

RESEARCH PROTOCOLS



Standardized Operational Protocol for the China Human Brain Bank Consortium

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ABSTRACT

The study of post-mortem human brains is fundamental to the discovery and diagnosis of most neurological diseases and an in-depth understanding of brain structure, function and disorders. Human brain banks use a standardized protocol to collect, process, and store post-mortem human brains and related tissues, together with relevant clinical information, and to provide the tissue samples and data to foster neuroscience research. A Standardized Operational Protocol (SOP) that is approved by and can be abided by all of the human brain banks in the China Human Brain Bank Consortium is critical to developing brain research in China. The first SOP of human brain banking in China was published in 2017 following the foundation of the China Human Brain Bank Consortium. So far, 20 members from different regions in China have joined the consortium, forming a nationwide collaboration network of human brain banks. To provide brain tissue samples of good quality and consistency to meet the increasing demand for neuroscience research, a revised SOP was drafted by experts from the China Human Brain Bank Consortium. Significant improvements in this new version of SOP include strengthened ethical guidelines, recruitment of matching controls, and more brain regions to be sampled for neuropathological evaluation.

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Introduction

In the 21st century, a prolonged life span increases the morbidity of neurological disease, and more research focuses on deep-understanding of brain functions and diseases. Considering the difference between animal and human brains, the need to validate the results from animal experiments, and the different disease prevalence among races, such as amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD) (Mehta et al., 2018; Pringsheim, Fiest, & Jette, 2014; Tsai, Wang, Hwang, Lee, & Lee, 2015), human brain tissues with reliable medical records and clinical data are invaluable for neuroscience research (Bell et al., 2008; Bigio, 2013; Mighdoll & Hyde, 2018; Tang et al., 2020). Human brain banks use a standardized protocol to collect, process, and store post-mortem human brains and related tissues, together with relevant clinical information, and to provide tissue samples and data to foster neuroscience research. Human nervous tissue and other samples such as cerebrospinal fluid, meninges and blood vessels collected during neurosurgery and other clinical operations can serve as critical supplementary resources to a brain bank if provided with ethical approval, informed consent and detailed medical records. Human brain banks have been well established in developed countries such as the USA and Netherlands (Kretzschmar, 2009). Standardized procedures of human brain banking were established and adopted to ensure the quality of tissue samples and support collaborative studies worldwide. Standardized construction of human brain banks in China started around 2012 (Yan, Ma, Bao, Wang, & Gai, 2015; Zhang et al., 2018). The Chinese brain bank's first Standardized Operational Protocol (SOP) was published in 2017 (Committee, 2014; Qiu W, 2017; W. Qiu et al., 2019). Nowadays, the China Human Brain Bank Consortium has been established to meet the increasing need for human brain samples in neuroscience research around China. A practical SOP will help to keep the sample quality in different human brain banks.

Compared with the previous version (Qiu W, 2017; W. Qiu et al., 2019), this SOP for China Brain Banking emphasizes the informed consent with permission to use medical information of donors, strengthens the recruitment of matching control samples, and proposes the basic brain regions which need to be dissected for neuropathological

evaluation. Other issues, such as the spinal cord, were recommended only in particular cases, which are not included in this SOP.

1. Objective of human brain tissue collection

The general objective is to collect as much standardized brain tissue as possible to meet the need for neuroscience research in China and worldwide.

1.1 Overall objectives of human brain tissue collection

- (1) The human brain tissues collection should include brain with neuropsychiatric diseases as much as possible, the clinical data, social information and biological information should also be collected at the same time;
- (2) The human brain tissues collection should cover different ages, sex, medical history, level of education, nation, region, occupation and handedness et al., to analyze the risk factors of disease comprehensively;
- (3) The human brain tissues collection should contain genetic diseases, especially the donors of neurological diseases, which also include children and fetuses;
- (4) The human brain tissue collection should comprise brains of donors who died of normal aging without traumatic brain injury, alcoholism, carbon monoxide poisoning, and obvious neurological or psychiatric diseases.

Notice:

- (1) The human brain tissues collection should be guided by the principle that minimizing the post-mortem delay (PMD). The states of the brain and the activity of biological macromolecules in the brain are fragile, so PMD of less than 24 hours is recommended for brain banks in this project. Less than 12 hours is better for those well-conditioned brain banks;
- (2) The human brain tissues collection should cover as completely as possible, which can consist of not only brain tissues but also cerebrospinal fluid (CSF), blood, and skin are recommended for future research;

- (3) The human brain tissues collection for genetic diseases as well as neonatal and fetal brain tissues, harvesting blood samples of parents is recommended.

1.2 Diseases-specific brain tissue collection

Members of the China Human Brain Bank Consortium can collect disease-specific brain tissues based on their conditions, i.e. harvesting brain tissues with kinds of neuropsychiatric diseases and matching control brain tissues. The following donor information includes medical histories, types and duration of diseases and medication et al. of neuropathological diagnoses and diseases.

