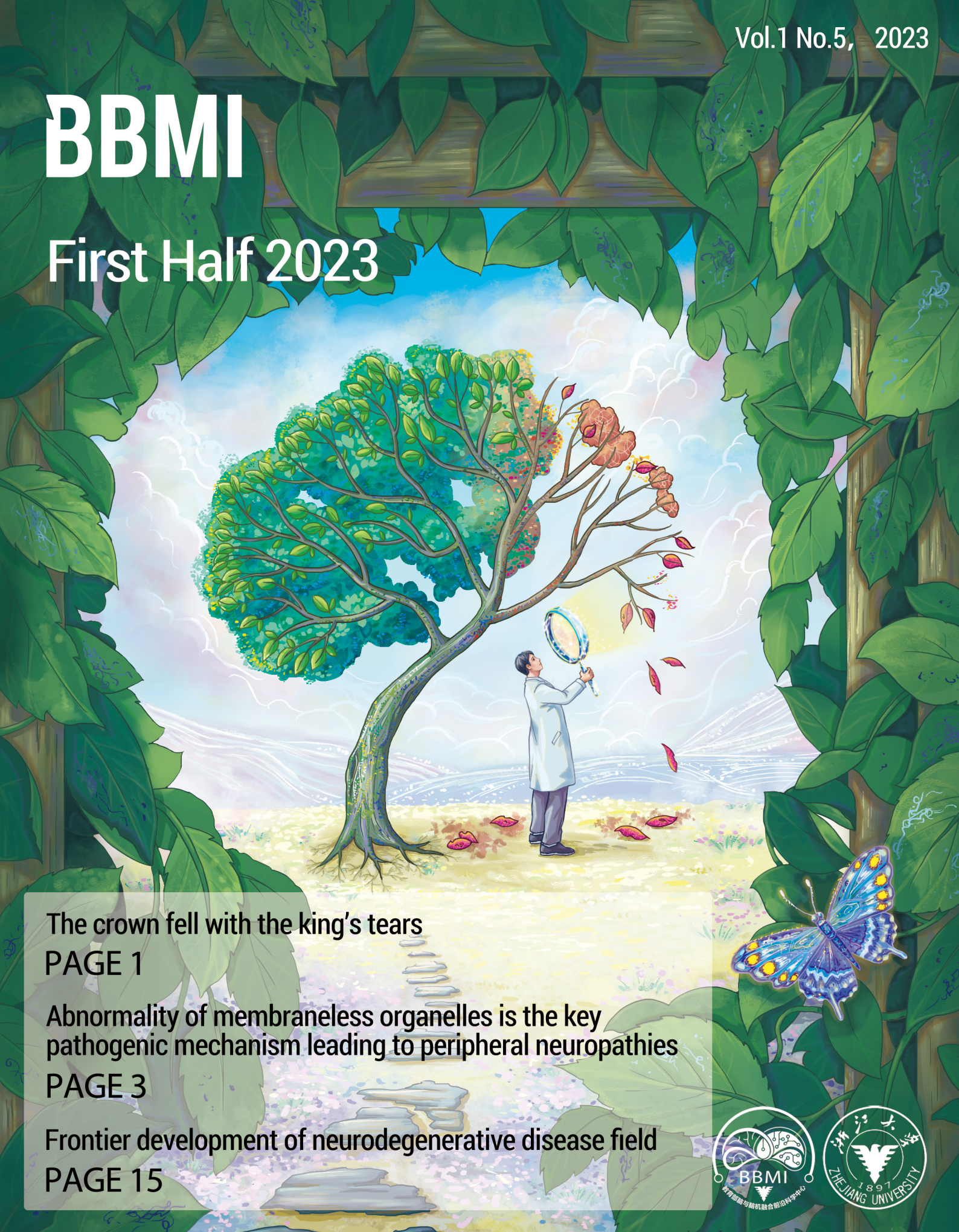
Vol.1 No.5, 2023

BBMI

First Half 2023




BBMI
教育部脑与脑机融合前沿科学中心

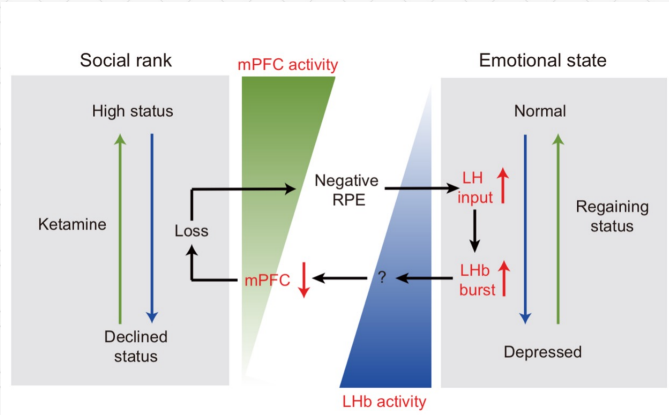
Research Progress

BBMI 2023

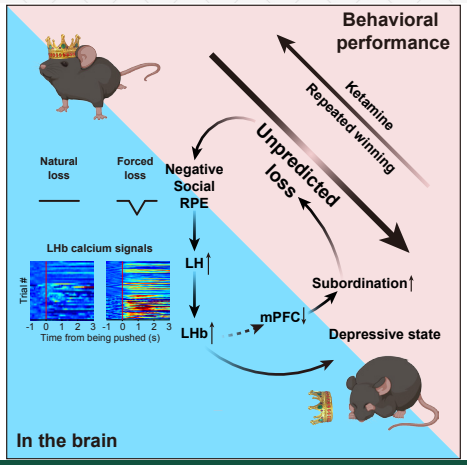
Frontier Development Of Neurodegenerative Diease Field

The crown fell with the king's tears

Neural mechanisms behind depressive-like behaviors following a decline in social status reveal reciprocal interactions between the social and emotional brain.



Reciprocal interactions between mood and social rank



Graphical abstract of this study

Downward social mobility is a well-known mental risk factor for depression, but its neural mechanism remains elusive. Here, by forcing mice to lose against their subordinates in a non-violent social contest, we lower their social ranks stably and induce depressive-like behaviors. These rank-decline-associated depressive-like behaviors can be reversed by regaining social status. *In vivo* fiber photometry and single-unit electrophysiological recording show that forced loss, but not natural loss, generates negative reward prediction error (RPE). Through the lateral hypothalamus, the RPE strongly activates the brain's anti-reward center, the lateral habenula (LHb). LHb activation inhibits the medial prefrontal cortex (mPFC) that controls social competitiveness and reinforces retreats in contests. These results reveal the core neural mechanisms mutually promoting social status loss and depressive behaviors. The intertwined neuronal signaling controlling mPFC and LHb activities provides a mechanistic foundation for the crosstalk between social mobility and psychological disorder, unveiling a promising target for intervention.

Fan, Z. et al. (2023) 'Neural mechanism underlying depressive-like state associated with social status loss', ***Cell***, 186(3). doi:10.1016/j.cell.2022.12.033.

HAILAN HU'S RESEARCH GROUP

For social animals, emotions and health are regulated by various social behaviors. Hailan Hu's group is dedicated to studying the neural basis and plasticity mechanisms of emotion and social behavior. They use cutting-edge techniques including imaging, electrophysiology (both *in vitro* and *in vivo*), molecular genetics, and optogenetics to conduct deep analysis of emotion- and social behaviors- and their related neural circuits.

What's the difference between the depressive behavior induced by loss of social status and that induced by other methods (such as chronic restraint, social defeat, etc.)? Also, if a depressive phenotype is induced in mice via these other methods, would they therefore also lose social status?

Zhengxiao Fan (first author): The main difference between the "forced loss" paradigm and other models is the risk factors of inducing depression. In the "forced loss" paradigm, mice are non-violently degraded to a lower position in the hierarchy. This is a more naturalistic setting related to the descent of human social rank which, in modern societies, does not typically result from physical fighting. Behaviorally, this psychological-stress-based depression model also induces behavioral despair and anhedonia just as also noted in other depression models. Mechanistically, all these models converge in information flow from upstream to the lateral habenula, leading to the development of depressive states. However, whether stress induced by other models reduces the social status has remained unexplored.

If the mice regain victory in the tube test paradigm with the help of artificial interference, would they also regain their lost social status?

Zhengxiao Fan: In this, we optogenetically activated the prefrontal cortex during the tube tests. By doing so we were therefore able to successfully restore the social status of rank-declined mice. Notably, mice that regained their dominant ranks showed reduced depressive-like phenotypes compared with the control group.

Is there any difference in lateral habenula in individual mice before forming a stable social hierarchy?

Zhengxiao Fan: Whether there is difference of the basal activity of LHb in difference mice without stable social rank has remained unexplored. It may be a promising question for the future.

Would stimulating LHb or inducing depressive state through behavioral methods lead to loss of rank in dominant mice individuals?

Zhengxiao Fan: To answer this we optogenetically activated LHb or the terminals of its upstream brain area, lateral hypothalamus, and successfully induce retreat and loss-of-rank in tube test. We are also now trying to figure out whether other behavioral models of depression can lower the social status. For example, we previously tried testing with the model of chronic restrain stress. However, this was difficult to analyze as mice that had previously experienced the chronic restrain stress then displayed reluctance to move within the tube, possibly by association between the similar circumstances of the narrow restraint of the restrain stress and the narrowness and confining nature of the tube in the tube test.

Do individual mice with different ranks in the same group display different susceptibility to depression?

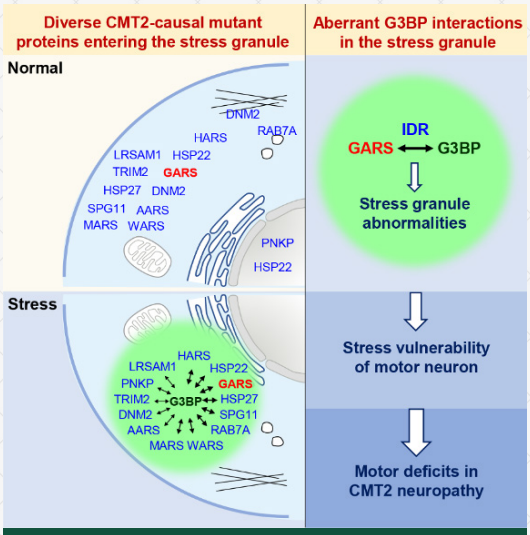
Zhengxiao Fan: For dominant animals, which have a wealth of winning experience, victory in competition becomes the default expectation. So, it is easier to induce a negative social prediction error in dominant mice, and such mice are therefore more susceptible to forced loss. Consistently, dominant animals are also more vulnerable to depression induced by chronic social defeat stress (CSDS, during which mice or rats are exposed to attacks by an extremely aggressive opponent for multiple days) than their subordinate cagemates (Larrieu et al., 2017).

Is it possible to raise the social rank of low rank mouse in depressive state through forced winning or mPFC stimulation?

Zhengxiao Fan: Previous Science articles from our lab have reported that activation of dmPFC instantaneously induced winning against previously dominant opponents with a 90% success rate, and dramatically changed the competitive strategies of such mice. We also hypothesized that forced win or activation of dmPFC could also increase the social status in depressed mice.

Abnormality of membraneless organelles is the key pathogenic mechanism leading to peripheral neuropathies

Complex diseases often involve the interplay between genetic and environmental factors. Charcot-Marie-Tooth diseases (CMT) are a group of inherited peripheral neuropathies with a prevalence of ~1:2500. According to various causal genes, CMT can be classified into many different subtypes. Despite similar clinical presentation among different CMT2 subtypes, their causal mutant proteins are highly varied in their cellular localizations and functions and It remains unclear whether there is a molecular link across different CMT-causal mutant proteins.



Abnormality of membraneless organelles is the key pathogenic mechanism leading to peripheral neuropathies

On February 3, 2023, Prof. **Ge Bai**'s research group from the School of Brain Science and Brain Medicine, Zhejiang University School of Medicine, and LI Jinsong's research group from the CAS Center for Excellence in Molecular Cell Science, Chinese Academy of Sciences published a cover article in ***Cell***, entitled *"Diverse CMT2 Neuropathies are Linked to Aberrant G3BP Interactions in Stress Granules"*. This study reveals that stress granule abnormality is the common pathogenic mechanism underlying different subtypes of CMT2 diseases. This finding establishes an important theoretical basis for the development of uniform treatment for multiple CMT subtypes, and also provides a new conceptual framework for understanding similar aspects of genetic heterogeneity in other diseases.

