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PERSPECTIVE

Standardized Operational Protocol for Human Brain Banking in China

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With the progress of society, there is an increasing need to tackle disorders of the central nervous system. Human brain tissue, unlike animal tissues, is an irreplaceable resource for the study of neurological diseases [1]. Aimed at scientific research and education, the roles of human brain tissue repositories are to acquire brain tissue from donors, prepare, process, and preserve collected samples, provide tissue to specific eligible facilities, and determine the characteristics of each tissue sample. The construction of human brain banks is highly valued by neurologists. Related academic achievements have promoted the understanding of the relationship between brain structures and functions, as well as the pathological features, etiology, and pathogenesis of neurological diseases. Meanwhile, human brain banking plays an important role in developing effective methods for preventing and treating these diseases [2].

However, the quantity and scale of brain banks in China have lagged behind those of many other countries, which hinders the related basic and clinical research [3]. International symposia on China brain bank building was held in

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2014 and 2016. By the joint efforts of ten medical schools and universities, the China Human Brain Bank Consortium was finally set up in May 2016. In order to guarantee the consistency of brain sampling among consortium members and make full use of these resources, we have borrowed valuable ideas from practical experience [2, 4–8], gathered critical information by examining the current status of existing human brain banks in China [3, 9, 10], and proposed a protocol for the standardization of human brain banking.

Objectives of Human Brain Tissue Collection

The overall objectives of brain tissue collection are:

(1) To build a collection of brain tissue that covers as many neurological or psychiatric diseases as possible;
(2) To collect brain tissue from donors differing in age, sex, ethnicity, location, handedness, educational level, and profession, among others, to help studies on risk factors of particular diseases through biometric analysis and big data mining;
(3) To collect brain tissue of donors with familial hereditary diseases, especially those with neurological diseases. Brain tissues from families with hereditary diseases (including fetal death and children) are a part of this effort;
(4) To collect brain tissue at different stages in the normal aging process (from individuals without obvious neurological or psychiatric diseases, traumatic brain injury, alcohol addiction, or carbon monoxide poisoning), and analyze the brains of matched patients and controls for confounding factors.

Notice:

(1) Postmortem delay should be minimized when collecting brain tissue, so that the state of macromolecules is better preserved;
(2) Brain tissue should be collected as completely as possible, with blood and cerebrospinal fluid also collected whenever possible. For hereditary diseases and fetal and neonatal brain tissue, it is particularly important to retain blood samples.

Members of the Brain Bank Consortium can carry out disease-specific brain tissue collection, i.e. collecting tissue associated with one or more neurological or psychiatric diseases and normal brain tissue. The medical histories of donors, the type of disease, its duration, and other characteristics should be recorded. Samples should be collected and classified according to the type of disease and neuropathological diagnosis.

Ethical Review of the Human Brain Bank

Ethical Requirements

Human Brain Banks should be equipped with independent or shared Ethics Review Boards to handle the ethical review of brain tissue collection and sample applications. Donors should be pre-informed and sign a consent form in writing.

Requirements of Ethical Review for Applicants Using Tissue Samples

The procedures for using human brain tissue include: application, independent review by experts, termination of review, suspension or termination of experiments, or education. After an applicant submits formal documents, including experimental methods, education programs, or research plans, the Ethics Committee of the brain bank decides whether to accept the application. An examination process should be organized within one month after the application is received. The applicant should be supervised by the Ethics Committee during the processes.

The ethical review process can follow normal procedures or simplified procedures. For the normal procedure, a majority of the committee members including at least one non-medical professional should be present. All members present at the meeting have the right to vote, but those who participate in the experiments, or have a conflict of interest in experiments should avoid voting. The plan must be approved by a majority of the members and the approval of at least one non-professional member is also required.

A simplified procedure may be used to allow some important individual projects to be carried out in a time-sensitive manner, and the scheme may be examined by the Chair of the Ethics Committee alone, or the examination may be appointed by the Chair to at least one experienced member. All members shall be informed of the program after the examination and approval.

Sources of Brain Tissue

At present, there are mainly two sources of human brain tissue in China: body donation and surgical sampling. Both should be carried out under the laws of China and social morals, and the donors or their next-of-kin who are

authorized by donors or by law shall sign the informed consent form, which should be notarized if necessary.