2. Legal and ethical review of the human brain bank

2.1 Legal requirements

The human brain bank must run under the relevant laws and regulations in China, including the Chinese Civil Code, Biosecurity Law of the People's Republic of China, and the People's Republic of China Regulations on the Administration of Human Genetic Resources, etc.

2.2 Ethical requirements

The human brain banks should have an ethical review board that can be independent or shared of its supporting institution to handle ethical reviews of human brain tissues, including collection and sample applications. Donors and/or immediate family should read and sign the informed consent carefully. The informed consent should tell volunteers the objective and significance of collecting brain tissue together with their right to quit at any moment without giving a reason. Human Brain Bank should also obtain agreements from volunteers and/or immediate family for brain autopsy, for using the material in medical education and research, and permission to copy their medical records.

2.3 Requirements of ethical approval for applicants using tissue samples

Human brain tissues applicant should be Principal Investigators from research institutes, universities or colleges. The applicant should submit ethical

approval for using human tissue in this project with a sample application form. An independent scientific committee should supervise the applicants during the process.

3. The source of brain tissues

Currently, body/organ/brain donation and surgical sample preparation are China's main sources of human tissues. All donation and operation procedures should comply with the laws and ethics of China. The donors and their immediate family or relatives given authority by donors or laws must sign the informed consent form, and it is better to notarize based on the local policies.

3.1 Voluntary whole-body or organ donation

The whole body donated by the donors to the body donation station, together with clinical information, will be used for teaching and researching. The human brain tissues collected mainly include the cerebrum, cerebellum, brain stem, cervical spinal cord, cerebrospinal fluid, pituitary, and peripheral ganglia.

Donation steps:

- (1) Donors and his/her immediate family should sign the "informed consent of voluntary whole-body/organ donation" and take it back to the body donation station to complete the donation registration procedure;
- (2) The body donation station should provide donation cards or certificates indicating the registration number to the donors, together with the additional materials, including contact information of donation and the donation procedure;
- (3) The body donation station staff should be notified by the donor's family or relatives when the donor is dying or dead. The team decides whether to receive the body of donors based on the donor's past medical history, causes of death and current situation of the human brain bank;
- (4) The body donation station staff should clarify the donation procedure and follow-up matters with the next of kin or executors of the donors and make sure they have signed the informed

consent form before transporting the donor to the donation station;

- (5) Donors and their next of kin have the right to withdraw from the donation program without providing a reason.

3.2 Surgical samples donation in the hospital

Surgical sample preparation is regularly conducted in neurosurgery operations, which mainly includes preparation in neurosurgery operations, including tumor tissues and normal brain tissues acquired through inevitable surgical removal.

Donation procedures for surgical samples:

- (1) Patients or their next of kin should sign the informed consent forms to allow samples to be collected and used for clinical teaching and scientific research before the operation.
- (2) Sample preparation region must be limited to the clinically necessary removal site.
- (3) Pathological diagnosis results of donors should be provided directly to the patient or via the clinician who is taking care of the patient.
- (4) Brain tissues from surgical operations and relevant clinical data, together with pathological diagnosis results should be preserved in the human brain bank.

4. Clinical data collection

4.1 Obtained during donation registration

The body donation staff should obtain the data and information about donors' basic demographic (including but not limited to gender, birth date, etc.) and medical information (including but not limited to medical history, medication, etc.) if possible by asking donors or their next-of-kin.

4.2 Cognitive function evaluation and head imaging examination after registration, if possible

Neuropsychological scales including the 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), et al, are recommended to use by neuroscientist for evaluating the cognitive function of volunteers after their registration. These neuropsychological scales are currently used in assessing early cognitive changes including Alzheimer's disease (AD), vascular dementia (VD), dementia with Lewy body (DLB), and frontotemporal dementia (FTD), and can also be applied to screening tests or diagnostic tests (Sachdev et al., 2014; Tay et al., 2015). The MMSE scale is recommended to finish first if possible, and other scales which are alternatives could be completed as far as possible. It should be noted that donors with standard scores probably have no cognitive impairment, but this does not exclude the situation that donors have a very early-stage disease. Neuroscientists can make a more detailed assessment of the brain's cognitive status if there is objective evidence of the donor's cognitive impairment.

Suppose donors have not received a whole brain MRI examination. In that case, it is suggested that the human brain bank can arrange a free whole brain MRI examination for donors during their life based on the conditions of the brain bank. The entire brain MRI examination sequences should comprise at least the conventional T1 and T2 weighted images. In contrast, other sequences such as enhanced imaging and FLAIR imaging are depend on the needs of the clinical diagnosis. The human brain bank should collect and preserve all donors' head/brain image data and physical examination results.

If the donors did not receive cognitive function tests or head imaging examination before his/her death, family members of donors could fill in the ECOG scale (Farias et al., 2021; Tomaszewski Farias et al., 2011), which is a feasible way to determine whether the donors had cognitive impairment or not. ECOG scale is particularly recommended because it is relatively simple and practical, and this score is strongly correlated to the pathological score of Alzheimer's disease (W. Y. Qiu et al., 2018; Yang et al., 2019).

