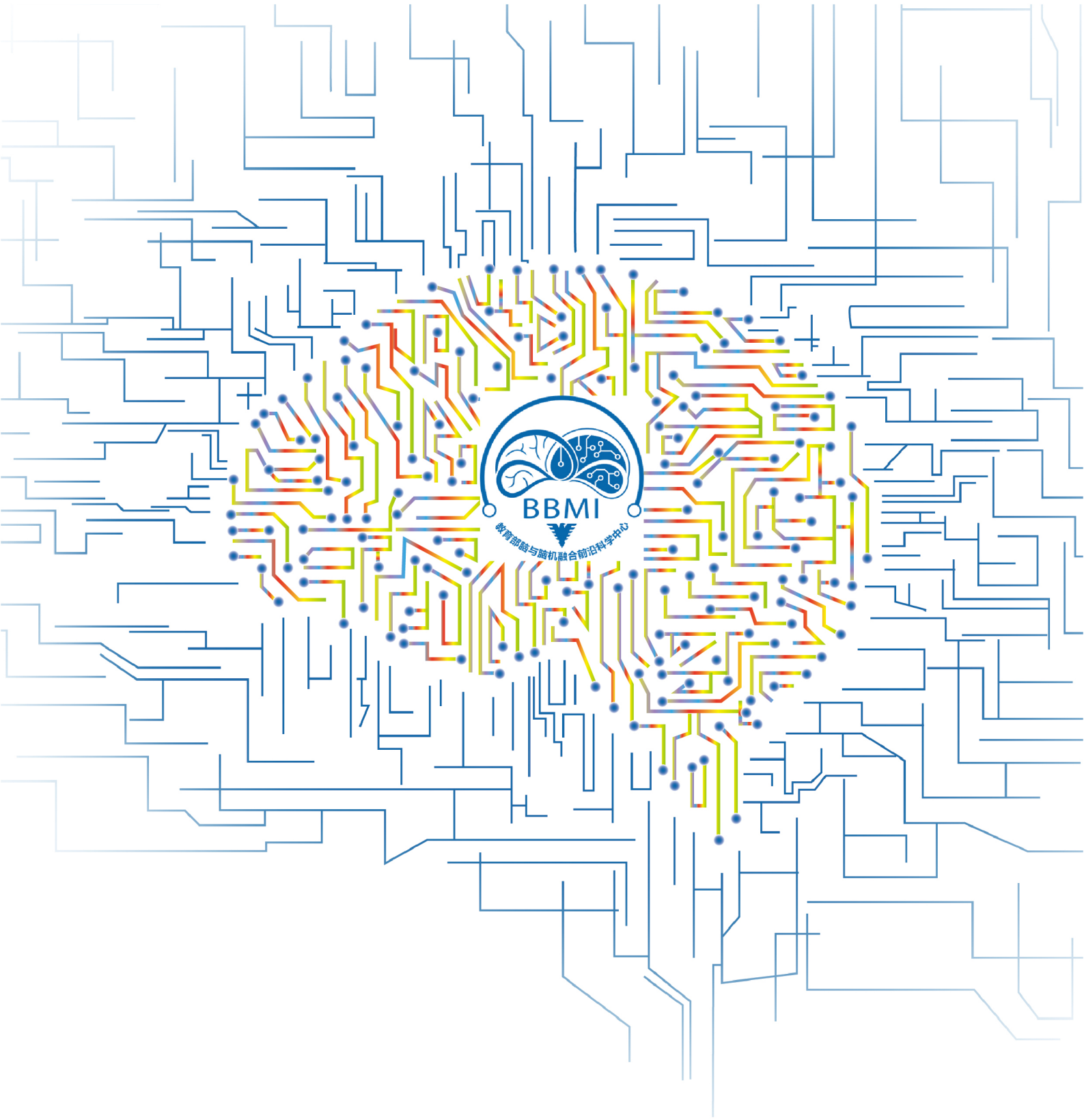
September 2021, Vol. 1 No. 1

BBMI Quaterly

Fall 2021

BBMI 教育部脑与脑机融合前沿科学中心

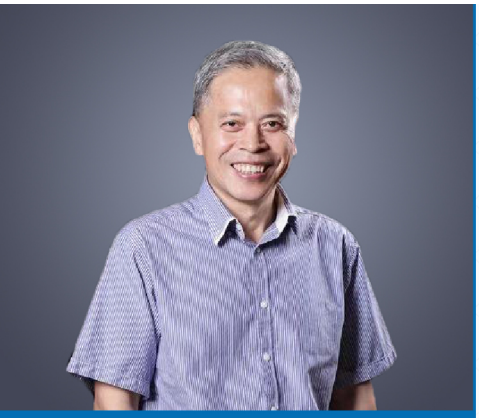
浙江大学 ZHEJIANG UNIVERSITY 1897


BBMI
教育部脑与脑机融合前沿科学中心

September 2021, Vol. 1, No. 1

RESEARCH DISCOVERIES

FRONTIER OBSERVATION



Brain science is one of the ultimate fields of human understanding and is among the most important frontier disciplines of this century. The MOE Frontiers Science Center for Brain Science and Brain-machine Integration (also known as BBMI Center) of Zhejiang University continuously promotes interdisciplinary convergence with a forward-looking perspective and innovative developments. At the BBMI center we are committed to exploring the unknown secrets of human intelligence by combining brain science with research and technology relating to artificial intelligence.

The center has made great achievements in the first quarter of this year including the discoveries of the neural circuitry that regulates social behavior; the brain regulation mechanism for anger and aggression; and the mechanism of brain network plasticity fluctuation during mesoscale analytic learning and memory. We have also elucidated the three-dimensional structure of the hypothalamic-neuropituitary endocrine system, as well as the structure of the pleasure neurotransmitter dopamine receptor.