Over the past decades, there has been an emerging consensus that most, if not all, neurological diseases involve interplays between genetic predisposition and environmental stressors. The formation of stress granules (SGs) is an important anti-stress mechanism that occurs in response to various aspects of environmental insult. Once formed, SGs help avoid protein mistranslation and effectively organize various signaling molecules and energy resources in cells, thereby improving cell survival under stress conditions. When the environmental stress disappears, the SGs are disassembled, and the translational machinery and various signaling molecules quickly resume their normal functions.

In this study, researchers began with glycyl-tRNA synthetase (GlyRS), the causal protein of the CMT2D subtype. When motor neurons were exposed to adverse environmental stimuli, the mutant GlyRS proteins translocated from the cytoplasm to the newly formed stress granules where they aberrantly interacted with the SG core protein, G3BP.

Using techniques including live cell imaging, proximity labeling, quantitative mass spectrometry, and STORM super-resolution imaging, the researchers found that the abnormal interaction between mutant GlyRS and G3BP had no effect on SG dynamics but significantly perturbed the G3BP-centric core SG network. This resulted in over-sequestration of many non-SG components and disrupted the SG-mediated stress response leading to increased stress vulnerability for motor neurons. The researchers also found that the aberrant interaction between mutant GlyRS and G3BP proteins was mediated by an intrinsically disordered region (IDR). Mutations in this region were then discovered to eliminate the influence of mutant GlyRS upon SGs, and alleviate motor deficits in a CMT2D mouse model.

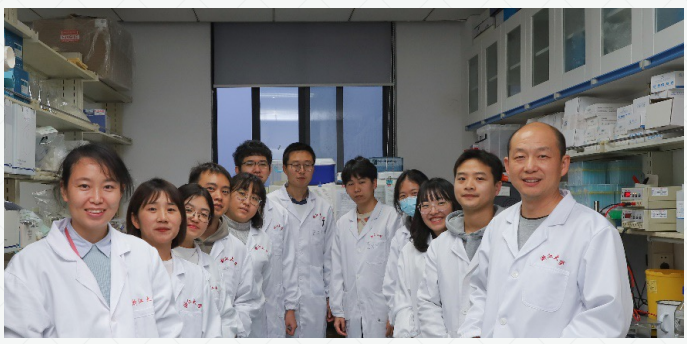
Furthermore, the researchers found that this mechanism could be extended to other CMT2 subtypes. By testing more than 20 different CMT2-causal mutant proteins, the researchers found that, upon stress, most of these proteins can enter SGs and aberrantly interact with G3BP. This led to SG abnormalities, and disturbed the stress response in motor neurons. These findings suggested that SG abnormalities may represent a common pathogenic mechanism underlying different subtypes of Charcot-Marie-Tooth diseases.

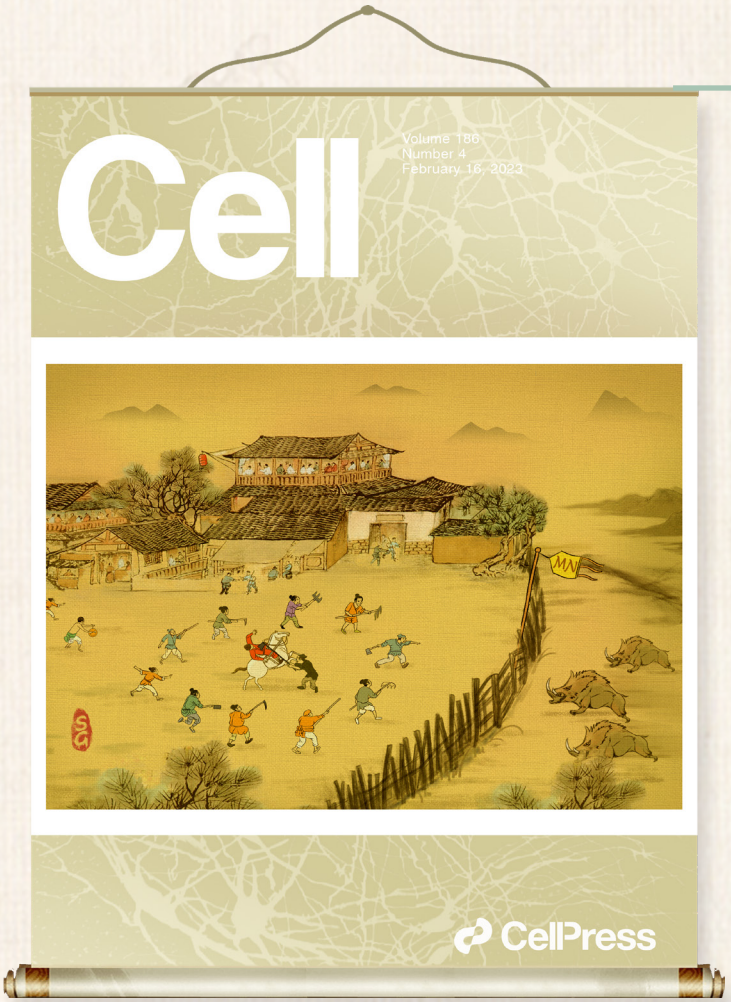
Overall, the researchers found that upon environmental stress, many CMT2-causing mutant proteins adopt similar properties by entering stress granules (SGs) where they aberrantly interact with G3BP and integrate into SG pathways. This disrupts SG-mediated stress responses, leading to increased stress vulnerability in motoneurons. These findings reveal a stress-dependent molecular link across diverse CMT2 mutants and provide a conceptual framework for understanding genetic heterogeneity in light of environmental stress.

Cui, Q. et al. (2023) 'Diverse CMT2 neuropathies are linked to aberrant G3BP interactions in stress granules', ***Cell***, 186(4). doi:10.1016/j.cell.2022.12.046.

GE BAI'S RESEARCH GROUP

The long-term objectives of Prof. Ge Bai lab research are: (1) To study the molecular mechanisms controlling the assembly of motor circuits; (2) To understand how neural miswiring and degenerative disorders affect motor circuit function and behavior; (3) To develop new therapeutic strategies for effective maintenance and repair of motor circuits under pathologic conditions.





Cover image legend

Charcot-Marie-Tooth type 2 neuropathies (CMT2) are a group of genetically heterogeneous disorders, in which similar peripheral neuropathology is inexplicably caused by various mutated genes with diverse cellular locations and functions. In this issue, Cui et. al. demonstrate that, upon environmental stress (beast attack), these CMT2 mutant proteins (man in black) adopt similar activities by entering the stress granule (crowd annotated with the “SG” seal) and aberrantly interacting with SG core protein, G3BP (horse rider), thereby disrupting the stress response in motor neurons (fenced village annotated with the MN flag). The cover image design is inspired by the ancient Chinese painting (Riverside Scene at Qingming Festival); the background is adapted from the drawing of Santiago Ramón y Cajal (motor neurons in the spinal cord). Artwork by Bai lab.

Prof. Boxun Lu

School of Life Sciences, Fudan University

Organelles serve as the fundamental functional and structural components of cells and their shape, composition, quantity, and other attributes are intricately linked to cellular physiological function. Among these organelles, RNA granules represent a distinct type of membraneless organelle composed primarily of RNA and RNA-binding proteins. These exist in a state of liquid-liquid phase separation within cells. In contrast to well-studied membrane-bound organelles, such as mitochondria or lysosomes, our understanding of membraneless organelles remains in its nascent stages. Despite this, such organelles are now known to play a pivotal role in various diseases, including degenerative conditions, and the study of RNA granules is therefore emerging as a prominent focus area within the field of international biomedicine.

RNA granules in cells can be divided into different types, among which stress granules (SGs) and Processing bodies (P-Bodies) are among the most widespread. SGs are granule-like structures in the cytoplasm and are formed by cells under stress. When the cell faces external stimuli, the ongoing protein translation on the ribosome is temporarily suspended, and components such as mRNA, RNA binding protein, and translation complex that occur on it are separated from the ribosome and combined with SG core proteins such as G3BP1/2, TIA1, and CAPRIN, through liquid-liquid phase separation to form particles with a size of about 0.1-2 μm. Once

▶ Next page

Prof. Beisha Tang

Department of Neurology
Xiangya Hospital, Central South University

Charcot-Marie-Tooth disease (CMT), also referred to as hereditary sensory and autonomic neuropathy (HMSN), is one of the most prevalent types of hereditary peripheral neuropathies encountered in a clinical setting. The primary clinical manifestations of CMT include progressive muscle weakness and muscle atrophy (mainly affecting the distal limbs), along with other sensory impairments. Based on distinctive neuropathological characteristics, CMT can be broadly classified into two types: CMT1 (demyelinating type) and CMT2 (axonal type). As of now, there is no known clinical treatment capable of reversing the progression of CMT. Current management primarily focuses on symptomatic and supportive care, encompassing rehabilitation therapy, orthopedic surgery, and other interventions.

CMT diseases exhibit a significant degree of "genetic heterogeneity," with CMT2 alone currently associated with over 30 distinct disease-causing genes. Understanding the mechanistic connection between the various subtypes of CMT has long posed a challenge due to the diverse intracellular localization and functions of these disease-causing proteins. Research conducted by the team led by Ge Bai and Jinsong Li has now revealed that while these pathogenic proteins associated with CMT2 may appear unrelated under normal physiological conditions, they exhibit distinct similarities when exposed to environmental stress conditions. Specifically, they accumulate within stress granules, engage in abnormal interactions with G3BP, and thereby lead to disruptions in stress granule dynamics. This groundbreaking study suggests that aberrant stress granules could serve as a shared pathogenic mechanism among diverse subtypes of CMT2 which significantly enhances our comprehension of the "genetic heterogeneity" conundrum within this field and offers a novel avenue for the development of broad-spectrum therapeutic drugs targeting rare nervous system disorders like CMT.

▲

▶ **Continued** the environmental stress is alleviated, the SG within the cell undergo depolymerization. The mRNA molecules and diverse components of the translation complex are then transported back to the ribosomes, allowing protein translation to resume. This dynamic process of assembly and depolymerization of SGs serve a crucial role in preventing protein mistranslation during times of stress. This enables cells to efficiently adapt to adverse environmental stimuli and helps ensure their ability to cope with such challenges effectively.

Over recent years, mounting evidence has highlighted the significant involvement of SGs in various neurodegenerative diseases. One notable example is amyotrophic lateral sclerosis (ALS), a prominent clinical motor neuron disorder. Here, unfavorable environmental stimuli, genetic mutations, and other factors can induce abnormal aggregation and protein misfolding of disease-associated proteins like TDP43 and FUS within SGs, which occur in a specialized microenvironment characterized by liquid-liquid phase separation. These protein abnormalities ultimately contribute to motor neuron degeneration. However, the role of SGs in certain neurological diseases, including CMT, where obvious protein aggregation is not observed, remains unclear and the understanding of how SGs function in these specific diseases has been limited.

The research conducted by Ge Bai and Jinsong Li's research group has discovered a distinct characteristic in CMT2 pathogenic proteins that sets them apart from neurodegenerative diseases like ALS. Unlike ALS, the pathogenic proteins associated with CMT2 do not influence the dynamic assembly disassembly of SGs or lead to pathological protein aggregation. However, these proteins disturb the SG core and exhibit abnormal interactions with the protein G3BP. This disrupts the network of SG core proteins, compromising the stress-responsive function of SGs. In comparison to neurodegenerative diseases like ALS, CMT demonstrates distinct characteristics such as early onset and slow progression. Consequently, a plausible hypothesis emerges suggesting that abnormalities in the SG core protein network and function may exist in other neurodegenerative disorders, including ALS. In this way, the formation of SGs could be an overlooked early pathological event the occurrence of which, coupled with subsequent protein aggregation, could potentially contribute to the rapid advancement and widespread dissemination of diseases like ALS. Conversely, since CMT pathogenic proteins typically do not form protein aggregates, the disease progression tends to be relatively gradual. This study introduces a novel mechanism that highlights the involvement of SG in peripheral nerve diseases, thus also broadening our understanding of the role played by membraneless organelles in neurodegenerative disorders. It also offers fresh insights for the development of drugs targeting these related diseases, presenting new avenues for therapeutic intervention.