4.3 Donors' medical records

The human brain bank should obtain the photocopy medical records of donors and store these records

in dataset for each donor. In the future, the connection of data banks between human brain banks and hospitals may be incorporated in the plan that a resource-sharing platform will be built, which allows researchers from human brain banks have permission to visit the electronic medical record system of the hospital, including extract the hospital diagnoses, treatment procedures and medical imaging of donors from structured electronic medical records, to realize integrated management including registration, disease diagnosis and treatment, brain donation and donor information feedback.

5. Sample preparation process of the whole brain

5.1 Confirming and recording the donor's information including the agonal state, causes of death, time of death, medical records and demographic information (such as age, gender and education level, etc.). Evaluating risk factors (Infectious diseases, etc.) after obtaining the family members' informed consent.

5.2 Sample preparation sites can be set in the pathology department or the dissection room. Degenerative processes in the brain start at death, so there is an excellent concern that during this period, enzymatic degradation and protein denaturation may affect parameters measured subsequently in the brain tissue. However, much evidence indicates that the agonal status (that causes a decrease of brain pH) of the brain impacts mRNA integrity more than the post-mortem interval (Bahn et al., 2001; Preece & Cairns, 2003; Ross, Knowler, & McCulloch, 1992; Vonsattel, Amaya Mdel, Cortes, Mancevska, & Keller, 2008).

5.3 Sample preparation process of the brain tissues: (1) Check and confirm the time of death; (2) If there is an extensive craniocerebral injury of the donor, we should not preserve the brain; (3) Incise the scalp along the coronal from the ear to the top of the skull then turn over the skin and expose the skull; (4) Carry out craniotomy, cut apart the skull at the ring line which begins at the level of 1 cm above the eyebrows to the occipital protuberance, to avoid excessive damage to brain tissues; (5) Peel the dura; (6) Complete removal of the cerebrum, cerebellum, brain stem (note that the pituitary stalk should carefully sectioned

while lifting the brain a little bit in order to leave the bottom of the hypothalamus intact) and part of the cervical cord, and sample the skin, scalp, blood, cerebrospinal fluid (CSF), bilateral carotid artery (CA), bilateral trigeminal ganglion (TG), cerebral dura mater (CDM), as well as antepituitary (A.P) and post-pituitary (P.P), the above all materials will be stored as shown in Table 1; (7) Take pictures and label the system number; (8) Staff in human brain bank record the post-mortem delay time after the dissection is complete and the sample preparation process, then sign.

6. Brain tissue dissection and preservation

A macroscopically detailed examination of the brain after removal is an integral part of the neuropathological diagnosis. After taking out the brain, the whole brain is weighed and photographed; the anteroposterior diameter, the left to the right diameter, as well as the height are measured; the symmetry, visible damage, infarction, hemorrhage, atherosclerosis, are noted down, and all infarcts and hemorrhages observed should be documented by a description of location, size, and chronicity. In addition, the pH value of cerebrospinal fluid (CSF) and brain tissue should be measured with a pH meter.

In general, one hemisphere should be frozen, while the contralateral hemisphere should be fixed, alternating left and right hemispheres. But if the post-mortem delay is more than 24 hours or the two cerebral hemispheres are affected by different diseases or to different degrees, or the sample requires bilateral pathological diagnosis, or certain diseases infect the patient, both sides should be fixed.

Before the two hemispheres separated, the cerebral arterial circle (of Willis) should be removed (cutting along the loop: anterior cerebral artery (ACA) - middle cerebral artery (MCA) - posterior cerebral artery (PCA) - vertebral artery (VA)) and stored in 10% neutral buffered formalin (10% NBF). Besides, optic chiasm, bilateral olfactory bulb (OB), cerebral pia mater (CPM), choroid plexus of the lateral ventricle (CPLV) and the pineal gland should also be removed and stored according to the method shown in Table 1.

The cerebrum, cerebellum, and brain stem are incised along the sagittal axis on the midline.