In addition, we have vigorously promoted multidisciplinary cooperation, talent exchange, and resource sharing and outreach. Academicians Muming Poo, Mingjie Zhang, Qingming Luo, and other experts and scholars to have been actively participating in academic exchanges with outstanding young researchers. At the same time, we have incorporated many young talents, inviting their effort and inputs to build the BBMI Center into an international center of excellence for brain science, leading the scientific community in novel aspects of research and development.

The future of brain science will entail multidisciplinary convergence and multi-technology crossover fusion. We sincerely and warmly invite you to engage with us in this multidisciplinary academic exchange where we can join together to promote the development of brain science.

SHUMING DUAN, CHIEF SCIENTIST
The Frontier Science Center for Brain Science and Brain-Machine Integration

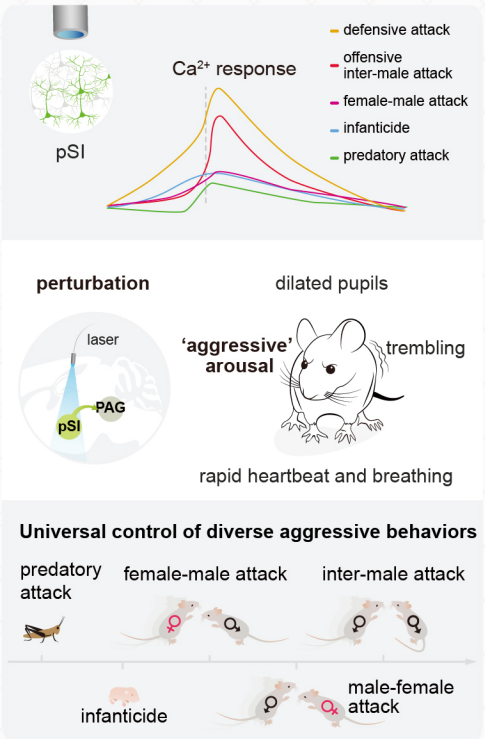
Revealing the brain-switch that controls rage

In the popular documentary "Animal World", lively commentary vividly displays various fights and conflicts occurring in animal societies. As we watch, we realize that many scenes seem familiar. Aggression and conflict, provocation with an angry response, threat and self-defense, failure and frustration, these and many other dramas are so reminiscent of the similar triggers and responses that occur in cases of human aggression.

