews document the severity and duration of these agonal states.

The Mount Sinai School of Medicine Alzheimer's Disease and Schizophrenia Brain Bank Perspective—Future Directions

Anticipating Brain Banking Trends

Technical and conceptual advances in neurobiology have grown exponentially during the past few decades. Given the relatively slow rate of accrual of brain specimens, banks must anticipate the needs that will arise in the years to come and collect, process, and store brain specimens to accommodate those needs, techniques, and concepts that are yet to be determined; however, predicting the future is an impossible task. Nevertheless, some needs are invariant and some trends are evident. For example, the study of gene expression is likely to continue for years to come and the use of recently developed techniques such as laser capture microscopy are likely to become more common. Accommodating these approaches requires a greater emphasis on aseptic techniques and better approaches to tissue preservation than those that were employed previously. Similarly, neuroanatomic study approaches have become increasingly quantitative with strong emphasis on stereologic sampling techniques. Brain bank tissue dissection and sampling techniques must be adapted to accommodate these increasingly sophisticated and quantitative approaches (46).

Expansion of Brain Tissue Resources

Postmortem brain tissue from well-characterized donors is a scarce commodity, and brain banks cannot afford to distribute whole tissue blocks for all meritorious study requests when the proposed studies require frequently requested brain regions. For example, requests for brain specimens to accommodate DNA-based studies of SNPs or copy number variations or methylation/acetylation status are exploding. Distributing whole brain tissue blocks for each such study can deplete banked tissues faster than they can be replenished. Brain banks may need to expand their mission by isolating and banking tissue derivatives such as DNA, RNA, and protein that can be multiply aliquoted and stereology relevant section series that can be separately distributed to maximize the usage of each specimen banked. Such an expansion of mission away from traditional brain banking approaches will allow each specimen to serve many different important studies. However, this same expansion of mission will place significant financial burdens on programs that are already being forced to do more with significantly less and require retooling by the banks not only with respect to capital resources but also technical and intellectual expertise.

The MSSM-BB demonstrates success in prospectively following and recruiting brain donations for which ample antemortem data are readily accessible. As a result, the MSSM-BB demands rigor in the clinical characterization on every case. Going forward, the MSSM-BB anticipates the need for increasing adaptability in how tissue is stored, preserved, dissected, and prepared for study. Brain banks may need to consider creating aliquot banks of DNA, RNA, protein, and other derivatives, such as presectioned and slide-mounted specimens to adapt to the

increasing demand for larger sample sizes and more numerous and varied tissue requests.

Discussion

The NIMH-BTC, HBTRC, and MSSM-BB offer three different approaches to brain tissue acquisition for neuropsychiatric research, each demonstrating the relative successes of these methods, i.e., unregistered donations at autopsy, nationwide networking (both preregistered and unregistered), and primarily prospective, preregistered collection, respectively. The three tissue acquisition methods yield somewhat different samples with respect to demographics. For example, NIMH-BTC has the advantage of younger cases but tends to have longer PMI, an increased incidence of suicide and substance abuse in its psychiatric cases, and possibly more severely ill cases, given sampling from medical examiners. Because of its tissue source, NIMH-BTC places great emphasis on directed supplemental toxicological analysis for medical examiner-derived samples, as donors are relatively young and illicit substance abuse is prevalent, and for patients when little information is known about psychiatric drug compliance.

The HBTRC may be the most well-known and prolific bank, acquiring up to 300 cases annually. The incidence of substance abuse in the collection is quite low, as many of its psychiatric cases die via natural causes (thus, it may sample less severe forms of schizophrenia and bipolar disorder, i.e., outpatients and/or those with family support who initiate brain donation); however, the HBTRC tends toward slightly older cases. Because its core mission is providing samples to the neuroscience community, the HBTRC is a leader in accessibility on all levels—from tissue disbursement to web-based data accessibility.

The MSSM-BB has the advantage of assessing the majority of donors psychiatrically and neuropsychologically while living, removing the confounds of retrospective clinical diagnosis; however, preregistered donation is extremely labor intensive with regard to tracking donors and its yield is directly proportional to funding support and can therefore be lower than other donation methods.

Despite disparate methods and missions, there are several areas of agreement among the three brain collections (Table 2). First, employment of diverse strategies for tissue acquisition are necessary, all of which rely upon strong working relationships and networking with respective tissue sources. These key relationships may start with medical examiners' offices, the local community, grassroots organizations such as the National Alliance on Mental Illness and other patient advocacy groups, or by national or international reputation, but all of these relationships rely upon the generosity of donating families who believe in each bank's research mission. Increasing collaborations between brain banks and organ donor networks as seen with the HBTRC may be useful in increasing the number of nonpsychiatric control specimens, especially in younger age groups.