| After taking out brain | Frozen at -80°C | | Fixed in 10% NBF | |
|---|--|--|---|--------------|
| | In cryogenic vials | | In cassettes | In tube |
| Region | Skin, scalp, blood, CSF, CA-L, TG-L, CDM, A.P, P.P, BA | | Skin, scalp, CA-R, TG-R, BA, pineal gland | Willis |
| Before separating brain | Frozen at -80°C | | Fixed in 10% NBF | |
| | In cryogenic vials | | In cassettes | |
| Region | CPM, optic chiasm, OB-L, CPLV | | OB-R, CPLV, pineal gland | |
| After separating brain | Frozen at -80°C | | Fixed in 10% NBF | |
| | In cryogenic vials | | In cassettes | In cassettes |
| ACA | √ | | | √ |
| MCA | √ | | | √ |
| PCA | √ | | | √ |
| Sample preparation of HP | | | | |
| Amy | | | √ | √ |
| Hippocampus. ant | | | √ | √ |
| Hippocampus. mid | | | √ | √ |
| Hippocampus. post | | | √ | √ |
| Sample preparation of dominant hemisphere | | | Frozen at -80°C | |
| Region | | | In cryogenic vials | In cassette |
| Frontal pole of cerebrum | | | √ | √ |
| Superior, middle, and inferior frontal gyrus | | | √ | √ |
| Temporal pole of cerebrum | | | √ | √ |
| Superior, middle, and inferior temporal gyrus | | | √ | √ |
| Precentral and postcentral gyrus | | | √ | √ |
| Supramarginal gyrus, angular gyrus, superior parietal lobule | | | √ | √ |
| Superior occipital gyrus | | | √ | √ |
| Occipital pole of cerebrum | | | √ | √ |
| Insula | | | √ | √ |
| Occipital cortex at the level of gyrus of calcarine sulcus | | | √ | √ |
| Anterior and posterior cingulate gyrus | | | √ | √ |
| Cingulate gyrus at the level of the lateral ventricle | | | √ | √ |
| Hypothalamus | | | √ | √ |
| Subthalamus | | | | |
| Thalamus | | | | |
| Caudate nucleus, putamen, globus pallidus, internal capsule | | | √ | √ |
| Gray matter and white matter | | | √ | √ |
| Superior colliculus and inferior colliculus | | | √ | √ |
| Substantia nigra and red nucleus, locus coeruleus | | | √ | √ |
| Medulla oblongata, spinal cord | | | √ | √ |
| Superior and inferior semilunar lobe and vermis of cerebellum | | | √ | √ |

Table 1. Sample regions needed for frozen tissues. **Note:** CSF: Cerebrospinal Fluid; CA-L: Carotid Artery-Left; CA-R: Carotid Artery-Right; TG-L: Trigeminal Ganglion-Left; TG-R: Trigeminal Ganglion-Right; CDM: Cerebral Dura Mater; A.P: Ante-Pituitary; P.P: Post-Pituitary; BA: Basilar Artery; CPM: Cerebral Pia Mater; OB-L: Olfactory Bulb-Left; OB-R: Olfactory Bulb-Right; CPLV: Choroid Plexus of Lateral Ventricle; ACA: Anterior Cerebral Artery; MCA: Middle Cerebral Artery; PCA: Posterior Cerebral Artery; Amy: Amygdala; HP. Ant: anterior of Hippocampus; HP. Mid: middle of Hippocampus; HP. Post: posterior of Hippocampus.

The midbrain, the brainstem and cerebellum nearby are removed at the level of superior colliculi or sectioned at the site just posterior to the mammillary bodies. The brainstem and cerebellum are separated at the level of cerebellar peduncles. One hemisphere, including the cerebral, cerebellum and brainstem, should be put into 10% NBF, where the samples must be fixed for 2 to 4 weeks for further neuropathological assessment. The other hemisphere will be dissected, as shown below.

6.1 Sample preparation of frozen hemisphere

The unfixed hemisphere should be dissected immediately on cold metal trays, and pieces of brain tissue should be prepared according to the proposed standard set of brain regions (Table 1). Note that hippocampus (HP), including the amygdala, is taken out along the collateral sulcus and cut coronal into 12 pieces (about 0.5 cm thick), marked from 1 to 12, and frozen in flat trays to keep morphology. The cerebellar hemisphere is cut into 1 cm-thick sagittal slices, dentate nucleus, superior and inferior semilunar lobe, and vermis of cerebellum cortex are taken and frozen. The brain stem is cut horizontal cross-slices, the superior and inferior colliculus, substantia nigra and red nucleus, locus coeruleus, medulla oblongata and spinal cord are dissected and frozen. All the samples should be preserved in cryogenic vials or cassettes (morphology should be preserved) at -80°C for future molecular biological research. After quick-freezing, the remaining slices should be pictured and packaged into the labeled self-sealing bags and kept in a frozen storage box at -80°C .

6.2 Sample preparation of fixed hemisphere

The whole brain or hemisphere is taken out after at least 2 weeks' fixation and examined if there is atrophy, infarction, bleeding, and other general pathological manifestations. Meninges and blood vessels are removed and kept in the cassette.

First, midbrain, connected brainstem, and cerebellum are separated from the hemisphere along the superior colliculus or incised at the site just posterior to the mammillary body. The brainstem and cerebellum are separated at the level of the cerebellar peduncles. The cerebellar hemisphere is cut into 1 cm-thick sagittal slices,

and cerebral hemispheres are cut into coronal slices from the frontal to the occipital lobe, numbered in sequence and pictured on both sides. The brain stem and spinal cord are cut cross with the thickness of about 0.5 cm, numbered from cranial to caudal, and pictured. Samples of different brain regions should be taken according to the proposed standard for neuropathological assessment. (Table 2 and Figure 1).