Willed Whole-Body Donation Station

Cadavers donated to the body donation station with the necessary clinical information can be used for education or research, and tissues to be collected include but are not limited to the cerebrum (including pituitary), cerebellum, brain stem, spinal cord, cerebrospinal fluid, and peripheral ganglia. Well-equipped brain banks can also be regarded as separate donation stations for brain tissues.

Donation steps:

(1) Donors should sign the "informed consent form of willed whole-body donation" and send it back to the body donation station affiliated with the Red Cross for registration.
(2) Based on the conditions of the brain bank, certain clinical services such as cognitive function assessment can be provided for specific groups of patients.
(3) Donation stations should provide donation cards, showing the application form number, contact information, and the procedure of donation, to be taken away and kept by the donors.
(4) When a donation is available, the staff of the donation station may decide whether it can be accepted based on the donors' past demographic and medical information, time of death, cause of death, and the current situation of the brain bank.
(5) Next-of-kin should sign the informed consent form again before the cadaver is transported to the body donation station.
(6) Donors and their next-of-kin have the right to withdraw from the donation program at any time.

Surgical Sampling in Hospital

Surgical sampling is regularly conducted in the Department of Neurosurgery and other related departments, where tumor tissues and potentially some surrounding normal brain tissues are acquired through inevitable surgical removal.

Donation procedures:

(1) Patients and their next-of-kin should agree to the removal of samples, and sign informed consent before the operation.
(2) The sampling site should be strictly limited to the necessary removal site.
(3) Pathological diagnosis results should be provided by the clinician to the patient and the next-of-kin after the operation.
(4) Brain tissues, relevant clinical data and the pathological diagnosis results should be preserved in the brain bank.

Clinical Data Collection

Obtained During Donation Registration

Registrars should obtain the donors' basic demographic and medical information by asking the donors or their next-of-kin.

Cognitive Function Evaluation and Head Imaging Examination After Registration if Possible

A set of accurate and easy-to-use neuropsychological scales are used to evaluate cognitive function after registration. The neuropsychological scales currently used to detect early cognitive changes in conditions such as Alzheimer's disease, vascular dementia, dementia with Lewy bodies, and frontotemporal dementia, include Mini Mental Status Evaluation (MMSE), Montreal Cognitive Assessment (MoCA), Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog), Clinical Dementia Rating (CDR), Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE), and Everyday Cognition scale (ECog), which can be applied as screening or diagnostic tests. It is strongly recommended to finish the MMSE if possible, while the others are optional. Scores within normal ranges suggest that there is probably no cognitive impairment, but this cannot rule out disorders at very early stages. If there is objective evidence of cognitive impairment, further testing is required.

If there is a more definite medical history of neuropsychiatric disorder, the receiving organization needs to make a clear diagnosis in accordance with the DSM-V diagnostic criteria [11, 12], mainly focusing on Alzheimer's disease, substance dependence, depression mania, post-traumatic stress disorder, and schizoaffective disorder.

If donors have not received whole-brain MRI examination within five years after registration, and the conditions of the human brain bank allow, a free whole-brain MRI is recommended, and its sequences should include at least the conventional T1 and T2 weighted images. According to the needs of clinical diagnosis, other sequences can be added, such as enhanced imaging, FLAIR (fluid-attenuated inversion recovery) imaging, or nuclear magnetic resonance spectroscopy. All previous head image data for the treatment of disease or from the physical examination are stored in the same data format under the corresponding ID number in the dataset of human brain bank donors.

If the donors did not receive cognitive function tests before death, family members of donors can fill in the ECog scale [13, 14], which is a feasible means of determining whether the donors had cognitive impairment. The ECog scale is particularly recommended because it is relatively simple and practical, and the score is strongly correlated with the pathological score of Alzheimer's disease [15].

Medical Records of Donors

Photocopies of the medical records of donors should be stored in the dataset for each donor in the human brain bank.

Family members are advised to request a corresponding copy of the medical records from the hospital. Based on the current relationship between the human brain bank and the hospital, the database of the human brain bank may be considered to be connected with the electronic medical record system of the hospital, allowing researchers to extract the hospital diagnoses, treatment processes, and data from medical imaging from structured electronic medical records.

In future, the construction of a resource-sharing platform is recommended to coordinate integrated management, including registration, diagnosis and treatment, brain donation, and donor information feedback.