Mapping structural and functional brain alterations in drug-naïve obsessive-compulsive disorder

INTERDISCIPLINARY INTEGRATION

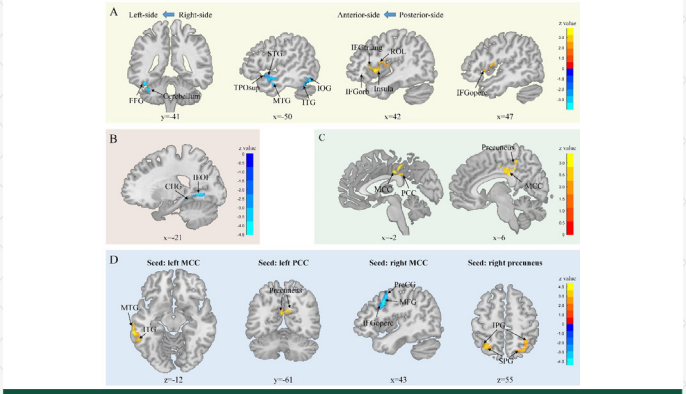
Interdisciplinary integration is an important driving force for promoting scientific innovation and development. The interdisciplinary collaborative innovation between medicine and engineering is a particularly exciting emerging interdisciplinary field. Here, incorporating the interdisciplinary collaboration of engineering science and psychiatry, this study was guided by clinical demand and applied multimodal ultra-high field MRI technology to the diagnosis of mental disorders. Such interdisciplinary collaboration and communication improves the quality of clinical research and empowers both health and well-being within society.

Obsessive-compulsive disorder (OCD), the fourth most common psychiatric disorder, is a common chronic mental disorder characterized by obsessions and compulsions. As a refractory, highly comorbid and disabling disease, OCD has a lifetime prevalence of 2%-3%. The main characteristics of OCD include repeated occurrences of uncontrollable thoughts (obsessions) and behaviors (compulsions). Residual symptoms, easy recurrence and protraction of the disease course not only seriously affect the quality of life and social functions of patients, but also increase the economic burdens arising from treatment. Therefore, OCD has become one of the top ten disabling diseases. However, the complex etiology and pathological mechanism of OCD is not yet fully understood.

Over recent years, magnetic resonance imaging (MRI) has been developed and increasingly used as a noninvasive and nonradioactive tool to reveal brain abnormalities in OCD. Previous MRI studies have demonstrated both structural and functional alterations of the cortico-striato-thalamo-cortical (CSTC) circuits consisting of the orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), caudate, putamen, globus pallidus, thalamus and subthalamic nucleus, which may play vital roles in the pathogenesis of OCD. Additionally, recent studies have found abnormal brain function in regions outside the CSTC circuits including the amygdala-hippocampus pathways, and in frontal-parietal and cerebellar-default mode network connectivities. However, these findings were inconsistent across studies due to several limitations, including small sample sizes, different inclusion/exclusion criteria, varied demographic characteristics, varied symptom dimensions, comorbidity, and variable medication status. In this way, prominent and consistent neuroimaging biomarkers remain to be discovered.

Using 7.0T multimodal magnetic resonance imaging, Prof. **Hsin-Yi Lai**, Prof. **Tao Li** and Prof. **Wenxin Tang** have collaborated to explore structural and functional alterations in drug-naïve obsessive-compulsive disorder patients. Their findings were published in the internationally renowned journal ***Asian Journal of Psychiatry*** in December. 2022. This study comprehensively revealed not only the classical cortico-striatal-thalamic-cortical (CSTC) circuit, but also clear aspects of the limbic system, and those of the default, visual, language, and sensorimotor networks, relevant to the pathogenesis of OCD.

Drug-naïve OCD patients exhibited significantly increased gray matter



Abnormalities in the structural and functional networks of the brain in patients with obsessive-compulsive disorder.
(A) Gray matter volume (B) White matter fractional anisotropy (C) Amplitude of low frequency fluctuation (D) Seed-based functional connectivity.

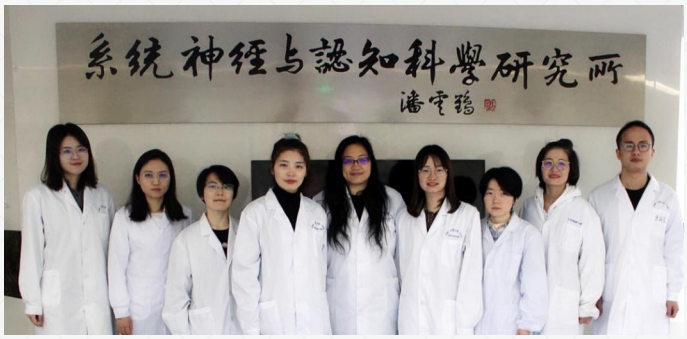
volume in the frontal cortex, especially in the orbitofrontal cortex, as well as volumetric reduction in the temporal lobe, occipital lobe and cerebellum. In addition, the integrity of white matter in the cingulate and inferior fronto-occipital fasciculus was compromised. Increased neural activities were observed in the cingulate gyri and precuneus. Increased temporal-middle cingulate and posterior cingulate-precuneus functional connectivity and decreased frontal-middle cingulate connectivity were further detected. Decreased fractional anisotropy values were found in the cingulum-hippocampus gyrus and inferior fronto-occipital fascicle in OCD patients. Moreover, significantly altered imaging features were related to OCD symptom severity. The severity of obsessions could be explained by abnormal brain activities in the CSTC circuit, DMN, limbic system and visual network, whereas the severity of compulsions could be explained by gray matter structural impairments in the DMN, limbic system, visual, language and sensorimotor network as well as white matter microstructural deficits.

In summary, the multimodal imaging information in our findings acts in a complementary manner to provide increased evidence that structural and functional abnormalities in multiple functional circuits could be regarded as major contributors to the neural mechanisms of OCD. This is the first 7.0T MRI study to explore brain structural and functional alterations of drug-naïve OCD, and the findings presented herein may promote future studies using 7.0T to identify the most prominent and replicable neuroimaging alterations of OCD. In the future, we will expose high specific and sensitive image markers for different dimensions of OCD by using brain-inspired artificial intelligence and further investigate the mechanism of occurrence and development and efficacy assessment for OCD. We also hope to propose intervention strategies for precise treatment of OCD patients.

Tang, W. et al. (2023) 'Exploring structural and functional alterations in drug-naïve obsessive-compulsive disorder patients: An ultrahigh field multimodal MRI study', ***Asian Journal of Psychiatry***, 81, p. 103431. doi:10.1016/j.ajp.2022.103431.

HSIN-YI LAI'S RESEARCH GROUP

The goal of Hsin-Yi Lai's group is devoted to developing and applying cutting-edge brain-machine fusion technology for investigating brain function and brain diseases. Our current research interests include: 1) developing an optical, electrical, magnetic, and acoustic multimodal fusion technology for recording and modulating the brain, 2) early detection of brain diseases using ultra-high field multimodal MRI. Our team has achieved innovative results in brain imaging technology, brain information processing, and brain reading and writing to core devices that provide important support for brain science and brain disease research.

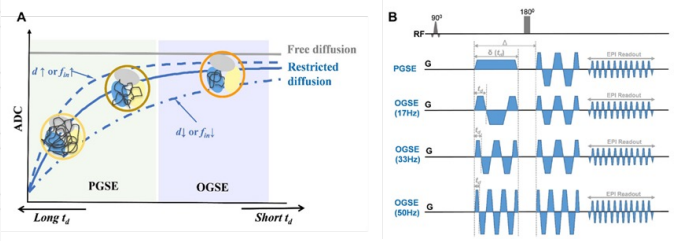


Histological and molecular classifications of pediatric gliomas with time-dependent diffusion MRI based microstructural mapping

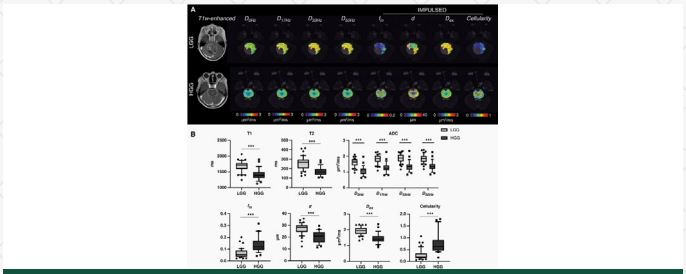
INTERDISCIPLINARY INTEGRATION

The success of this study is attributed to the collaboration among experts in biomedical engineering, radiology, and pediatric oncology. By combining diverse perspectives, methodologies, and expertise, we were able to pioneer new avenues in the field of CNS tumor research. This interdisciplinary synergy enabled us to develop novel approaches and gain insights into the microstructural characteristics of pediatric gliomas. The integration of advanced imaging techniques, such as t_d-dMRI, with histological and molecular analyses played a pivotal role in unraveling the intricacies of these tumors. This collaborative effort not only deepened our understanding of CNS tumors but also underscored the significance of interdisciplinary cooperation in addressing complex medical challenges.

Central nervous system (CNS) tumors are the most common solid tumors in children and a leading cause of mortality. Among CNS tumors, gliomas are the most prevalent type which are histologically classified into low-grade gliomas (LGG) and high-grade gliomas (HGG), with the latter having a long-term survival rate of less than 10%. The diffuse midline glioma (DMG) is a particularly devastating category within pediatric gliomas, typically located in critical areas such as the thalamus, brainstem, or spinal cord, making surgical



(A) Schematic diagram of time-dependent diffusion magnetic resonance imaging technique.
(B) Schematic diagrams of PGSE (Pulsed Gradient Spin Echo) and OGSE (Oscillating Gradient Spin Echo) sequences used in the experiment.



(A) Microstructural parameter maps obtained using the td-dMIRI technique for representative patients from histological groups.
(B) Intergroup differences of various MRI indices between LGG and HGG.

intervention impractical. These DMGs have an extremely low survival period of only 9-11 months. Regardless of the histological characteristics, patients with DMG carrying H3K27 alterations exhibit significantly lower overall survival. Thus, H3K27 alteration serves as a crucial molecular biomarker for DMGs in the 2021 World Health Organization (WHO) classification of CNS tumors. In this way, accurate histological and molecular grading is essential for both prognostic determination and for the choice of treatment strategies in children with DMG.

On January 7, 2023, Prof. **Dan Wu**'s team from the College of Biomedical Engineering and Instrument Science at Zhejiang University, in collaboration with Dr. **Hongxi Zhang**'s team from the Department of Radiology at Zhejiang University Children's Hospital, published a paper titled "*Histological and Molecular Classifications of Pediatric Glioma with Time-dependent Diffusion MRI based Microstructural Mapping*" in the journal ***Neuro-Oncology***. The team introduced a non-invasive microstructural mapping technique based on the theory of using time-dependent diffusion MRI (t_d-dMRI) to quantify tumor cell characteristics. They were able to demonstrate its initial efficacy in histological grading and H3K27 molecular subtyping of pediatric gliomas.

t_d-dMRI, based on diffusion time, exhibits unique advantages in mapping the cellular microstructure. By utilizing specialized diffusion encoding schemes, it captures restricted diffusion across multiple diffusion times. This time-dependent diffusion measurement can then be used to construct biophysical models for quantifying microstructural features. t_d-dMRI -based microstructural imaging is therefore considered a step towards achieving "virtual pathology."

The team initially recruited 75 pediatric patients and acquired t_d-dMRI images of gliomas using the house-made oscillating gradient (OGSE) and pulsed gradient spin echo (PGSE) sequences. Subsequently, this data was fitted to the IMPULSED model and correlated with pathological slices.