Areas with pathological manifestations are recommended to be taken in addition to the conventional sites recommended previously.

| | |
|----|--|
| 1 | Precentral and postcentral gyrus at the same tissue block |
| 2 | Meninges |
| 3 | Hypothalamus |
| 4 | Superior and middle temporal gyrus at the same tissue block |
| 5 | Middle frontal gyrus. |
| 6 | Cingulate gyrus |
| 7 | Caudatum and putamen at the same tissue block |
| 8 | Putamen, globus pallidum and insula at the same tissue block |
| 9 | Amygdala and entorhinal cortex* |
| 10 | Hippocampus ant.* |
| 11 | Hippocampus mid.* |
| 12 | Hippocampus post.* |
| 13 | Thalamus |
| 14 | Inferior parietal lobule |
| 15 | Superior parietal lobule |
| 16 | Occipital cortex |
| 17 | Midbrain including substantia nigra |
| 18 | Colliculus inferior/pons |
| 19 | Locus coeruleus/pons |
| 20 | Cerebellum including dentate nucleus |
| 21 | Medulla oblongata |
| 22 | Spinal cord C |
| 23 | Cerebral arterial circle of Willis including basilar artery* |
| 24 | Olfactory bulb* |
| 25 | Frontal lobe white matter |

Table 2. Block sample preparation sites of fixed tissue. Note: *These block sites have been sampled and fixed in 10% NBF in fresh tissue sample preparation; select them out.

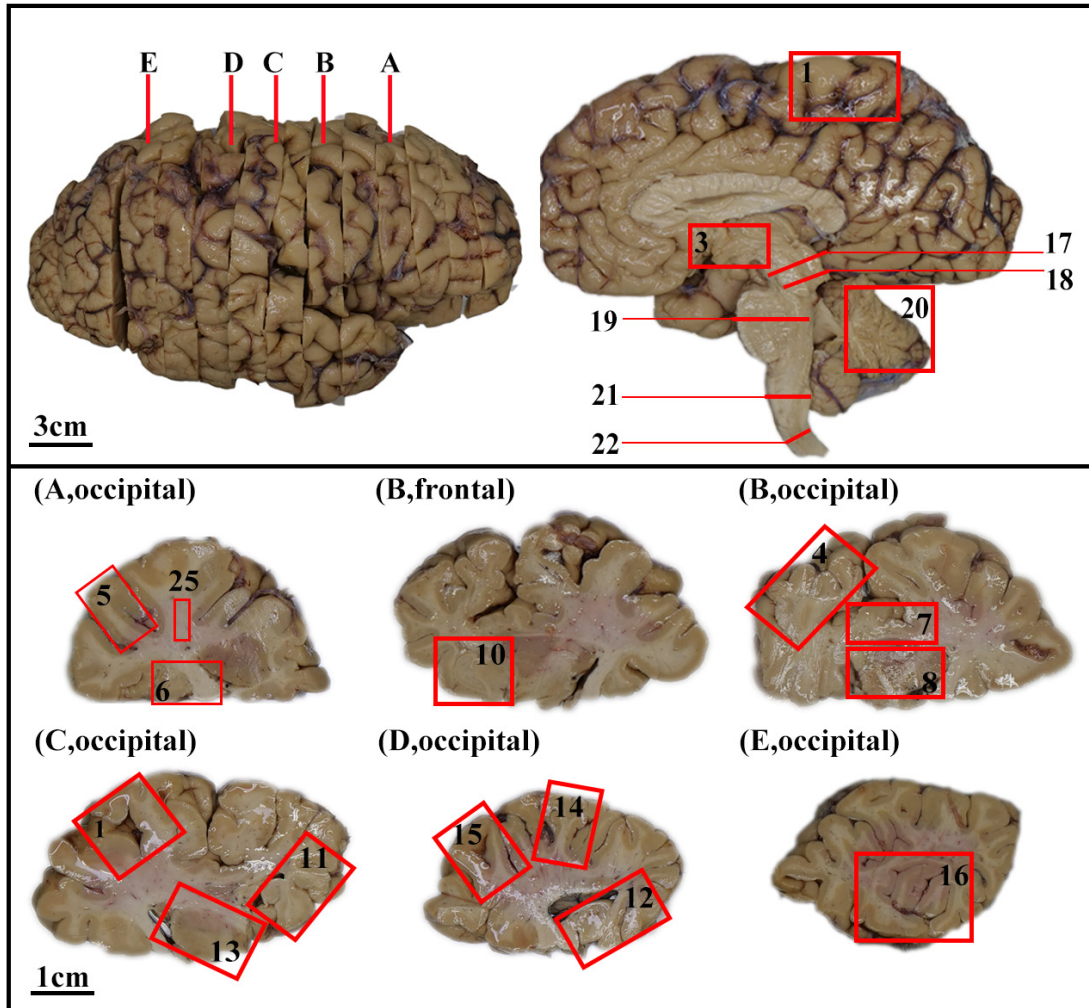


Figure 1. Illustration showing where to collect some blocks listed in Table 2. Each brain slice was sectioned from the frontal pole to the occipital level and marked with A-E to indicate the specific slices with sampling sites of the blocks.