In studying the neural circuitry underlying aggression and anger, the team of Shumin Duan and Yanqin Yu highlighted a key brain region for its regulation - the posterior substantia innominata (pSI). In mouse-based studies, this region became abnormally active when the mice started a fight or even made a threatening gesture prior to conflict initiation. Conversely, the previously combative and irritable mice immediately became perfect gentlemen when this brain region was suppressed!

This finding, published in the journal Neuron in 2021, provided new evidence on a key brain region involved in emotional regulation. Though this brain area had remained unnoticed for quite a while, it has gradually come to be understood that the pSI is a defined region located at the brain's emotional center - the extended amygdala. Duan and Yu's team's findings went further. They confirmed that the pSI is not simply mysterious like its name – an unknown area, but is a critical brain region regulating various aggressive behaviors in mice.

In the face of different intensities of threat or stimuli, ranging from cricket predation to conspecific threats, pSI neurons in mice were seen to dynamically increase their activity. Temporal and magnitudinal responses in neuronal dynamics are highly efficient and accurate and often used for predicting and evaluating various internal states and intensities of behaviors. Such observations revealed that the pSI is not isolated, and show that the pSI projects into the midbrain periaqueductal gray (PAG), an area responsible for motor control in social interactions. The activation of pSI-PAG neurons instantly switched the mice from a peaceful to a combative state. The mice then exhibited dilated pupils, notably increased respiration and heart rates, body tremors, and were primed to attack at the slightest provocation. Regardless of the initial state of the mouse, activating the pSI made the mouse instantly enter such an aggressive state. It was noted that these stimulation-induced physiological changes and motor outputs highly reflect many aspects of human anger. The pSI-PAG circuit was therefore concluded to be closely related to anger and generalized aggressive control for mice in a manner that could also relate to human aggression.

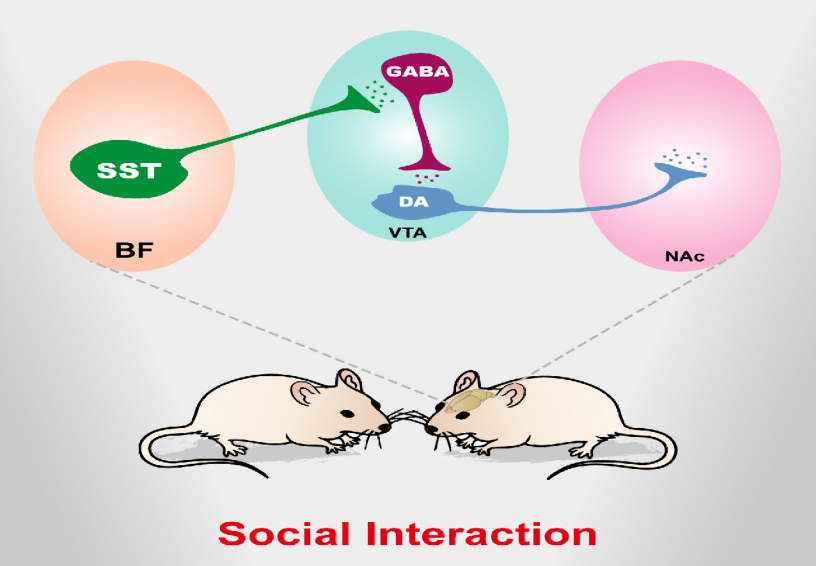


Schematic diagram of this study

Alleviating pathological aggression in humans is a major challenge that relates to the whole of society. Understanding how inappropriate aggressive behaviors are linked to specific brain circuits or gene targets is a critical path for next-generation therapy. The next goal for the team is to study how individual neurons in the pSI encode internal states related to specific aggressive behaviors in rodents. Moreover, studies have shown that pan-amygdala lesions can effectively control severe and excessive violence in humans. The team is also interested in testing whether alterations in the human pSI could be used to curb attacks from these clinical cases. New imaging techniques with the high spatial and temporal resolution are now able to be applied to this question.

Novel neural circuitry mechanisms for regulating social behavior

When we see someone in trouble, we want to step forward and help. When we experience something pleasant, we want to share it with our friends. When our own abilities are insufficient to complete a task, we want to seek aid from others. Satisfying such social behaviors is the cornerstone of normal life. Abnormal social behavior is often highlighted when such tendencies seem somehow absent, distorted, or disordered, and often relate to psychiatric conditions such as autism or social phobias. These in turn may relate to the mechanisms of the neural circuitry of social behavior, many aspects of which are little understood.