The team discovered that, for the histological grading of HGG, cell densities and intracellular fractions were significantly higher than those of LGG, whilst those of the cell diameter, diffusion coefficients, T1 and T2 values were significantly lower. Overall, cell density showed the best classification performance. Regarding molecular subtyping, H3K27-altered tumors showed significantly lower T1, T2, apparent diffusion coefficients (ADC), extracellular diffusion coefficients, and cell diameters, as well as higher intracellular fractions and cell densities compared to wild-type DMG. Among these features individually, cell diameter showed the best classification performance. However, the combination of cell diameter and extracellular diffusion coefficient achieved the highest overall performance improvement. By correlating model parameters with pathological images obtained from H&E-stained sections, the team were able to demonstrated high level of consistency.

As emphasized by the WHO 2021 guidelines, combining histological and molecular information is crucial for accurate prognosis and optimal treatment selection in CNS tumors. The team designed a prospective study to explore the clinical application of newly proposed cellular microstructural markers based on t_d-dMRI theory in the histological and molecular identification of pediatric gliomas. Preliminary results indicated that the cell volume index exhibited favorable performance in histological grading, while the cell diameter index demonstrated high discriminatory power in distinguishing H3K27-altered and wild-type DMGs, underscoring the value of these non-invasive microstructural features in pediatric gliomas. Particularly, distinct microstructural characteristics were observed among different histological and molecular subtypes, highlighting the importance of utilizing pathology indicates that specifically reflect cellular microstructure rather than relying solely on simple ADC measurements. Such a desired accurate picture can be provided by t_d-dMRI technology.

Zhang, H. et al. (2023) 'Histological and molecular classifications of pediatric glioma with time-dependent diffusion MRI-based microstructural mapping', ***Neuro-Oncology***, 25(6), pp. 1146–1156. doi:10.1093/neuonc/noad003.

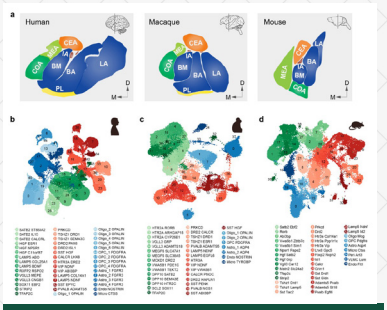
DAN WU'S RESEARCH GROUP

The Advanced Magnetic Resonance Imaging and Technology (AMRIT) Laboratory is dedicated to brain science research and innovative imaging technology. The team conducts a range of pioneering studies such as high-resolution rapid imaging, microstructural imaging based on diffusion MRI, and imaging of fetuses and infants. The original research has been published in top-tier journals within the fields of ***PNAS, Radiology, NeuroImage,*** and more.

Investigating the evolution of the amygdala across species to explore the origin of emotion

The research team led by Prof. Xiaoming Li has recently published an article entitled *"Molecular and cellular evolution of the amygdala across species analyzed by single-nucleus transcriptome profiling"* in *Cell Discovery* on Feb. 14th. This research uncovered both evolutionary conservation and divergence of the amygdala across species and laid a solid foundation for future studies of the amygdala's function at the cell-type level.

The amygdala, or an amygdala-like structure, is found in the brains of all vertebrates and plays a critical role in survival and reproduction. Damages or functional disruptions of the amygdala have been implicated in various neurological diseases, especially neuropsychiatric disorders including schizophrenia, anxiety, and bipolar disorder. Structurally, we also know that the amygdala is a heterogeneous complex that includes multiple subnuclei and neuronal cell types originating from cortical and subcortical territories. In this way it represents a mosaic-like structure with multiple embryonic origins. However, the picture and details, beyond these observations, soon becomes rather more opaque. Despite two hundred years having passed since we first described this almond-shaped mass of gray matter, our understanding of the cellular composition and gene expression patterns of the amygdala remains highly limited. In particular, the lack of cell-type-specific studies has been a major obstacle to further dissecting amygdala function and understanding amygdala-related disorders.



Profiling of amygdala cell types across mammals by snRNA-seq.

Recent advances in single-cell and single-nucleus RNA-sequencing (scRNA-seq/snRNA-seq) technologies have facilitated the molecular characterization of diverse cell types in different brain regions across species. To characterize cell types of the amygdala at the transcriptomic level in mammals and sauropsids (the most diverse and successful group of terrestrial vertebrates encompassing both birds and reptiles), our researchers applied snRNA-seq assays (Chromium v3) using human, macaque, mouse, and chicken samples. This work generated snRNA-seq data for more than 200,000 cells in the amygdala for each of these groups. An unprecedented number of mammalian amygdala neuronal and non-neuronal cells were captured in these datasets. Strikingly, the vast majority were neuronal cells, among which excitatory neurons and inhibitory neurons accounted for about 50% each. Hybridization (ISH) data from the Allen Brian Atlas and RNAscope analysis showed that neurons in each cluster exhibited spatial distribution specificity and were each primarily confined to one particular subnucleus of the amygdala. Cross-species analysis revealed that inhibitory neurons and inhibitory neuron-enriched subnuclei of the amygdala were well-conserved in cellular composition and marker gene expression, whereas excitatory neuron-enriched subnuclei were relatively divergent. Furthermore, $LAMP5^+$ interneurons were much more abundant in primates, while $DRD2^+$ inhibitory neurons and $LAMP5^+$ $SATB2^+$ excitatory neurons were particularly dominant in the human central amygdalar nucleus (CEA) and basolateral amygdalar complex (BLA), respectively. This work also identified CEA-like neurons (lateral ganglionic eminence-derived (LGE), LGE-derived) and their species-specific distribution patterns in chickens. Based on these observations, our researchers hypothesized that amygdala subnuclear specialization in chickens may be incomplete due to the widespread distribution of LGE-derived interneurons. On the other hand, the dramatic increase in MGE/CGE-derived (medial ganglionic eminence/caudal ganglionic eminence) neurons in mammals may be a driving force for specialization of amygdala subnuclei, replacing the function of LGE-derived neurons in the pallial amygdala during evolution.

This research highlights the extreme cell-type diversity in the amygdala and reveals the conservation and divergence of cell types and gene expression patterns across species that may contribute to species-specific adaptations.

Prof. Xiaoming Li from Zhejiang University School of Medicine is the main corresponding author. Prof. Xiaoqun Wang and Prof. Qian Wu from Beijing normal university are co-corresponding authors. Dr. Bin Yu, Qianqian Zhang and Lin Lin are co-first authors. This work was supported by Science and Technology Innovation 2030 – the "Brain Science and Brain-Inspired Technology" Major Project, the Major Program of the National Natural Science Foundation of China, the Strategic Priority Research Program of the Chinese Academy of Sciences, the Key-Area Research and Development Program of Guangdong Province, the Key R&D Program of Zhejiang Province, the Fundamental Research Fund for the Central Universities, and the CAMS Innovation Fund for Medical Sciences.

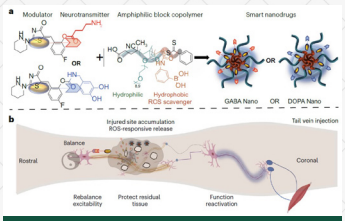
Yu, B. et al. (2023) 'Molecular and cellular evolution of the amygdala across species analyzed by single-nucleus transcriptome profiling', ***Cell Discovery***, 9(1). Doi:10.1038/s41421-022-00506-y.

XIAOMING LI'S RESEARCH GROUP

Xiaoming Li's research group is dedicated to the study of different synapses and neural circuits, aiming to find molecular targets for treating neuropsychiatric disorders such as anxiety, depression and schizophrenia, and to provide potential treatment strategies. His main research includes: 1) neural circuits of emotion and affective disorders; 2) the pathogenesis and mechanisms of anxiety, depression, schizophrenia, and other neuropsychiatric diseases.

Drug therapy activates residual neural circuits to facilitate spinal cord injury recovery

Spinal cord injury (SCI) can lead to irreversible loss of central nervous system tissue, resulting in partial or complete loss of sensation and motor function below the level of injury. The treatment of SCI has always been a major scientific challenge in the fields of neuroscience and regeneration. Early and accurate rehabilitation therapy can effectively improve the cure rate, reduce disability rate, restore limb function, and improve the quality of life for patients. In fact, the majority of SCI patients do not experience complete spinal cord injury, having still retained some intact residual neural circuits. However, at least two major pathological processes hinder the function of such residual neural circuits. Firstly, trauma-induced cell death and vascular rupture triggers inflammation and the production of cytotoxic factors (such as reactive oxygen species), a process known as secondary injury, which further damages the remaining spinal cord connections and exacerbates their functional deficits. Secondly, SCI induces significant excitatory changes that disrupt the overall balance of the damaged spinal cord neural circuits. Over recent years, brain-spinal cord interface devices have achieved some success in rehabilitation therapy. However, these brain-spinal cord interface devices still face several major challenges in the process of clinical translation: 1) expensive cost, making large-scale application difficult; 2) inability to be applied in the acute phase of SCI treatment; 3) inability to provide neuroprotection during the acute phase of injury to help more neural circuits survive.



Design and mechanism of intelligent nanodrugsfor SCI treatment

As early as 2018, Academician Zhigang He from Harvard Medical School and Academician Xiaosong Gu from Nantong University collaborated on a study published in the journal ***Cell***. They showed that the expression of KCC2 can reduce the excitability of inhibitory interneurons, activate dormant residual tissue during spinal cord injury, and promote functional recovery after SCI using the KCC2 agonist CLP257. However, this method also has some limitations, such as the inability to target specific neural circuits, low bioavailability of the drug, and the inability to provide neuroprotection.

In the face of the remaining significant medical needs of spinal cord injury, one particular challenge is to activate residual neural circuits after injury through drug therapy in order to repair spinal cord function and restore patients' motor abilities. On June 12, 2023, Prof. **Xuhua Wang** and his team from the School of Medicine at Zhejiang University published a research paper titled "*Controlled delivery of a neurotransmitter-agonist conjugate for functional recovery after severe spinal cord injury*" in the journal ***Nature Nanotechnology***. This study developed a dual-functional intelligent nanodrug that can be administered intravenously and which efficiently and specifically targets specific neurons, exerting dual effects of neuroprotection and neural regulation, and promoting the integrity and functional recovery of residual neural circuits after spinal cord injury.

To achieve targeted delivery, Xuhua Wang's team utilized the biological characteristics of the body and "disguised" the drug as a neurotransmitter, successfully achieving targeted delivery functionality. In order to promote the potential accumulation of the drug in tissues, the research team designed a reactive oxygen species (ROS)-responsive release nanocarrier to encapsulate the hydrophobic coupled neurotransmitter CLP-257, thereby creating an intelligent nanodrug. This nanodrug can be administered through minimally invasive intravenous injection, respond to ROS at the site of spinal cord injury, and release the drug. After targeted treatment, GABA Nano can efficiently target GABAergic neurons and exhibit dual actions in the injured spinal cord: clearing accumulated reactive oxygen species in damaged tissues to protect residual neural circuits and targeting inhibitory interneurons to promote the integration of residual neural circuits into the host spinal cord. By preserving residual neurons as much as possible and successfully modulating neuronal excitability, the recovery of hindlimb function in SCI rats was significantly improved.

These research results present a new approach to the development of spinal cord injury treatment drugs involving enhancing targeted neural regulation and combining it with neuroprotection to promote spinal cord function recovery. This may be the most appropriate approach to developing more affordable and universally effective drugs for efficient spinal cord injury treatment.