North America may benefit from following the lead of Australia and Europe, where brain banking networks or globalization have recently been established to combine resources across countries for identifying, collecting, and sharing specimens (10,48,49). It would appear to be a logical next step to network among the established banks such as NIMH-BTC, HBTRC, and MSSM-BB to pool resources to standardize tissue acquisition and clinical characterization methods, which would, in turn, reduce the "noise" in any given assay by limiting the variability in these controllable methodological variables. However, an official

Table 2. Summary Recommendations for Psychiatric Brain Banking**Networking**

- Establishment of close working relationships with a variety of sources for donation (medical examiners, families of individual donors or prospective donors, grassroots community organizations/patient support/advocacy groups, and organ donation programs), leading to increased sample sizes (particularly for younger healthy control subjects), to maintain large cohorts of high-quality subjects
- Development of a North American brain banking network with the goals of pooling resources and standardizing all aspects of tissue processing (reducing variability in tissue acquisition, clinical characterization, and tissue processing methods)

Rigorous Characterization

- Dedicated trained professionals to determine antemortem or retrospective diagnoses of donors through detailed clinical diagnostic review process of all available data
- Directed toxicologic screening of all cases in addition to medical examiner toxicology, particularly for cases donated through medical examiners' offices (i.e., for younger donors, where illicit drug abuse is more prevalent and when less is known about psychiatric medication compliance)
- Well-screened nonpsychiatric control subjects across the life span (negative for psychiatric or substance abuse history, negative toxicology for all illicit drugs including marijuana)
- Neuropathological examination to both confirm for and screen out neurological and neurodegenerative diseases (e.g., neuritic pathology, cerebrovascular disease)
- State-of-the-art stereological methods for tissue dissection to enable quantitative assessment and detection of subtle abnormalities, avoid false positive or negative findings due to variable sampling

Accessibility

- A streamlined, web-based process for requesting tissue
- Interactive web-accessible annotated databases for investigators and collaborators that capture demographic, clinical, neuropsychiatric, and neuropathological data, as well as digital photography of samples and genomic data, with public access to some results in the future (all while maintaining donor confidentiality)
- Access for investigators and collaborators to adequate sample sizes and resources for studying tissue
- Maintain a central registry of data derived from tissue samples supplied by each brain bank, permitting novel interactive analyses
- Make data available to the general scientific community after a set period of time, deposited into an organized web-based system, to facilitate dissemination of data sets and encourage new statistical and theoretical approaches

Adaptability

- Adaptability in how tissue is acquired, processed, stored, and dissected
- Adaptability or "re-tooling" of how tissue is aliquoted and disseminated (DNA, RNA, protein) to maximize the use of each individual donation
- Adaptability in scientific methods for study (e.g., the shift from case-control studies to high throughput gene expression studies) and/or to anticipate novel and future tissue uses

North American brain banking network may not be realistic given the diverse infrastructure of many of the US and Canadian banks (i.e., with varied funding sources, including the federal government, Veteran's Administration, or disease-specific patient advocacy groups) and even among the three collections described here. At the same time, one must use caution before pooling tissue from varied sources such as those of NIMH-BTC, HBTRC, and MSSM-BB, where cases may differ significantly in age, socioeconomic background, PMI, illness severity, or comorbidities. Subtle differences in demographics may lead to variance and thus type II errors. However, in a recent study using microarray techniques in human brain by Oldham *et al.* (50), it was demonstrated that efforts can be made to offset such variance by statistically normalizing to reduce batch effects resulting from combined datasets.

Second, all three of the brain collections agree that rigor in tissue and diagnostic characterization are essential to a successful bank, whether it be detailed clinical diagnostic information gathered antemortem or postmortem, toxicologic analysis of medical examiner-derived cases, neuropathological examination of all cases to screen for neurological diseases, or adoption of stereological tissue sampling methods.

Third, the importance of sample accessibility has been underscored. Accessibility can mean a number of things, beginning with access to gathering large numbers of well-characterized cases to ensure adequate tissue for dissemination, as well as individual investigators' accessibility to these tissue resources, by way of tissue disbursement. Data accessibility is also critical to postmortem brain studies, particularly through investigator databases in various stages of development seen at all three banks,

whether for demographic or clinical data, neuropathological data, tissue tracking, genomic data, or other archival datasets.

Lastly, the overall adaptability of brain banks is critical to the success and future of psychiatric brain banking in psychosis. Banks must be adaptable not only in how tissue is acquired, preserved, dissected, and stored, but also in how tissue is aliquoted and distributed. Furthermore, the investigators conducting research must also be adaptable through continual application of innovative scientific approaches to the study of brain tissue (e.g., microarray techniques, cell culture, and laser capture microscopy).

Conclusions

The study of schizophrenia and related disorders is actually the study of brain disease. Accordingly, although blood, urine, cerebrospinal fluid, lymphocytes, and fibroblasts have utility, ultimately, there is no substitute for brain tissue. Given the limitations of in vivo neuroimaging, postmortem human brain tissue may be essential for uncovering the cellular and molecular mechanisms for neuropsychiatric disorders. Despite the divergent methods for tissue acquisition and each bank's particular research focus, all three collections have a proven track record for successfully acquiring well-characterized brain tissue samples of psychotic disorders and nonpsychiatric controls subjects. All three brain collections strive to apply ever-increasing rigor, not just for diagnostic determination of cases, increasing sample sizes, or screening and tissue characterization processes but also to the forward-thinking scientific approaches in studying brain

tissue with the mutual goal of improving treatments for schizophrenia and related illnesses.

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