After sampling the blocks of brain tissues recommended above, the dehydration and paraffin embedding procedures should be performed for pathological diagnoses. The remaining tissues are preserved in a 10% NBF fixing solution, which needs to be replaced every two years.

7. Pathological diagnoses of brain tissues

7.1 Brain tissue preparation, preservation, and staining

- (1) Fixed brain tissues can be embedded in paraffin by hand or using automatic dehydration embedding machines.
- (2) Slice thickness of paraffin section of brain

tissues should be 5 ~ 10 μm according to different staining, and the slices need to be baked at 60°C overnight.

- (3) Routine HE staining, Hago silver staining (M-Ag staining), Gallyas staining, modified Bielschoesky staining (Garvey), Congo red staining, Kluver staining, and immunohistochemical staining is carried out for the pathological diagnoses as Table 3. Additional staining regions are required according to medical history and previous findings, shown in Table 4 and Table 5.
- (4) The remaining block, after being marked, is stored at room temperature.

| | Brain region | Special staining | | | | | Immunohistochemistry | | | | | | |
|----|-------------------------------------|------------------|-------|------|--------|---------|----------------------|-------|---------------|-----------|-------|------|------|
| | | H&E | Congo | M-Ag | Kluver | Gallyas | Garvey | p-Tau | α -syn | A β | TDP43 | &p62 | #FUS |
| 1 | G. pre + postcentral | √ | | √ | | | √ | | | | | | |
| 2 | Meninges | √ | √ | | | | | | | √ | | | |
| 3 | Hypothalamus | √ | | | | | | | | | | | |
| 4 | Superior & middle temporal gyri | √ | √ | √ | | √ | √ | √ | | | | √ | √ |
| 5 | Middle frontal gyrus | √ | √ | √ | | √ | √ | | | √ | | | √ |
| 6 | Gyruscinguli | √ | | | √ | | | | | | | | |
| 7 | Caudatum / Putamen | √ | | | | | | | | | | | |
| 8 | Putamen/ Pallidum/ Ins. | √ | | | | | | | | | | | |
| 9 | Amy + ent. cort. | √ | | √ | | √ | √ | | √ | | √ | √ | |
| 10 | Hippocampus ant. | √ | √ | √ | | | √ | √ | | | | | √ |
| 11 | Hippocampus mid. | √ | √ | √ | | √ | √ | √ | | | √ | √ | √ |
| 12 | Hippocampus post | √ | | | | | | | | | | | |
| 13 | Thalamus | √ | | | | | | | | | | | |
| 14 | Lob. par. inf. (cerad) | √ | | √ | | √ | √ | √ | √ | √ | | | |
| 15 | Parietal lobe | √ | √ | | | √ | √ | | | | | | |
| 16 | Occipital cortex (BA 17&18) | √ | √ | √ | | √ | √ | | | | | | |
| 17 | Midbrain including Substantia nigra | √ | | | | | | | √ | | | | |
| 18 | Colliculus inf. / pons | √ | | | | | | | | | | | |
| 19 | Loc. Coeruleus / pons | √ | | | | | | | | | | | |
| 20 | Cerebellum / dent. | √ | | | | | | | | | | | |
| 21 | Medulla oblongata | √ | | | | | | | √ | | | | √ |
| 22 | Spinal cord C | √ | | | | | | | | | | | |
| 23 | *Basilar Artery | √ | | | | | | | | | | | |
| 24 | Olfactory bulb | √ | | | | | | | | | | | |
| 25 | Frontal lobe white matter | √ | | | | | | | | | | | |

Table 3. In routine cases, special staining and immunohistochemistry (IHC) staining of different brain regions. **Note:** Garvey staining could be used instead of M-Ag and Gallyas methods while the number of brain regions should not decrease; \$: Congo red staining of meninges and occipital cortex should be done in all cases, regions marked with \$ should be stained if one of previous two regions appears positive; &: region should be stained by IHC anti p62 in cases with spinocerebellar ataxia (SCA) and ” ’Huntington’s disease (HD) history or neuropathological manifestations; #: region should be stained by IHC anti-FUS in cases with frontotemporal lobar dementia (FTLD) and amyotrophic lateral sclerosis (ALS) history or neuropathological manifestations; *: region not required for all brain banks.