The basal forebrain (BF) promotes social behavior via its neural circuitry mechanism

A team led by Professor Han Xu from the School of Brain Science and Brain Medicine, Zhejiang University, has just published in their latest research in PNAS in 2021. In this, the neural circuitry that regulates social behavior in the basal forebrain (BF) was revealed for the first time. In addition, it was observed that inhibitory projections from the BF to the ventral tegmental area (VTA) could regulate social behavior by disinhibiting dopamine (DA) neurons.

As the first author of the study, Associate Professor Jun Wang, reports, "The understanding that the vTA-NAc DA reward pathway can regulate social behavior has been widely recognized and has been the focus of many previous publications. 'Social reward theory' was then proposed based on such observations. Our study further explored the activation of the upstream brain region of DA neurons, an important complement to 'social reward' theory, revealing an entirely new function of the BF, which is a traditionally researched brain region, to regulate the behavior of social interactions."

To explore the relationship between the BF and the regulation of social behavior, the research team used a three-chambered social approach test to directly measure the activity of BF neurons via fiber-optic calcium recording. In this the activity of BF to VTA projection neurons is concurrently regulated by light through photogenetic pathways. GABAergic neurons expressing somatostatin in BF (BF-SST) were seen to be strongly activated during social interactions in mice. However, inhibition of the BF-SST to VTA projection pathway led to a breakdown in social behavior for these animals, suggesting that the BF-SST to VTA projection pathway is necessary for normal social behavior.

Based on these results, the team further explored how SST neurons work through the VTA. The reward-related DA neuron activity in the VTA is regulated by tight feedforward control inhibition of local GABAergic neurons. By combining in vitro electrophysiology and optogenetics to investigate, the research team found that activating the BF-SST to VTA projection led to a recording of the postsynaptic inhibitory current from a larger proportion of VTA-GABA neurons. Upon such activation, the amplitude of the inhibitory current was also noted as significantly larger in GABA neurons than in DA neurons. These observations indicate that SST neurons are less likely to directly act on VTA-DA neuons but tend to project onto the local GABAergic neurons of the VTA, thereby relieving their inhibitory effects on DA neurons and thus regulating social behavior.

Our study revealed the neural circuitry of the BF which regulates social interactions under normal physiological conditions. As for the next research plan, Jun Wang suggests, "Social deficits are conmmon symptoms in many mental illnesses. Whether our newly discovered neural circuit is involved in the occurrence of social behavior disorder in mental illness will be an important research direction for the future. In addition to exploring the specific mechanisms by which the BF neural circuit regulates social disorders in animal models of mental illness, we will also work closely with experts from multiple clinical disciplines including psychology and psychiatry. Further validation of such research from functional brain imaging and targeted physical interventions in the disease population are expected to lead to the development of new strategies for clinical intervention for a number of social disorders related to mental illness."

Analysis of the structural code of the "pleasure neurotransmitter" receptor

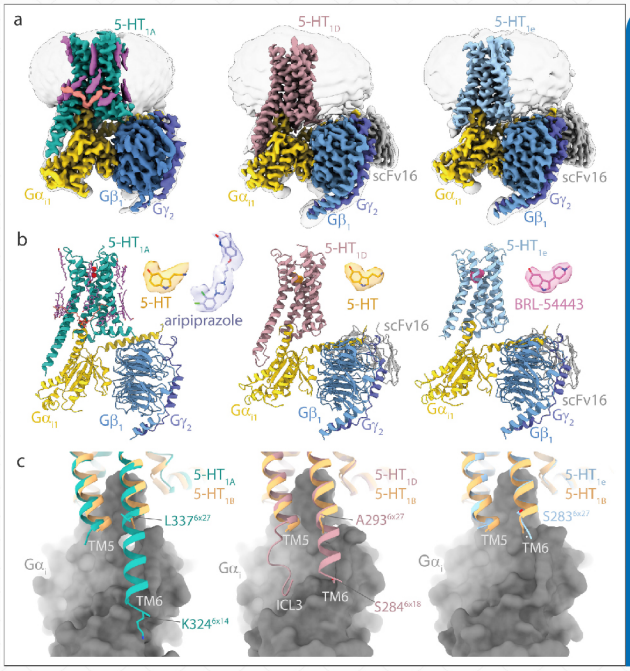
In our brains, a pleasure neurotransmitter called serotonin (5-HT) helps us feel happy, relaxed, and confident, and is linked to our sense of satisfaction. Most of the physiological functions of 5-HT are mediated by G-protein-coupled receptors (GPCRs) on the cell membrane. Studies have shown that 12 GPCRs mediate serotonin function in humans. These receptors are important targets for the treatment of psychiatric disorders such as depression, schizophrenia, and migraine. Although the function of serotonin and its receptors have been a long-term focus for neuroscience, many aspects having been deeply explored, there are still many unsolved problems and questions regarding its function and molecular regulation mechanisms.