Brain Tissue Sampling Process

Confirm and record the agonal state, time and cause of death; obtain informed consent from family members; collect and document medical records; and evaluate risk factors (e.g., infectious diseases).

Sampling sites can be in the Department of Pathology or the dissection room. If the sampling process cannot be carried out in a timely manner, cadavers should be preserved at low temperature.

Sampling process for a donated brain: (1) Confirm the time of the brain collection; (2) Incise the scalp along the coronal plane from the ear to the top of the skull then turn over and expose the skull; (3) Carry out a craniotomy, cut apart the skull at the ring line which begins at the level 1 cm above the eyebrows to the occipital protuberance, to avoid excessive damage to brain tissue; (4) Peel the dura; (5) Remove the cerebrum, cerebellum, brain stem, and part of the cervical cord, and sample the blood and cerebrospinal fluid; (6) Temporarily preserve the sample in crushed ice, then capture images and label the system number; (7) Staff in the human brain bank confirm the data and brain collection process, and sign.

Brain Tissue Management and Preservation

A macroscopically detailed examination of the brain is an integral part of the neuropathological diagnosis. After removing the brain, it is weighed; the anteroposterior diameter and the left-to-right diameter are measured; the symmetry, visible damage, infarction, hemorrhage, or atherosclerosis, are observed. If the postmortem delay is > 24 h, or the two cerebral hemispheres are affected by different diseases or to different degrees, or the sample requires bilateral pathological diagnosis, or the patient is infected by certain pathogens, both hemispheres should be fixed; otherwise the dominant hemisphere should be frozen, while the contralateral hemisphere is fixed.

Fresh Frozen Steps

(1) Incise the cerebrum, cerebellum, and brain stem along the mid-sagittal axis; weigh and photograph the fresh hemisphere.

(2) Remove the midbrain, brainstem, and cerebellum at the level of the superior colliculi or section at the site just posterior to the mammillary bodies, and separate the brainstem and cerebellum at the level of cerebellar peduncles.

(3) Take two small pieces of tissue ($\sim$1 g) from the frontal cortex and place them in a refrigerator at –80°C for DNA and RNA extraction and pH measurement.

(4) Make 1 cm-thick coronal slices of the fresh cerebral hemispheres, sagittal slices of the cerebellar hemispheres, and horizontal cross-slices of the brain stem.

(5) Arrange the slices in fronto-occipital order and take photographs. After quick-freezing, the samples are packaged into labeled self-sealing plastic bags, put into a frozen storage box, and stored at –80°C. The samples are retrieved to extract the required substances when needed. Brain banks can also take the tissues from the following anatomical sites during the process, and then mark and quick-freeze them separately, which allows convenient access for further research. Recommended sites on the fresh hemisphere (*indicates sites on the fixed hemisphere that must be sampled for basic neuropathological diagnoses) include:
the trigeminal nerve, pituitary gland, optic nerve, olfactory bulb, pineal gland, occipital pole, cerebellum*, temporal pole, medulla oblongata*, spinal cord, substantia nigra*, locus coeruleus*, anterior and posterior central gyri, superior, middle* and inferior frontal gyri, superior*, middle and inferior temporal gyri, marginal gyrus, angular gyrus, apical

lobules*, choroid plexuses, hippocampus*, amygdala*, paraventricular zone, superior colliculus, inferior colliculus, hypothalamus, thalamus, caudate nucleus*, putamen, globus pallidus*, gray matter, white matter, and meninges*.

Fixation Steps

(1) The hemisphere or the whole brain, and the spinal cord to be fixed, are placed into phosphate buffer solution (pH 7.2–7.4) consisting of 6% neutral buffered formalin (NBF), and transferred to 4% NBF after 3 days, where the samples need to be fixed for more than 2 weeks.
(2) Take out the fixed whole brain or hemisphere and remove the meninges together with the meningeal vessels. Examine where there is atrophy, infarction, bleeding, or other general pathological manifestations.
(3) Remove the midbrain and connected brainstem and cerebellum above the superior colliculus or incise at the site just posterior to the mammillary body.
(4) Separate the brainstem and cerebellum at the level of the cerebellar peduncles; carry out sagittal slicing on the cerebellar hemisphere.
(5) Prepare coronal slices of the cerebral hemispheres at a thickness of 1 cm, from the frontal to the occipital lobe, number in sequence, and take photographs.
(6) Prepare cross-sectional slices of the brain stem and spinal cord at a thickness of $\sim$0.5 cm, number in sequence, and take photographs.
(7) Sample the specific area with pathological manifestations in addition to the conventional sites recommended previously.
(8) Complete the sampling process according to the sampling sites recommended above, and then dehydrate and embed for pathological diagnoses. The remaining tissue is preserved in 4% NBF fixative, which needs to be replaced every two years.