| | Brain region | Special staining | | | | | | Immunohistochemistry | | | |
|----|-------------------------------------|------------------|----------------|------|--------|---------|--------|----------------------|---------------|-----------|-------|
| | | H&E | Congo | M-Ag | Kluver | Gallyas | Garvey | p-Tau | α -syn | A β | TDP43 |
| 1 | G. pre + postcentral | √ | | √ | | | √ | | | | |
| 2 | Meninges | √ | √ | | | | | | | √ | |
| 3 | Hypothalamus | √ | | | | | | | | | |
| 4 | Superior & middle temporal gyri | √ | ^s √ | √ | | √ | √ | √ | | √ | |
| 5 | Middle frontal gyrus | √ | ^s √ | √ | | √ | √ | √ | | √ | |
| 6 | Gyruscinguli | √ | | | √ | | | | | | |
| 7 | Caudatum / Putamen | √ | | √ | | | √ | √ | | √ | |
| 8 | Putamen/Pallidum/ Ins. | √ | | √ | | | √ | √ | | √ | |
| 9 | Amy + ent. cort. | √ | | √ | | √ | √ | | √ | | √ |
| 10 | Hippocampus ant. | √ | ^s √ | √ | | | √ | √ | | √ | |
| 11 | Hippocampus mid. | √ | ^s √ | √ | | √ | √ | √ | | √ | √ |
| 12 | Hippocampus post | √ | | | | | | | | | |
| 13 | Thalamus / subthal. | √ | | | | | | | | | |
| 14 | Lob. par. inf. (cerad) | √ | | √ | | √ | √ | √ | √ | √ | |
| 15 | Parietal lobe | √ | ^s √ | | | √ | √ | | | | |
| 16 | Occipital cortex (BA 17&18) | √ | √ | √ | | √ | √ | √ | | √ | |
| 17 | Midbrain including Substantia nigra | √ | | √ | | | √ | √ | √ | √ | |
| 18 | Colliculus inf. / pons | √ | | | | | | | | | |
| 19 | Loc. Coeruleus / pons | √ | | | | | | | | | |
| 20 | Cerebellum / dent. | √ | | √ | | | √ | | | √ | |
| 21 | Medulla oblongata | √ | | | | | | | √ | | |
| 22 | Spinal cord C | √ | | | | | | | | | |
| 23 | *Basilar Artery | √ | | | | | | | | | |
| 24 | Olfactory bulb | √ | | | | | | | | | |
| 25 | Frontal lobe white matter | √ | | | | | | | | | |

Table 4. Special staining and immunohistochemistry (IHC) staining of different brain regions in AD cases (red √ for additional regions). **Note:** Red √ for additional regions.

7.2 Pathological diagnoses

Pathological diagnoses should be made after verifying the medical history, examining the gross pathological results and the histopathological results based on the internationally or nationally approved diagnostic criteria (Cairns et al., 2007; Deramecourt et al., 2012; Dickson et al., 2009; Schellenberg & Montine, 2012; Trojanowski, Revesz, & Neuropathology Working Group on, 2007; Vonsattel et al., 1985). Alzheimer's disease (AD), Lewy body disease (LBD),

cerebral vascular disease (CVD), hippocampal sclerosis (HS), frontotemporal lobar dementia (FTLD), primary age-related tauopathy (PART) and Parkinson's disease (PD) should be routinely assessed in brain banks which accept brain mainly from older "people's donation (Del Tredici & Braak, 2016; Dutra, Cortes, & Vonsattel, 2015; Jellinger & Attems, 2007; Mackenzie et al., 2010; McKeith et al., 2017; Nelson et al., 2011; Neumann et al., 2009). Recommended antibodies are listed in Table 6.

| | Brain region | Special staining | | | | | Immunohistochemistry | | | | |
|----|-------------------------------------|------------------|-------|------|--------|---------|----------------------|-------|---------------|-----------|-------|
| | | H&E | Congo | M-Ag | Kluver | Gallyas | Garvey | p-Tau | α -syn | A β | TDP43 |
| 1 | G. pre + postcentral | √ | | √ | | | √ | | √ | | |
| 2 | Meninges | √ | √ | | | | | | | √ | |
| 3 | Hypothalamus | √ | | | | | | | | | |
| 4 | Superior & middle temporal gyri | √ | √ | √ | | √ | √ | √ | √ | | |
| 5 | Middle frontal gyrus | √ | √ | √ | | √ | √ | | √ | √ | |
| 6 | Gyruscinguli | √ | | | √ | | | | √ | | |
| 7 | Caudatum / Putamen | √ | | | | | | | | | |
| 8 | Putamen/Pallidum/ Ins. | √ | | | | | | | | | |
| 9 | Amy + ent. cort. | √ | | √ | | √ | √ | | √ | | √ |
| 10 | Hippocampus ant. | √ | √ | √ | | | √ | √ | √ | | |
| 11 | Hippocampus mid. | √ | √ | √ | | √ | √ | √ | | | √ |
| 12 | Hippocampus post | √ | | | | | | | | | |
| 13 | Thalamus / subthal. | √ | | | | | | | | | |
| 14 | Lob. par. inf. (cerad) | √ | | √ | | √ | √ | √ | √ | √ | |
| 15 | Parietal lobe | √ | √ | | | √ | √ | | √ | | |
| 16 | Occipital cortex (BA 17&18) | √ | √ | √ | | √ | √ | | | | |
| 17 | Midbrain including Substantia nigra | √ | | | | | | | √ | | |
| 18 | Colliculus inf. / pons | √ | | | | | | | | | |
| 19 | Loc. Coeruleus / pons | √ | | | | | | | √ | | |
| 20 | Cerebellum / dent. | √ | | | | | | | | | |
| 21 | Medulla oblongata | √ | | | | | | | √ | | |
| 22 | Spinal cord C | √ | | | | | | | | | |
| 23 | *Basilar Artery | √ | | | | | | | | | |
| 24 | Olfactory bulb | √ | | | | | | | | | |
| 25 | Frontal lobe white matter | √ | | | | | | | | | |