In March 2021, the team of Yan Zhang (Zhejiang University) with Huaqiang Xu and Yi Jiang (Shanghai Institute of Materia Medica, Chinese Academy of Sciences) co-published important research in Nature. In this, they successfully analyzed five structures of three types of serotonin receptors. This study was the first to show how the phospholipid PI4P (PtdIns4P) and cholesterol regulate function of these receptors. It also revealed how the antipsychotic drug aripiprazole recognizes the serotonin-receptor to function. As a first-line drug, aripiprazole is clinically used in the treatment of schizophrenia, depression, bipolar disorder, autism, and other common psychiatric disorders. The results provided an important theoretical support for more precise use and treatment for this important drug.

A key focus was "What actually lies behind this "happy neurotransmitter", the serotonin receptor?". Using single-particle cryo-electron microscopy (SEM), the team were the first to analyze five near-atomic resolution complexes of the serotonin receptor and Gi protein (inhibitory G protein). They were able to newly identify a phospholipid molecule at the interface between the 5-HT1A receptor and Gi protein. This was named PI4P. PI4P was shown to significantly promote the activation of G protein. In addition to PI4P, the team also identified at least 10 cholesterol molecules at the receptor-cell membrane interface, confirming that these molecules play key roles in the regulation of receptor function.

Further investigation delved into the question of how serotonin receptors are so accurately and selectively identified by drugs to perform their unique work. The team uncovered key evidence in the case of aripiprazole. As a highly selective ligand for the 5-HT1A receptor, aripiprazole is 10 to 1,000 times less active against 5-HT1B, 5-HT1D, and 5-HT1E receptors than against 5-HT1A receptors. Structural analysis showed that the extracellular terminal of TM7 of the 5-HT1A receptor, the receptor that binds aripiprazole, had been shifted outward by 3 angstroms relative to other less selective receptor subtypes, resulting in a relatively larger ligand-binding pocket to hold the quinolinone group of aripiprazole. At the same time, a cholesterol molecule bound to the 5-HT1A receptor was implicated in the formation of the aripiprazole ligand-binding pocket, being able to maintain the TM1 and TM7 conformations near the quinolone group of the ligand. Taken together, these findings elucidated the mechanism of the high selectivity of aripiprazole to 5-HT1A receptors.

In this study, the structures of various serotonin receptors, their small molecular ligands, their therapeutic agents and their corresponding Gi protein complexes were analyzed for the first time using near-atomic resolution electron microscopy. Based on structural information and functional analysis, they were able to reveal the constitutive activation mechanism of 5-HT receptors and the molecular mechanism of selective recognition of serotonin receptor subtypes by antipsychotic drugs. The continuing unraveling of the code for the receptors of this "pleasure neurotransmitter" is a cornerstone that should soon enable scientists to pinpoint more precise and selective targets for mental illness, leading to increased drug efficiency and enabling increasing relief of patients suffering from such conditions.