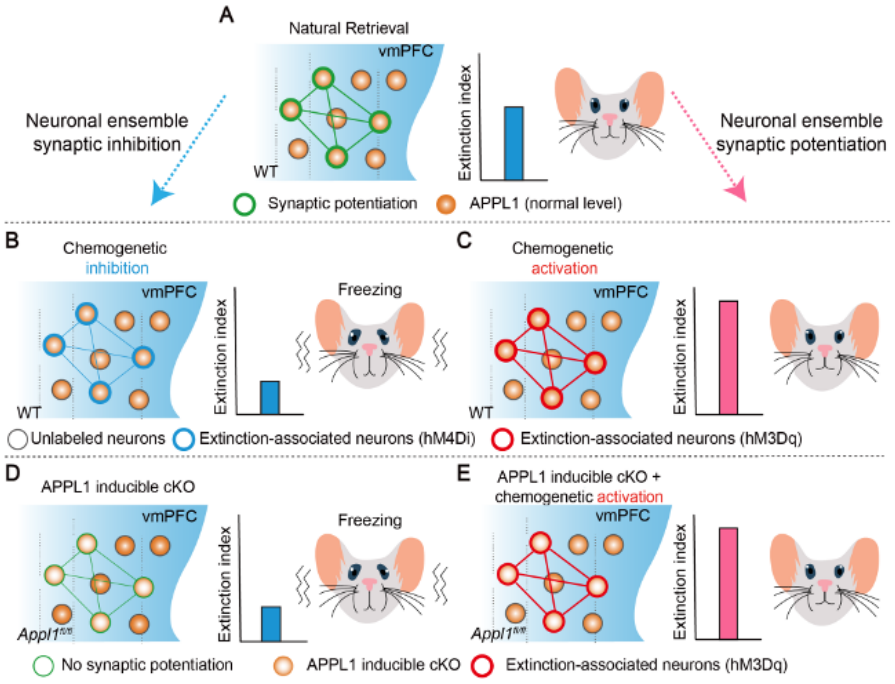
Zuo, Y. et al. (2023) 'Controlled delivery of a neurotransmitter–agonist conjugate for functional recovery after severe spinal cord injury', ***Nature Nanotechnology*** [Preprint]. doi:10.1038/s41565-023-01416-0.

XUHUA WANG'S RESEARCH GROUP

Xuhua Wang's group primarily focuses on drug and drug delivery system research for the treatment of central nervous system diseases. His main research interests include large language models, AI-based drug design, AAV gene delivery vector design and development, brain-machine interfaces, and tissue engineering techniques. Recently, Xuhua Wang's group independently developed the FeatNN drug screening platform based on machine learning. This achievement was published in the journal ***Briefings in Bioinformatics*** in January 2023. In order to find more efficient neural regulatory drugs, the team has used FeatNN to screen more efficient KCC2 agonists from millions of compounds. This new type of agonist is expected to further enhance the therapeutic effects of GABA Nano and achieve more efficient functional repair. In addition, Xuhua Wang's team is also dedicated to exploring drugs and methods for neural axon regeneration. They collaborated with Wang Zhiping's team to develop a class of UbV. E4B protein drugs, which successfully promote cortical spinal axon regeneration. This achievement was published in ***iScience*** in December 2022.

Stay away from fear

A neuronal ensemble in the vmFPC stores fear extinction memory



Schematic diagram of this study

The research team led by Prof. Shuang Qiu has recently published an article titled *"NMDAR-dependent synaptic potentiation via APPL1 signaling is required for the accessibility of a prefrontal neuronal assembly in retrieving fear extinction"* in *Biological Psychiatry* on Feb 24th. This research identified a cellular and molecular mechanism underlying fear extinction, which provides potential therapeutic targets to treat PTSD.

'Fear extinction' refers to a process whereby there is a decline in conditioned fear responses in the absence of adverse events. The ventromedial prefrontal cortex (vmPFC) has been viewed as a locus active in the storing, recalling memory and of the process of fear extinction. However, the synaptic and cellular mechanisms underlying this process have remained elusive. Prof. Shuang Qiu's research group combined transgenic mice, electrophysiological recording, activity-dependent cell labeling, and chemogenetic manipulation to analyze the role of the adaptor protein APPL1 in the vmPFC in fear extinction retrieval. They found that both constitutive and conditional APPL1 knockout decreases NMDA receptor (NMDAR) function in the vmPFC and impairs fear extinction retrieval. Moreover, APPL1 undergoes nuclear translocation during extinction retrieval. Blocking APPL1 nucleocytoplasmic translocation reduces NMDAR currents and disrupts extinction retrieval. This research further identified a prefrontal neuronal ensemble that is both necessary and sufficient for the storage of extinction memory. Inducible APPL1 knockout in this ensemble abolishes NMDAR-dependent synaptic potentiation and disrupts extinction retrieval, while simultaneously chemogenetic activation of this ensemble rescues the impaired behaviors. Therefore, these results indicate that a prefrontal neuronal ensemble stores extinction memory, and APPL1 signaling supports these neurons to retrieve the extinction memory via controlling NMDAR-dependent potentiation.

Hua, S.-S. et al. (2023) 'NMDA receptor–dependent synaptic potentiation via APPL1 signaling is required for the accessibility of a prefrontal neuronal assembly in retrieving fear extinction', ***Biological Psychiatry***, 94(3), pp. 262–277. doi:10.1016/j.biopsych.2023.02.013.

SHUANG QIU'S RESEARCH GROUP

Shuang Qiu's group mainly focuses on identifying synaptic, cellular, and circuitry mechanisms underlying learning and memory, as well as those behind some neuropsychological diseases, such as anxiety disorder. Her lab is also interested in deciphering the neural circuit participating in feeding behavior. Over the recent 5 years, Prof. Qiu's lab has published a series of articles including those published by ***Nature Communications*** (2018, 2020), ***JMCB*** (2021), and ***Biological Psychiatry*** (2023).

The BBMI Academic Reports

2023 First Half

Professor Bo Li

- Chair professor

The Cold Spring Harbor Laboratory

20th Feb. 2023

Dissecting the neural circuit of motivational behaviors

Professor Tianming Gao

- Director

Guangdong-Hong Kong-Macao Greater Bay Area Center for Brain Science and Brain-Inspired Intelligence

3rd Apr.2023

Advances in Novel Targests for Antidepressant

Professor Keqiang Ye

- Endowed Professor and Department Chair

Faculty of Life and Health Sciences
Shenzhen Institute of Advanced Technology
Chinese Academy of Sciences

27th Apr.2023

Alzheimer's Disease:
Novel Mechanisms and Drug Development

Professor Xinhong Zhu

- Professor

School of Biomedical Science and Engineering
South China University of Technology

10th May. 2023

Emotion-Cognation Interface

Professor Tian Xue

- Chair Professor

Department of Life Sciences and Medicine
University of Science and Technology of China

15th May. 2023

Light and Life

Professor Minghu Han

- Executive Director

Faculty of Life and Health Sciences
Shenzhen Institute of Advanced Technology
Chinese Academy of Sciences

28th Feb. 2023

The Formation and Neural Mechanisms of Stress Resilience

Professor Yizheng Wang

- Director

National Clinical Research Center for Geriatric Diseases
Huashan Hospital

- Chief Scientist

China National Clinical Research Center for Neurological Diseases

4th Apr.2023

Brain Injury and Brain-Inspired Perception

Professor Jing Yang

- Principal Investigator

School of Life Sciences
Peking University

9th May. 2023

Neuroimmune Regulation in Physiology and Disease

Professor John Pham

- Editor-in-Chief

Cell

12th May. 2023

Scientific publishing in service to science

Professor Yi Zhang

- Fred Rosen Professor

Harvard Medical School and
Boston Children's Hospital

18th Jul. 2023

Understanding the cellular, molecular, and circuit bases of brain rewardand its dysfunction in neuropsychiatric diseases

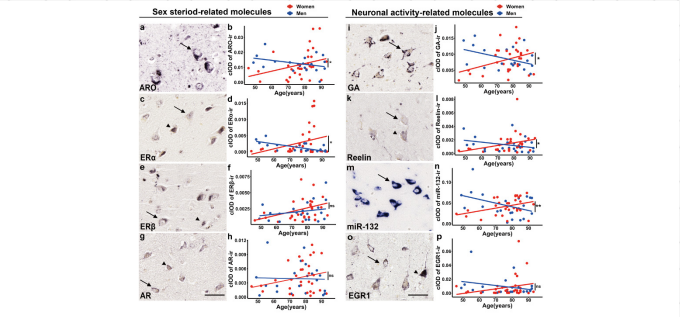
Analyzing the sex differences in the risk of developing Alzheimer's disease

Brains adopt sexually dimorphic strategies to maintain cognition during aging

Alzheimer's disease (AD) is the most prevalent type of dementia in the elderly. It is initially characterized by symptoms of impaired short-term memory and sleep disorders with subsequent increase in global memory loss, aphasia, agnosia, and incontinence. In this way AD seriously affects the health of patients, the life of the family, and puts a heavy burden on society. According to data published by the World Alzheimer's Association in 2022, dementia will eventually affect 139 million people worldwide. AD is a multifactorial disease in which age, genetic factors like APOE-ε4, female gender, obesity and hypertension are important risk factors. However, there are also factors postponing AD, such as being bilingual, having a good schooling, a demanding job and an active lifestyle. AD occurs in 9.4% of patients over the age of 65 years with its prevalence exponentially increasing with age. It is notable that women have a higher prevalence of AD than men, and when affected by AD, female AD patients show a faster decline of cognitive functions. While such sex differences in AD are proposed to be closely related to decline of estrogens in menopause, the mechanisms involved in the onset and progression of AD remains ambiguous. This became one of the aims of the team led by Prof. **Ai-Min Bao**, from the School of Brain Science and Brain Medicine of Zhejiang University, to study the reason why women are more vulnerable than men for AD using postmortem human brains.

In recent postmortem human brain research, this team, in close collaboration with Prof. Dick Swaab in Amsterdam, focused on the entorhinal cortex. This is a brain area crucial for memory and one of the earliest structures affected in AD. They had previously observed that in the entorhinal cortex of donors with intact cognition there was a higher level of hyperphosphorylated Tau (pTau), one of the typical neuropathological markers of AD, in elderly women than in well-matched men (Hu et al.). This implied that the entorhinal cortex was an excellent structure for further studying the mechanisms underlying the remarkable susceptibility of the entorhinal cortex for AD in women.

Based on this finding, the research team further explored the mechanisms behind the increased risk of AD in women during aging, and published the article entitled *"Sexually dimorphic age-related molecular differences in the entorhinal cortex of cognitively intact elderly: Relation to early Alzheimer's changes"* in ***Alzheimer's & Dementia*** (March 24, 2023). Researchers measured the changes in 12 characteristic molecules in relation to age by quantitative immunohistochemistry or in situ hybridization in the postmortem entorhinal cortex of cognitively intact elderly women and well-matched men (46-93 years of age). The molecules were arbitrarily grouped into: i) sex steroid-related molecules, ii) markers of neuronal activity, iii) neurotransmitter-related molecules, and iv) cholinergic activity-related molecules. Most notably, the molecular changes in relation to age indicated increasing local estrogenic and neuronal activity, accompanied by a higher and faster hyperphosphorylated Tau accumulation in women's entorhinal cortex versus a mainly



Sex differences in age-related changes of sex steroid-related molecules and markers of neuronal activity in the entorhinal cortex of cognitively intact elderly.

unaltered local estrogenic/androgenic and neuronal activity in men's. This indicated that the entorhinal cortex employs a different neurobiological strategy in women versus men to maintain cognitive function. Unfortunately this seems to function at the cost of an earlier start of AD in women. In other words, the activation in the elderly women's entorhinal cortex may not only be a compensatory response to the altered brain functions related to age but also be 'the beginning of the end' in terms of AD.

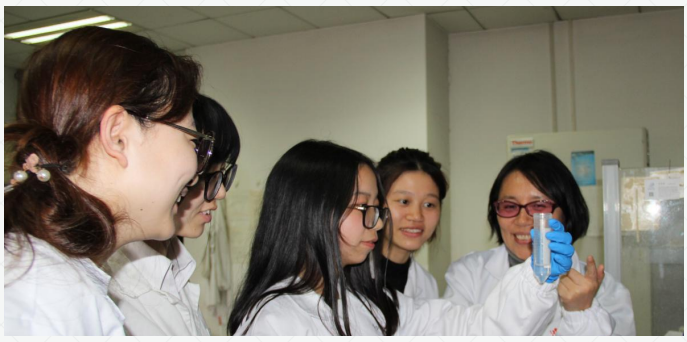
These significant findings are encouraging the team to continue to explore the sex differences in the human brain during the development of AD and to examine related mechanisms, with the purpose of uncovering the overall perspective of the occurrence and development of AD during aging.

Hu, Y. et al. (2021) 'Sex differences in the neuropathological hallmarks of Alzheimer's disease: Focus on cognitively intact elderly individuals',***Neuropathology and Applied Neurobiology***, 47(7), pp. 958–966. doi:10.1111/nan.12729.