Table 5. Special staining and immunohistochemistry (IHC) staining of different brain regions in PD cases (red√for additional regions). **Note:** Red √ for additional regions.

| Manufacturer | Antibodies | Clone | Cat. No. |
|--------------|---|-----------|----------|
| Affinity | Phospho-TDP43 (Ser409/Ser410) antibody | pS409/410 | AF3832 |
| BD | Purified Mouse Anti-p62 Ick ligand (p62) | | 610833 |
| Thermo | Anti-Human Phospho-PHF-tau pSer202/Thr 205 Monoclonal Antibody (p-tau) | AT8 | MN1020 |
| Novocastra | NCL-L-ASYN (α -SYN) | KM51 | ASYN-L |
| Sigma | Monoclonal Anti- β -Amyloid antibody produced in mouse (A β) | 6F/3D | A3981 |
| Biolegend | Monoclonal Anti- β -Amyloid antibody produced in mouse (A β) | 6E10 | 803001 |

Table 6. SAntibodies are recommended for neuropathological assessment.

7.3 Pathological diagnoses report

Standardized, unified, accurate, and comprehensive neuropathological diagnosis should be formulated to ensure the consistency of quality standards and facilitate resource sharing among different human brain banks. The pathological diagnosis report of donors needs to be reviewed and signed by a licensed pathologist or a neuropathologist qualified as licensed physician. The neuropathological quality control team is obliged to conduct random on-the-spot checks on the consortium members' pathological reports, ensuring standardising the neuropathological diagnosis process. The pathological diagnosis result should be recorded in detail and preserved in the database.

8. DNA, RNA and protein analysis

The progression of the molecular analysis techniques, such as single-cell sequencing, proteomic analysis, DNA, RNA and protein analysis from the autopsy brain sections, could assist the diagnosis and mechanism research as well as the new biomarkers exploration. These data collections are recommended in a well-equipped brain bank.

9. Application and feedback of using brain tissues

Human brain tissues applicant should be Principal Investigators from research institutes, universities or colleges. The applicants should formally submit the application. The application should include experimental objectives and methods, a teaching program or research plan, funding support, ethical approval, etc. The Academic Committee of the individual brain bank should review the submission to decide if the samples can be provided. If the application is accepted, the applicant should sign the Sample Usage Agreement or Material Transfer Agreement (MTA) which asks the applicant to use samples according to the plan, keep safe of the models and should not give them to other researchers. Feedback on the quality of samples, research data and publications from these samples should be submitted to the brain bank.

Principles abided by applicants of human brain tissues:

- (1) The sample users of brain tissues and related materials from the human brain bank must be researchers listed in the application forms. Research sites where the researcher's conduct should also be consistent with that in the application forms.
- (2) Any information on the human brain tissues applied by researchers must be kept anonymization.
- (3) The published articles use human brain tissues should include acknowledgements to the human brain bank.
- (4) The sample use agreement shall become effective after being signed by all listed researchers and institute representatives of applicants, as well as the person in charge of the human brain bank.
- (5) The published research results using human brain tissues should be provided to the human brain bank by the applicants or researchers.

10. Information management of brain tissues

10.1 The preservation and management of brain tissue information

- (1) The paper materials and electronic archives of the basic information of donors, including name, age, sex, place of birth, place of residence, contact information, health status, medical history and medication, etc., needs to be created, the materials about the detailed knowledge of the brain tissues and informed consent on autopsy should also be created.
- (2) Record form of brain autopsy should be kept;
- (3) Photos of the front, side, and bottom of the brain, as well as the coronal and sagittal slices of the brain, should be taken for preservation;
- (4) Slice for neuropathological assessment should be scanned and preserved. Photos acquired through fluorescence staining, western blotting, or other experimental methods should be preserved;
- (5) Molecular analysis information (DNA, RNA, protein, etc.) should be kept;

- (6) Sample application and sharing record should be kept;
- (7) All the information should be collected as a unified database managed by a full-time staff; information backup also needs to be emphasized.

10.2 Database and website of the platform of regional brain bank collaboration network

- (1) Website hyperlinks of each brain bank are set up on the platform website;
- (2) Individual brain bank should upload relevant data and receive the inspection from the quality control group;
- (3) Brief information, such as brain bank ID, sex, age, PMD, etc. of each brain should be present on the website of the platform;
- (4) Applicants registered could browse and submit applying form online; the application is routinely reviewed by the academic committee of the individual brain bank; if one human brain bank cannot meet the requirement of the research, the academic committee of the project is responsible for the coordination of the brain tissue resources of various brain banks.

In conclusion, human brain banks in China are developing at high speed, and the number of members in China Human Brain Bank Consortium has increased from 10 in 2017 to 20. There will be more brain banks and brain donations in other regions of China. We hope that the standardized operational protocol greatly facilitates brain research in China and worldwide.

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