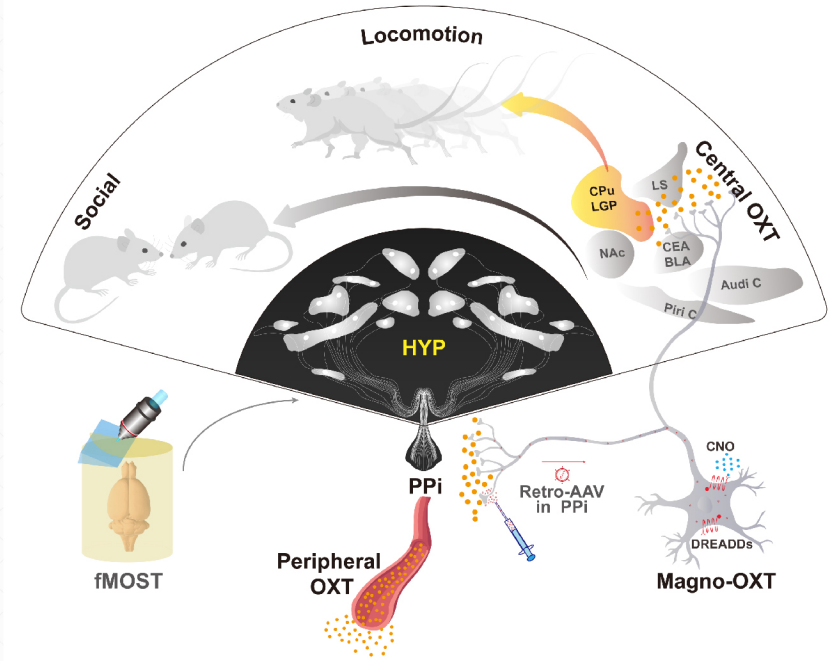
Cryo-EM structures of 5-HT1A, 5-HT1D and 5-HT1E receptors, ligands and Gi protein complexes

Projection mapping and functional analysis of endocrine neurons in the hypothalamo-neurohypophyseal system

Love, loyalty, and trust are linked to oxytocin neurons in the hypothalamo-neurohypophyseal system (HNS). The HNS is composed of magnocellular neuroendocrine cells (MNCs) and the posterior pituitary gland (also known as the neurohypophysis). MNCs send long axons into the posterior lobe of the pituitary gland where oxytocin (OXT) and arginine vasopressin (AVP) are released into the bloodstream to regulate reproductive behavior and water-salt balance. Emerging studies have shown that oxytocin and vasopressin also play important roles in the brain and directly regulate social and stress-related behaviors. However, after being released into the bloodstream, these hormones are unable to cross the blood-brain barrier and re-enter the brain. Therefore, how they enter the brain remains to be clarified.

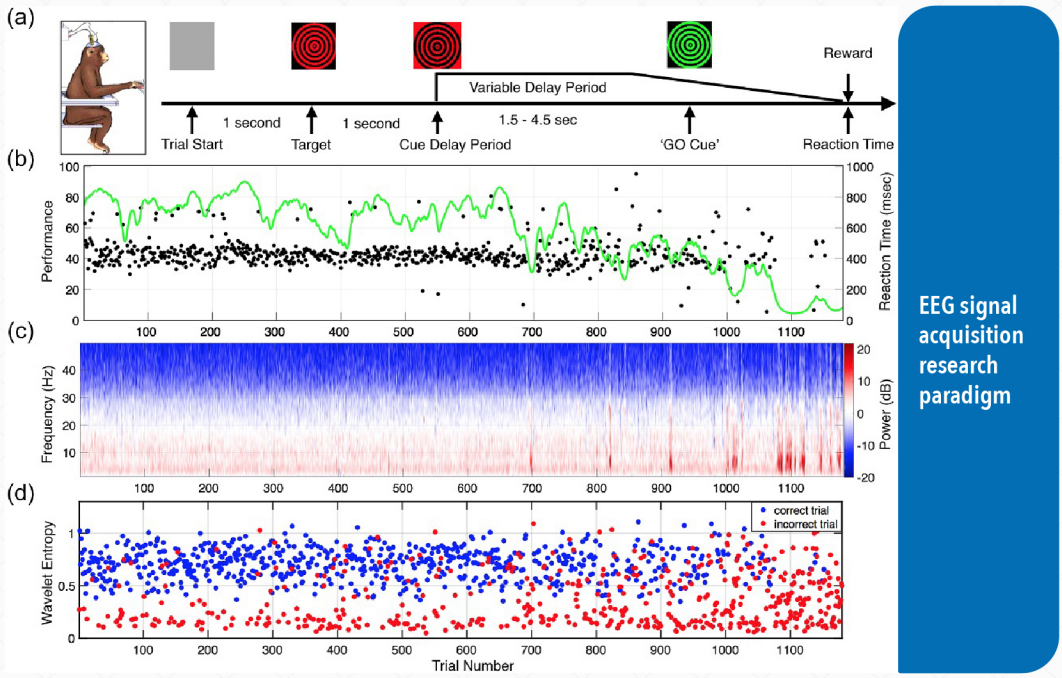
Using retrograde viral tracers and fluorescent micro-optical sectioning tomography (fMOST), Dr. Shumin Duan, Zhihua Gao and An'an Li's group managed to construct the first precise of 3D projection map of MNCs. They found that some oxytocinergic neurons (OXT-MNC) project not only to the posterior pituitary but also collaterally project to multiple brain regions including the amygdala and caudate putamen.