Pathological Diagnoses of Brain Tissues

Brain Tissue Preparation, Preservation, and Staining

(1) Fixed brain tissues can be embedded using automatic dehydration embedding machines.
(2) Paraffin sections: thickness, 5–10 μm, baked at 40°C for 2–3 days.
(3) Routine hematoxylin/eosin, silver, and immunohistochemical staining are carried out for the pathological diagnoses, and the remaining block, after being marked, is stored at room temperature.

Pathological Diagnoses

(1) Pathological diagnoses should be made after examining the donor's medical history and the results of gross pathological and histopathological examination. The diagnoses of neurological and psychiatric diseases, if applicable, should be performed following the international diagnostic criteria [16–21]. While not all neurological diagnoses need to be completed, efforts should be made to provide more accurate and comprehensive diagnoses as brain banks develop.
(2) The pathological diagnosis report should be reviewed and signed by qualified neuropathology specialists. The neuropathological quality-control team, composed of members of the Consortium Expert Committee, is obliged to conduct random spot-checks on the pathological reports of the consortium members, to standardize the neuropathological diagnosis process.
(3) Pathological diagnosis results should be recorded in detail and preserved in the information database.
(4) The results of pathological diagnoses can be given to the next-of-kin of donors by the clinician if requested.

Application and Feedback for Using Brain Tissue

An application for the use of brain tissue should be formally submitted by the applicants. The Ethics Review Board and the Brain Bank Management Committee should review the submission to decide whether any sample can be provided. After approval, users sign the agreement and acquire the brain tissue samples. Applicants should observe the following principles:

(1) Only researchers listed in the application form are authorized to use the samples. The place where the research is conducted must be consistent with that in the application form.
(2) Any information about brain tissue should be kept confidential.
(3) Acknowledgment to the human brain bank should be made in articles published.
(4) The agreement is only valid after being signed by all participating researchers, their institute representatives, and the person in charge of the human brain bank.

(5) Published research results should be provided to the human brain bank by researchers who used the brain tissue.

Information Management on Brain Tissue

Guidelines for the Preservation and Management of Brain Tissue Information

(1) An electronic archive of the donors' basic information needs to be created, and this also includes the information on the brain tissue donated to the brain bank;
(2) Photographs of the front, side, and base of the brain as well as the coronal and sagittal slices should be captured for storage;
(3) Images acquired through immunohistochemistry, fluorescence staining, western blotting, and other experimental methods should be preserved;
(4) Genetic information (such as DNA, RNA, and protein) based on the brain bank should be collected whenever possible;
(5) All the collected information is summarized as a unified database managed by full-time staff; information backup also needs to be emphasized.

Establishment and Management of Individual Brain Bank Websites

Brain banks are recommended to establish websites. The contents of the website may include a brief introduction, donor's page, scientific researcher's page, and databases.

(1) A brief introduction to the human brain bank includes the purpose, functions, types and numbers of the samples;
(2) In the donor's page, the prerequisites, procedures of donation, and details about the usage of brains and the benefits of brain donation should be indicated;
(3) In the scientific researchers' page, the requirements (responsibilities and obligations) for the research team, and downloading links to relevant application forms for brain tissues should be included;
(4) In the database page, a database should be established to record information about donors, such as age, sex, cause of death, and basic neuropathological assessments.

Integration and Management of the Brain Bank Consortium Website

(1) It is important to allocate resources to set up website hyperlinks for each member's brain bank. When such a national platform is available, members of Brain Bank Consortium can share information about brain samples;
(2) Users of brain tissue may apply through a member of the Consortium, and the examination and approval are conducted by the corresponding brain bank; if the bank cannot meet the requirement of the research alone, the person in charge of this bank can apply to the Brain Bank Consortium, which is responsible for the coordination of the brain tissue resources of various brain banks.

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