Chen, X. et al. (2023) 'Sexually dimorphic age-related molecular differences in the entorhinal cortex of cognitively intact elderly: Relation to early Alzheimer's changes', ***Alzheimer's & Dementia*** [Preprint]. doi:10.1002/alz.13037.

AIMIN BAO'S RESEARCH GROUP

Ai-Min Bao's group focuses upon the neurobiological pathogenesis of human brain disorders including mood disorders and neurodegenerative diseases, mainly based upon postmortem human brain samples. In addition, they are actively involved in the China Brain Bank. They have published more than 60 SCI papers in journals including in ***Alzheimer's and Dementia, Brain, Molecular Psychiatry,*** and ***Trends in Neurosciences***.



Dissecting the circuit mechanism behind the hyperexcitability of CA1 pyramidal neurons in an AD mice model

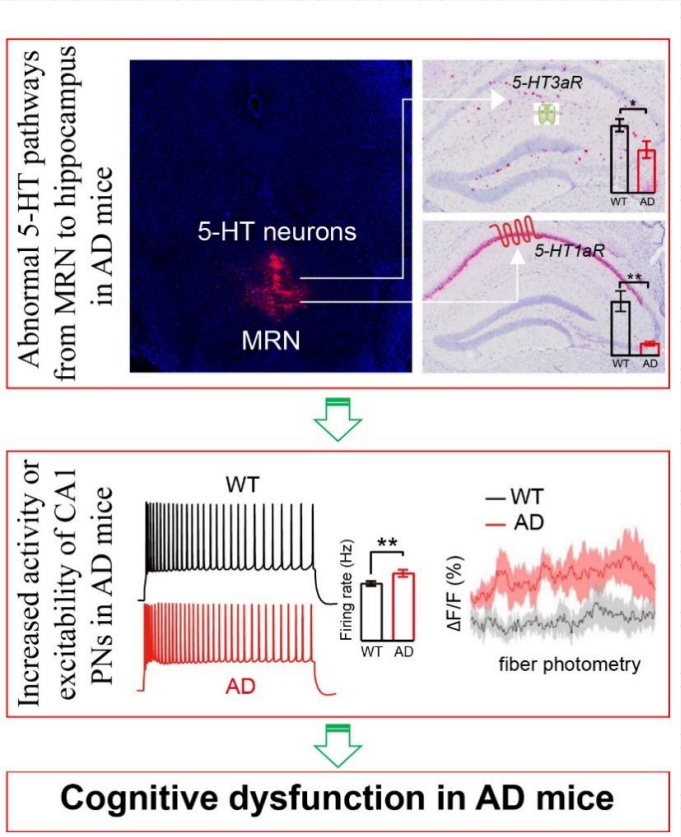
Alzheimer's disease (AD) is the most common neurodegenerative disease. Extracellular deposition of amyloid β (Aβ) and intracellular neurofibrillary tangles (NFT) have been long recognized as the major pathological hallmarks of AD. However, the wider pathogenesis of AD remains elusive. Disturbed excitabilities of pyramidal neurons (PNs) in CA1 or granule cells (GCs) in the dentate gyrus (DG) are early events of AD. These are associated with aberrant activity of neural circuits and may account for, at least partially, the resulting deficits in cognitive function. Despite this suggestion, the mechanisms underlying the abnormal excitability of CA1 PNs or DG GCs remain to be completely investigated.

Recently, an article entitled *"Aberrant serotonergic signaling contributes to the hyperexcitability of CA1 pyramidal neurons in a mouse model of Alzheimer's disease"* was published in ***Cell Reports*** from the laboratory of Prof. **Binggui Sun** at Zhejiang University School of Brain Science and Brain Medicine.

In this study, Wang et al. examined the intrinsic membrane properties of CA1 PNs in acute brain slices with patch-clamp recordings. They found that the excitability of CA1 PNs was increased in hAPP-J20 versus WT mice. Consistently, their fiber photometry recordings in freely moving mice revealed that CA1 PNs were hyperactive in hAPP-J20 mice. Combining anterograde/retrograde tracing with RNAscope, western blotting and HPLC, they showed that the serotonergic signaling pathway from the median raphe nucleus (MRN) to the hippocampus was suppressed in hAPP-J20 mice. Interestingly, chemogenetic activation of serotonin (5-hyProfoxytryptamine, 5-HT) neurons in the MRN attenuated the activity of CA1 PNs in hAPP-J20 mice by reducing the excitability or increasing the inhibitory synaptic transmission of CA1 PNs. Results of pharmacological experiments indicated that 5-HT3aR and/or 5-HT1aR play important roles in these processes. Furthermore, chemogenetic activation, but not inhibition, of MRN 5-HT neurons for 4 weeks improved memory of hAPP-J20 mice in contextual fear conditioning and novel object/position tests, with treatments with antagonists against 5-HT3aR and 5-HT1aR attenuating these effects. More importantly, although activation of 5-HT3aR or 5-HT1aR singly with their selective agonists for 4 weeks did not affect the memory of hAPP-J20 mice, combined chronic treatments with agonists for both 5-HT3aR and 5-HT1aR significantly improved the memory of these mice. However, modulating the serotonergic signaling was unable to significantly affect the deposition of Aβ, levels of soluble Aβ, and the proteolytic processing of hAPP in the hippocampus of hAPP-J20 mice.

In summary, this study identified the impaired 5-HT/5-HT3aR and/or 5-HT/5-HT1aR signaling as new pathways contributing to the hyperexcitability of CA1 PNs and the impaired cognition in hAPP-J20 mice.

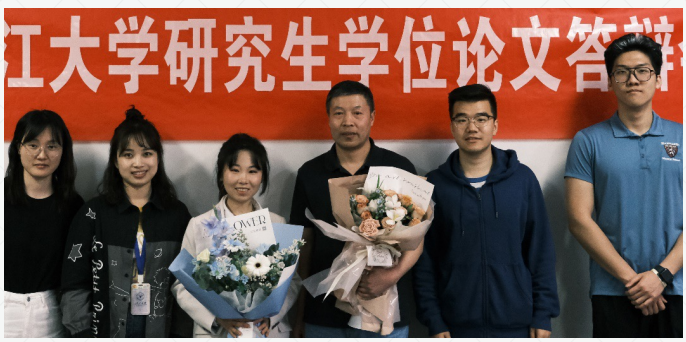
Wang, J. et al. (2023) 'Aberrant serotonergic signaling contributes to the hyperexcitability of CA1 pyramidal neurons in a mouse model of alzheimer's disease', ***Cell Reports***, 42(3), p. 112152. doi:10.1016/j.celrep.2023.112152.



Abnormal serotonergic pathways from the median raphe nucleus (MRN) to the hippocampus contribute to the hyperexcitability of CA1 pyramidal neurons and then lead to cognitive dysfunction in AD mice.

BINGGUI SUN'S RESEARCH GROUP

Binggui Sun's group is working on Alzheimer's disease. Currently, they apply multiple approaches including electrophysiology, fiber photometry, chemogetic and optogenetic modulation, cell and molecular biology, and behavioral tests to investigate the mechanisms underlying the aberrant activity/excitability of hippocampal neurons in mouse models of Alzheimer's disease.



In vivo imaging of the dynamic changes after cortical single microvessel occlusion

Cerebral cortical vasculature has been implicated in the pathological progression of brain function in many neurodegenerative and cerebral small vessel diseases. There is an increasing recognition that such dysfunctions of microvasculature may underlie many neurodegenerative diseases including vascular dementia, Alzheimer's disease (AD), 'silent' stroke, Amyotrophic lateral sclerosis (ALS), and psychiatric manifestations such as those noted in autism spectrum disorder. Despite the many studies on large-scale strokes, often linked to major vascular obstructions such as the blockage of major arteries characteristic of an ischemic stroke, little is known about how the far smaller lesions of microvasculature may have effects on vasodynamics and neural activity in surrounding tissues. The potential effects of laminar-specific occlusions that may lead to blood flow redistribution after pre-capillary arteriole occlusion also require further clarification.

Our research team of **Wang Xi/Anna Wang Roe** are therefore focusing on the functional role of cerebral vasculature and neurovascular coupling mechanisms. We hope to explore the link between microvascular function and neuropathy in normal brain physiology and various brain diseases, as well as the impact of cerebrovascular insufficiency on brain function. This may eventually provide new strategies for the treatment and prevention of related diseases.

A new precise ultrafast laser-induced photothrombosis (PLP) method that combines a 1070 nm laser with a spiral scanning method to stimulate a highly efficient, safe, and spatially accurate Rose Bengal light activation-induced photothrombosis technique was previously developed by the Wang Xi/Anna Wang Roe team (***Small methods***, 2023). In this new study, we constructed different degrees of local microischemia models using the PLP method to further investigate how microvascular ischemias affect cortical blood flow and neuronal functional changes, and to reveal the mechanisms of blood flow remodeling and functional changes under vascular dysfunction. This was published in ***Cell Reports*** in May 2023.

In this work, our team demonstrated a precise and reproducible technique for application of the PLP method into neuroscience research. This approach, combining two-photon microscopy, enables the quantitative mapping and characterization of vasodynamics in the microvascular network, segment by segment. With this technique we found distinct redistributions of blood flow in upstream verses downstream branches of the micro-occluded capillaries. We found that layer 4 exhibited a stronger reversal of downstream flow, indicating a greater ability to respond to vascular micro-ischemia, while layer 2/3 demonstrated a stalling of flow. Moreover, we revealed that these micro-occlusions had led to the degeneration of single local neurons. Overall, we were able to provide a new approach to address the hypothesis that neurodegenerative diseases may be based on microvascular ischemias.

The present study complements previous work focusing on large ischemic and hemorrhagic disruptions. The construction of a micro-occulsion model reveals new blood flow characteristics of the microvascular network and sophisticated connections between vessels and neurons. Understanding the underlying physiological or pathological cerebral perfusion system and its mechanisms, especially the acute growth evolution of microvascular lesions, is essential for resolving brain function and neurovascular coupling mechanisms in health and disease. It is also the key to understand the underlying mechanisms of altered neuroplasticity as a prerequisite for developing therapeutic modalities for blood flow control defects that occur after ischemic strokes, and that has many potential implications for their associated neurodegenerative diseases.

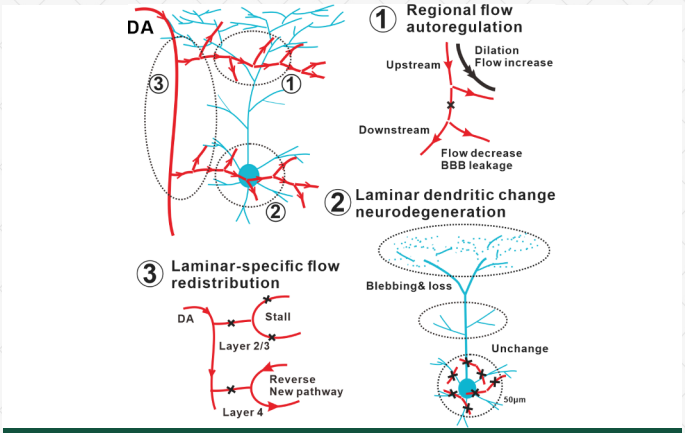


Figure: Microvessel occlusion induces local blood flow autoregulation and associated neurodegenerative changes.
1.Single-capillary occlusion leading to rapid regional blood flow autoregulation.
2.Focal capillary occlusions inducing signs of neuronal degenerative change.
3.Microvessel occlusions producing lamina-specific microvascular flow vasodynamics.

Zhu, L. et al. (2023) 'Single-microvessel occlusion produces lamina-specific microvascular flow vasodynamics and signs of Neurodegenerative Change', ***Cell Reports***, 42(5), p. 112469. doi:10.1016/j.celrep.2023.112469.

WANG XI/ANNA WANG ROE'S RESEARCH GROUP

1) Developing new imaging tools on 2P/3P platform to reveal the structure and function of the *in vivo* microvascular system, combining the advantages of multidisciplinary cooperation; developing new *in vivo* vascular system manipulation methods, such as ultrafast laser-induced precision thrombosis (PLP).