To further determine the functions of OXT-MNC, scientists used chemogenetic tools to selectively activate or inhibit OXT-MNC. They found that selective activation of OXT-MNCs not only increased oxytocin levels in the peripheral circulation, but also promoted social behavior in rats, mediated by OXT released from collateral projections. Conversely, inhibition of these neurons reduced blood oxytocin levels and inhibited social behavior in the animals. This suggests that endocrine neurons coordinate both peripheral and central activities by a twin release of hormones into the blood through main axons and also neuropeptides into the brain through collateral projection. These OXT-MNCs neurons therefore act as a two-headed sprayer with one nozzle directed towards the peripheral blood to regulate reproduction behaviors and the other directed towards the central nervous system to regulate emotions. These findings were published in *Neuron* in 2021.



The HNS of rats was reconstructed using the fMOST technique. Chemogenetic manipulation of OXT cells can affect the level of OXT in the peripheral blood and regulate the social behavior of rats.

Characteristics of brain electrical activity in mental fatigue



EEG signal acquisition research paradigm

For individuals with suspected brain injury or chronic mental fatigue, if signs or symptoms such as decreased vigilance are noticed at a sufficiently early stage, symptomatic treatment can be carried out in combination with a closed-loop neuro-regulation strategy. In a related study, EEG signals of mental fatigue from the brain cortex in non-human primates were collected through cortical electroencephalography (ECoG) by the Lin Yao team at Zhejiang University. Characteristic EEG signals that could be used to distinguish mental fatigue were identified within the spectrum of electrical signals received. The mental fatigue behaviors of different animals, or of the same animal with or without induced fatigue, were then predicted successfully. This provided a useful reference tool for further exploration of personalized therapeutic interventions for related conditions. This research was published in 2021 in the Journal of Neural Engineering.

In this study, a 10-channel epidural ECoG electrode array was implanted in the brain of macaques. This enabled the signal acquisition area to cover the occipital, temporal, and prefrontal cortex of the left and right brain. The monkeys were then trained on tasks that included a random delay of 1.5 to 4.5 seconds after the cue, which increased their attention expenditure but made them more prone to "mental fatigue" and increased the failure rate for the task. Recorded data were analyzed mainly based on the ECoG signals related to either "success" or "failure" behaviors in the task.

The research team selected signals defined as higher order spectral temporal (HOST) signals using the distinct characteristices of these as biological indicators of mental fatigue in macaques. Indicators, such as 'wavelet entropy' (a value representing the sequence of probability distribution with the larger the entropy value, the closer the probability distribution for the disorder), 'instantaneous amplitude', 'instantaneous frequency', and others, were used to predict the instantaneous parameters of gradient enhancement decision relating to "mental fatigue." Although there were significant differences between successful and failed power spectra, higher-order spectral feature information had natural advantages with better accuracy, sensitivity, and higher F1 scores (an indicator used to measure the accuracy of a dichotomous model) in classification results as compared with other competing techniques.