2) Neurovascular coupling mechanisms from mice to primates. Using mouse models to understand the association between cerebral small vessel diseases as well as neurodegenerative diseases; and the neurovascular coupling mechanism of BOLD signals, as detected by combined fMRI, in primate models.

A new technique in the early and precise diagnosis of neurodegenerative diseases: exosome nano flow detection technology

An Interview with Prof. Jing Zhang

Professor **Jing Zhang** holds the esteemed titles of National Overseas Distinguished Expert, National Second-Class Professor, and Doctoral Supervisor. Currently, he serves as the Director of the "National Health and Disease Human Brain Tissue Resource Bank" at Zhejiang University. In addition, he plays pivotal roles as the Deputy Dean of the Yuhang Branch at the First Affiliated Hospital of Zhejiang University School of Medicine, the Director of the Pathology Department within Zhejiang University Pathology Center, and the First Affiliated Hospital of Zhejiang University School of Medicine.

During his tenure in China, in the capacity of Chief Scientist, he has overseen numerous national and international collaborative projects, including major initiatives sponsored by the Ministry of Science and Technology, Beijing Municipal Science and Technology Commission (Brain Program), National Natural Science Foundation of China, and the Zhejiang Provincial Innovation and Entrepreneurship Team. Since 1984, he has amassed an impressive publication portfolio, contributing over 250 articles to esteemed journals like ***Acta Neuropathologica, Alzheimer's & Dementia, Annals of Neurology,*** and ***Brain***, among others.

Your team has made significant strides in developing exosome-based nanoflow detection technology. Could you explain the core principles and mechanisms underpinning this innovative approach?

Jing Zhang: Exosome research has gained remarkable prominence over recent years, extending its reach beyond neurobiology into domains such as tumor studies and even cosmetic medicine. However, transitioning these technologies to clinical applications poses a substantial challenge. The primary issue is that of poor reproducibility. For instance, in widely used immunoprecipitation experiments within exosome research, the amount of precipitated protein varies each time. While this variability might not impede research publication, it becomes a significant concern when attempting clinical testing. To address this hurdle, we embarked on a responsive strategy in 2021. Our approach involves exosome enrichment followed by fixation using paraformaldehyde, which generates cell membrane openings and facilitates antibody binding. Ultimately, we employ nanoflow cytometry for detection—a technique that rivals traditional immunoprecipitation methods and, by comparison, offers simplicity and heightened reproducibility.

What sets exosome-based nanoflow detection technology apart from other biopsy techniques when it comes to diagnose neurodegenerative diseases?

Jing Zhang: To illustrate, let's consider Alzheimer's disease. Detecting the disease's two key markers, Aβ and Tau protein, within blood encounters two main challenges. Firstly, not all of these proteins can enter the bloodstream, and their presence in the blood remains minimal. Moreover, some patients refrain from undergoing cerebrospinal fluid tests, preventing direct marker detection. Secondly, these two proteins may also be produced by other organs in the body. Thus, even if indicators are aberrant, it's uncertain whether they signify brain lesions.

In contrast, exosomes furnish a wealth of information, akin to distinctive fingerprints for each individual. Through exosome analysis, medical professionals gain precise insight into the tissue origin of the relevant protein. Additionally, they can differentiate between distinct protein types originating from various distinct brain regions and the exosomes derived from diverse cell sources. This implies that through exosome analysis we can attain a more nuanced understanding of cerebral changes, enabling pinpoint accuracy in monitoring brain fluctuations.

You've successfully employed this technology in the clinical diagnosis of Alzheimer's disease. How far in advance can this method detect the onset of Alzheimer's disease?

Jing Zhang: This technology can potentially detect Alzheimer's disease approximately 5-10 years before the emergence of evident symptoms. Prior to the manifestation of noticeable clinical signs, brain lesions have already taken root within the patient. The body's robust compensatory mechanisms often mask these lesions until the disease progresses and overcomes such compensation, only then producing symptoms. In this way clinical manifestations only arise when these compensatory mechanisms falter.

However, molecular markers have the ability to accumulate during the initial stages of the disease. Detecting these markers at this early juncture allows for the identification of the disease in its incipient phases. Consequently, early intervention becomes feasible, presenting an opportunity to mitigate the progression of the disease.

What is the current status of the clinical implementation of this technology?

Jing Zhang: Our aspirations to bring this technology into clinical practice are resolute, yet it remains relatively nascent. Hindered by regulatory considerations and other factors, extensive implementation remains challenging at this juncture. Presently, we have initiated limited-scale free testing within the hospital, offering voluntary participation to those interested. Our endeavor to propagate this innovation is ongoing. We've successfully gathered and analyzed hundreds of samples thus far, and our vision is to foster a wider acceptance of early diagnosis concepts in the future.

Additionally, we've leveraged clinical cohort data from the brain bank to further enrich our study by collecting blood samples for testing. Although the field is still evolving and we stand at a relatively pioneering juncture, numerous pressing challenges still necessitate resolution.

From your perspective, which technologies and directions in the realm of early neurodegenerative disease diagnosis warrant attention?

Jing Zhang: Currently, two pivotal issues plague neurodegenerative disease diagnosis. First, we face delayed detection. Brain lesions, as previously discussed, emerge early on, yet clinical diagnosis often transpires several years later. This temporal discrepancy significantly hampers effective disease treatment. Second, the conundrum of comorbidity looms largely. The elderly are susceptible to many underlying health conditions, and Alzheimer's disease frequently coexists with other ailments. This intricate interplay complicates clinical diagnosis and treatment.

To tackle these challenges, the foremost imperative is the advancement of precision medicine. Remarkable strides have already been made in this realm concerning malignant tumors. A detailed tumor classification based on imaging, gene sequencing, and tailored treatment strategies for diverse subtypes has become feasible. By contrast, current neurodegenerative disease detection methods often oversimplify the complex factors at play in their development, potentially impeding treatment efficacy. While some view Alzheimer's disease as untreatable, I contend that this viewpoint is incomplete. The current impediment largely stems from the absence of precise diagnosis and targeted intervention.

To facilitate breakthroughs in neurodegenerative disease diagnosis and treatment, the evolution of precision medicine is paramount. The burgeoning multi-omics technology landscape, coupled with the utilization of big data and artificial intelligence analysis, holds potential to screen and amalgamate markers, thereby fostering disease sub-categorization. Our exosome research further enriches diagnostic possibilities, constituting a focal point of our current investigative efforts.

Your recent efforts encompass the establishment of Zhejiang University's brain bank, alongside your research commitments and particular focus on human samples. In your view, how does China's current landscape fare in terms of human sample research and brain bank construction? Can you shed light on the progress achieved?

Jing Zhang: Zhejiang University's brain bank guided by the leadership of Academician Duan Shumin, has been a vanguard in national brain bank construction. Notably, our brain bank boasts a cadre of adept pathologists proficient in precise sample analysis and diagnosis. As mentioned earlier, neurological diseases often co-occur with high comorbidity rates. Consequently, meticulous pathological analysis by experts becomes paramount for in-depth sample classification, thereby offering scientific research teams more precise insights. Moreover, our focus extends to foster standardized procedures for human brain sample collection, utilization, and diagnosis. Our aspiration is to inspire other entities to establish brain banks conforming to these standardized protocols. This endeavor aligns with the overarching purpose of Zhejiang University's Brain Bank: to optimally serve scientific research and bolster the advancement of science within our nation.

What, in your view, are the strengths of conducting clinical trials within China? Could you elaborate on the current challenges you are contending with?

Jing Zhang: China's most significant asset lies in its vast patient population. However, translating these patients into effective research subjects remains a challenge. Our scientific research system, especially the development of clinical cohorts, still has considerable room for growth. In essence, China's clinical research harbors immense potential, yet it remains largely untapped. In my assessment, bridging clinical and basic research requires the establishment of collaborative mechanisms and dedicated scientific researchers to aid in clinical research design and execution.

What are your aspirations and recommendations concerning the collaboration between clinical and basic research within the bi-brain center framework?

Jing Zhang: Facilitating enhanced dialog and connectivity between clinical and basic research realms is imperative. Clinicians often grapple with unanswered clinical research queries, while scientists frequently lack a nuanced comprehension of specific clinical demands. In my research group, a robust synergy between clinical and basic research exists, granting me substantial insight into both domains. However, my aspiration is to proliferate this model and cultivate a culture of collaboration. Regular forums, such as salons, can furnish a platform for clinicians and scientists to exchange ideas. From my standpoint, scientific research should originate from the clinic, transcend clinical boundaries, and ultimately re-engage with the clinic. The completion of this "closed-loop trilogy" facilitates the dissemination and transformation of scientific findings and propels the advancement of neuroscience and neurological disease research. The Bi-Brain Center has demonstrated rapid growth in recent years, attaining noteworthy accomplishments. Leveraging this partnership, my proposition is to prioritize clinical considerations and augment clinical translational research endeavors.

Safeguarding cognitive function through fundamental research — Listening to the muted melodies of the deceased by scalpel and microscope

An Interview with Prof. Aiming Bao

Aimin Bao is a professor and doctoral supervisor of Zhejiang University. She currently holds the position of Executive Deputy Director of the National Health and Disease Human Brain Tissue Resource Center, as well as the role of Secretary-General of the International Neuroendocrine Federation. Additionally, she serves as the Deputy Director of the Human Brain Bank Research Branch of the Chinese Anatomy Society and holds the position of Deputy Director of the Science Popularization Committee of the Zhejiang Neuroscience Society. Aimin Bao also proudly represents as a valued member of the Program Committee for the Austria Bregenz International Symposium on Neurobiology and Neuroendocrinology of Aging.

Aimin Bao's research group has dedicated extensive efforts to investigating the neurobiological origins of depression and neurodegenerative disorders. Their primary focus lies in the meticulous analysis of postmortem human brain tissue samples, delving into the intricate details of the "stress hypothesis" concerning pathogenesis of depression, as well as the underlying mechanisms driving gender-related distinctions in this realm. As the first and/or corresponding author, she has authored over 50 impactful SCI papers in distinguished journals such as ***Brain, Molecular Psychiatry, Frontiers in Neuroendocrinology, Sleep, Cerebral Cortex***, and ***Trends in Neurosciences***.

In your research, you frequently utilize human brain samples. What underscores the significance of studying the human brain?

Aiming Bao: Over a century ago, Dr. Alzheimer encountered his first patient with what would later be known as Alzheimer's disease and forged a brain donation agreement. This marked the inception of Alzheimer's groundbreaking research and the profound significance of studying the human brain. Throughout the brain bank's establishment journey, we've consistently underscored this significance, that such an act can wield a substantial influence on the well-being of future generations. The inaugural specimen in our brain bank was contributed by Director Zhang Baorong, hailing from Zhejiang University's Second Affiliated Hospital. This altruistic gesture originated from a patient afflicted with Huntington's disease, who was touched by Professor Zhang Baorong's dedicated commitment to comprehending and combatting this ailment.

In our current research, we have utilized human brain specimens from the Dutch Brain Bank, founded in 1985 and currently housing a substantial repository of 5,000 to 6,000 samples. Rather than a mere repository, this brain bank resembles more of an active institution—akin to a bank—constantly receiving applications from researchers and distributing compatible samples, thus facilitating an ever-evolving scientific pursuit. Our own brain bank, a product of a decade of endeavor, has recently surpassed 500 cases. However, its representation across diverse diseases remains limited. Consequently, our current reliance on the Netherlands brain bank for human brain research persists. Looking forward, our aspiration is to eventually employ our own collection for research. With our populous base, envisioning a time when public awareness about brain donation proliferates, we anticipate a substantial expansion in the scale of our brain bank's samples.

Can you elaborate on the process the human brain undergoes, from extraction to research initiation?

Aiming Bao: Following brain donation, our procedure involves segmenting the brain by its respective regions. One portion is preserved in formalin, while the other is frozen. Simultaneously, we create pathological sections. These sections undergo pathological assessment using methods like HE (hematoxylin and eosin) staining, and in conjunction with the donor's essential medical history, a conclusive diagnosis is reached. Additionally, we allocate samples based on researchers' requirements, enabling one brain to contribute to diverse research projects. Our routine tasks encompass receiving, processing, and dispatching samples, constituting the core functions of the brain bank.

Your recent research has shed light on the underlying gender disparities in Alzheimer's disease. What prompted you to investigate the role of hormone levels in delineating the contrast between the disease's onset in men and women?

Aiming Bao: Dating back over a century, Dr. Alzheimer identified two distinct pathological changes in the brain tissue of his initial patient, now recognized as amyloid plaques and neurofibrillary tangles. Contemporary understanding attributes these formations to Amyloid (Aβ) and hyperphosphorylated Tau protein (pTau), respectively. Historically, we perceived these alterations as the primary drivers of Alzheimer's disease (AD). However, treatments targeting at these changes have persistently faltered. Moreover, instances of individuals harboring Aβ in the brain without conspicuous cognitive decline have surfaced. Contemplating the analogy of a cell to a room, cellular dysfunction analogous to a

room's malfunction can generate substantial "garbage." Aβ and pTau could potentially be manifestations of this cerebral "garbage." This notion, while plausible, fails to address the root causes of this waste production—an aspect we have long aspired to decipher.

Gender disparities in brain disorders hold great significance. Most cerebral conditions exhibit discernible gender-based differences, implying an inherent link between disease mechanisms and gender. This awareness must extend to animal research as well where the failure of certain translational endeavors may be attributable to gender distinctions. In the context of AD, women experience a notably higher incidence rate. Even after accounting for the longevity factor, women exhibit a greater vulnerability to AD. Additionally, cognitive decline following AD onset occurs more rapidly in women than in men.

Alzheimer's Disease (AD) possesses a unique attribute resembling an accelerated aging trajectory. In cases where other ailments do not prove fatal, they often ultimately culminate in premature aging. This premature aging process shares similarities with AD. Consequently, the pivotal question arises: What are the most pronounced gender-based distinctions in the aging process? Primarily, women experience menopause, a phase absent in men, marked by a sharp decline in peripheral hormone levels. Conversely, men encounter a more gradual hormonal descent. Early on, experiments explored supplementing sex hormones as an AD treatment, yielding varying outcomes—ranging from remarkable effectiveness to ineffectiveness, and even hastening disease progression in some cases. This prompted the realization of a potential therapeutic window, emphasizing the critical timing for intervention.

Intriguingly, we were motivated to probe whether alterations in peripheral hormones would reverberate within the brain. To investigate, we juxtaposed hormone levels in rats undergoing normal reproductive cycles against those subjected to stress. Notably, substantial differences surfaced in peripheral hormone levels. However, within the thalamus and hippocampus, brain regions linked to this analysis, sex hormone levels exhibited relative stability, hinting at a cerebral regulatory mechanism fostering hormonal equilibrium. Extrapolating this to humans, some women grapple with pronounced mood fluctuations during menopause, while others remain emotionally steady. This observation fuels our belief in an intricate connection between peripheral and intracerebral sex hormone levels, characterized not by a straightforward positive correlation, but rather a more nuanced interplay.

How does gender play a role in the context of Alzheimer's disease, and what led you to uncover its underlying mechanism?

Aiming Bao: The roots of this investigation trace back to a study published a few years ago, during which we examined 648 samples from the Dutch brain bank. In earlier research, samples were meticulously matched for variables like age and gender. However, at the outset of this study, we intentionally eschewed matching to observe gender differences across the entire population. Surprisingly, we observed that gender disparities in AD only became pronounced after reaching the age of 80. Subsequently, we meticulously selected a matched cohort from these samples.

Upon rigorous matching, we made a crucial discovery: in the entorhinal cortex, pTau levels were significantly higher in females than in males, while Aβ levels remained relatively stable. This revelation prompted a broader inquiry into the pathogenesis of AD in both genders and the manifestation of gender disparities. Our initial focus gravitated towards sex hormones. Regrettably, the paraffin section samples we employed couldn't directly gauge hormone levels. Instead, we aimed to infer estrogen levels through estrogen receptor measurements. Furthermore, we delved into the process of androgen conversion to estrogen, which necessitates aromatase catalysis (p450).

Recognizing the intimate link between learning, memory, and choline activity, we employed choline synthase (ChAT) and acetylcholinesterase (AChE) as indicators of choline metabolism. Additionally, we utilized staining techniques for the Golgi apparatus, glycoprotein reelin, early growth response factor (EGR1), and small RNA132 to illuminate cellular metabolic activity. The entorhinal cortex emerged as our target due to its susceptibility to initial pTau damage. Typically, the emergence of Aβ doesn't immediately impair cognitive function, but the advent of pTau corresponds to a gradual cognitive decline. Our overarching objective is to maintain patients in a stage of uncompromised cognitive function. Consequently, we focused on studying the entorhinal cortex, aiming to unearth the fundamental cause of cognitive deterioration.

We conducted an examination of the fluctuations in male and female sex hormone receptors across different age groups. Our observations unveiled an intriguing trend: as age advances, the female sex hormone system appears remarkably active. This is particularly evident in the heightened expression of aromatase, elevated estrogen levels, and increased androgen receptor presence. This confluence of factors suggests that these women possess substantial estrogen within their brain environments. Notably, this suggests that women with healthy cognitive function exhibit heightened estrogen activity. Intriguingly, we can speculate that an inactive estrogen system in women might correlate with the onset of AD—a subject warranting our future investigations.

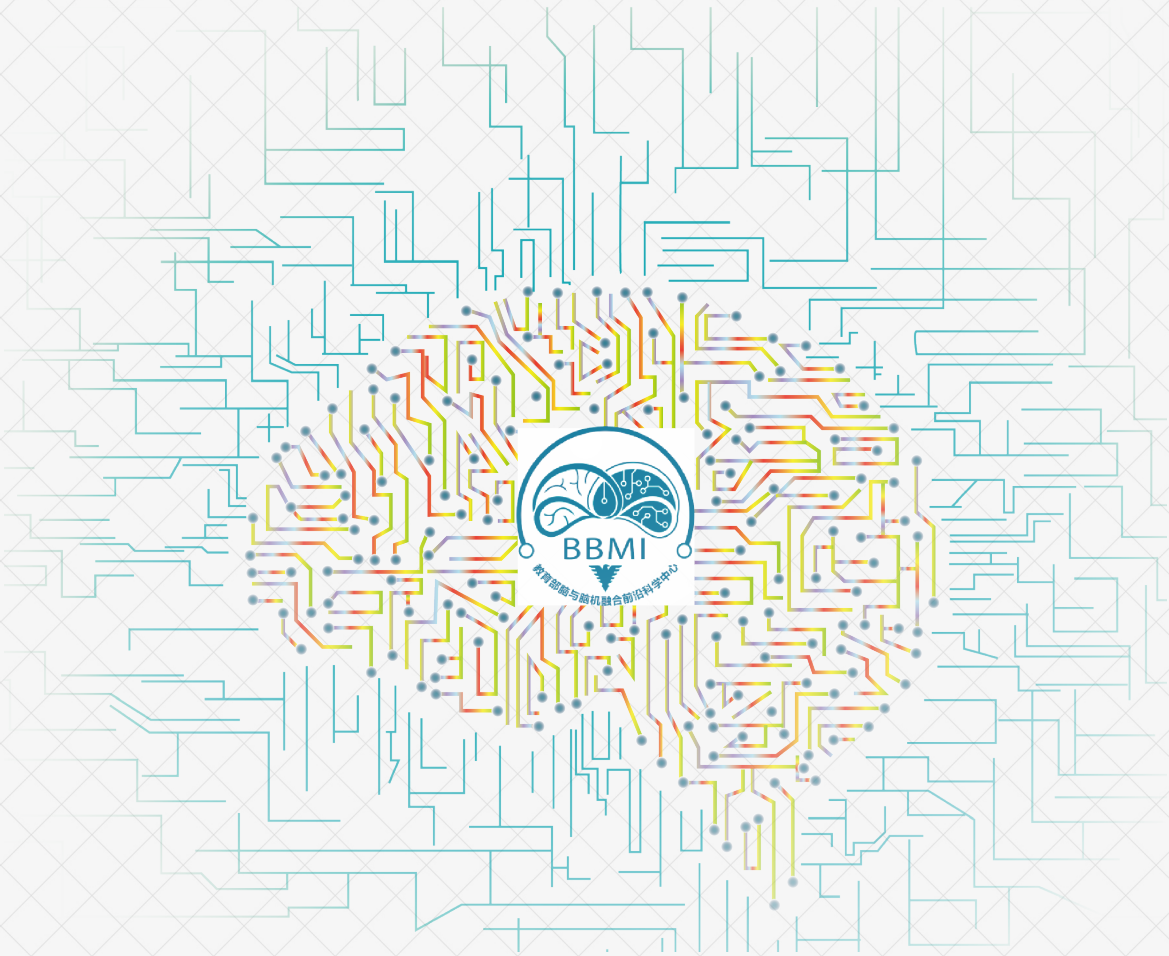
Conversely, a contrasting scenario emerges for middle-aged and elderly men. Their sex hormone levels maintain relative stability. This constancy, in our interpretation, reflects their lack of necessity for heightened hormonal surges to achieve equilibrium. In direct contrast, middle-aged and elderly women necessitate robust sex hormone secretion to maintain brain homeostasis. Consequently, we posit that men in these age groups possess heightened cerebral resilience, signifying augmented synaptic transmission capabilities, learning, and memory functions. This insight stands as the most significant revelation of our recent research endeavor.

How did you reconstruct the pre-mortem conditions from brain samples to discern the disease's causality?

Aiming Bao: Upon receiving donated brains, our objective mirrors that of a detective arriving at a crime scene—to expeditiously

retrieve and analyze the brain post-mortem, akin to deducing the culprit. Naturally, our task is highly intricate, given the marked diversity among each individual's brain composition. During academic conferences, scientists bring their distinct pieces of the puzzle to the table, endeavoring to collaboratively assemble the larger picture. Over time, we began discerning that metabolic disruptions that significantly contribute to AD progression. Diverse revelations emerged, spanning glucose and lipid metabolism irregularities, the influence of insulin treatment, and anomalies in lipid metabolism. Notably, carriers of apolipoprotein APOE4 exhibit significantly heightened AD susceptibility—a compelling observation given that cholesterol is a precursor to the sex hormones of our interest. Consequently, we questioned whether AD patients experience disrupted cholesterol synthesis, resulting in reduced sex hormone levels. Additionally, aromatase—a crucial element in the electron transport chain—also maintains close ties to cellular metabolism.

Contemplating the potential to rectify metabolic disorders amid the aging process, we embarked on exploring avenues for improvement. This entails not only energy supplementation but also the activation of energy metabolism functions. Addressing the pervasive insomnia seen in nearly all AD patients holds paramount importance due to its pronounced impact on homeostasis. Proposing practical measures, we advocate daytime sun exposure for the elderly and the use of relatively side-effect-free substances like melatonin at night to sustain circadian rhythms.



Our Vision

The BBMI center is one of the first six national frontier science centers launched by the Ministry of Education (MOE). The BBMI center capitalizes on the interdisciplinary scientific, medical, and engineering strength of Zhejiang University, and holds the mission to synergize brain science discoveries with brain-inspired intelligence advancement. Ultimately, this synergy shall be reinforced to pioneer new frontiers of fundamental neuroscience investigation, promote the development of novel therapeutics, and implement brain-inspired artificial intelligence.

"Innovate 2030" Plan

Launched by Zhejiang University, this plan aims to make full use of the comprehensive advantages of the various related disciplines to create a new high-water mark in cross-research innovation, promote the convergence of disciplines and cross-field fusion innovation, and foster a batch of world-leading research results and superior disciplines for the future.

How to submit & subscribe

Subscriptions to BBMI Quarterly are available at no charge in print or PDF form. To subscribe or submit story ideas and comments, send your e-mail to jieyingfu@zju.edu.cn.

BBMI Quarterly Academic Advisory Committee

The MOE Frontier Science Center for Brain Science and Brain-machine Integration of Zhejiang University

0571-87071107

http://www.bbmi.zju.edu.cn/zxjj