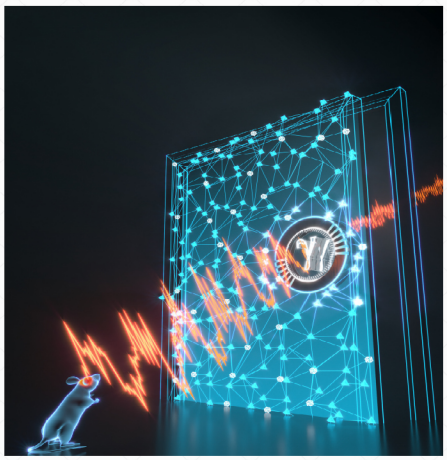
While the ECoG biomarker developed in this study proved to be a stable predictor of mental fatigue, the team noted that to apply this approach to patients with brain injury it would be necessary to adjust it to target the characteristic biomarkers of impaired cognitive function in humans. However, for the macaque training task, mental fatigue could be detected with impressive speed and efficiency, and the deep brain stimulator could be controlled to quickly recover the mental stress state of the monkeys. This should pave the way towards the provision of a convenient and efficient treatment approach to alleviate the symptoms of human chronic mental fatigue, to achieve adaptive closed-loop regulation, and to improve any intervention effect aimed at targeting the brain's fatigue or diseased state.

The molecular mechanism underlying brain network plasticity during learning and memory

Wonderful experiences can be stored in our brains as memories, thereby achieving "moments of permanence". From a neural perspective, this "permanence" depends on enduring remodeling for the connecting strength between nerve cells. This manifests itself in changes in syna, also known as 'long-term plasticity'. Long-term potentiation (LTP), an important form of long-term plasticity, has been studied for half a century and has shed light on many aspects of memory formation and retrieval. Professor Huan Ma has a long-term interest in this fundamental process as he feel that major questions still remain regarding how LTP is mediated during learning and memory.

"Thus far," he explained, "most LTP studies have focused on the projection of excitatory nerve cells to excitatory nerve cells (E-E). However, the projection of excitatory nerve cells to inhibitory nerve cells (E-I) is also crucial for neural networks and computation. Much less is known about the mechanism and significance of LTP E-I projection, which has become a key focus for us."

One key observation is that in E-E networks, LTP is mediated by the 'memory molecule' α CaMKII. However, α CaMKII is not expressed in inhibitory interneurons. This has led many scientists to debate the existence of LTP in E-I projections over the past



30 years." Dr. Xingzhi He from Ma Huan's group proposed a new idea. It came from the observation that broad-spectrum inhibitors of CaMKII could also impair the plasticity of inhibitory neurons. This suggested that there is a corresponding molecule, similar to α CaMKII, also existing for inhibitory neurons.

To explore this possibility, the RNAscope technique was used to screen for the gene expression of the CaMKII family in inhibitory nerve cells. It did indeed find that a subtype of the CaMKII family, γ CaMKII, was enriched in inhibitory nerve cells. Meanwhile, γ CaMKII knockout transgenic mice showed not only damage to LTPE-I, but also significant damage to their long-term fear memory.

Another member of the team, Dr Guangjun Zhou, then recorded the electrophysiology of mice in vivo. He found that learning and memory failed to enhance the power of gamma and theta oscillations in electrophysiologic recordings of the hippocampus of γ CaMKII knockout mice, indicating that the plasticity of the neural network was impaired. Together, these data demonstrated that neural network plasticity can be gated by synaptic plasticity. This study was published in the journal *Neuron* in 2021.

Professor Ma concluded, "From γ CaMKII to synaptic plasticity to neural network plasticity, our results showed that there are plasticity changes in the excitatory input received by inhibitory neurons during the processes of learning and memory. Furthermore, the role of inhibitory neurons may be to further boost EEG activity at certain wavelengths. Thus, in these studies, we have simultaneously elucidated a molecular coupling mechanism between neural cell and neural network plasticity as it is applied to learning and memory. This may further lead to novel therapeutic approaches for cognitive and intellectual disorders and for electrical stimulation of the brain."

[Mu-ming Poo]

[Shuming Duan]

[Hailan Hu]

AN OVERVIEW OF THE ANNUAL CONFERENCE OF THE BBMI CENTER

Liangzhu, China, April 17, 2021

The first **'Annual Conference of the MOE Frontier Center for Brain Science and Brain-machine Integration'** was successfully held at Liangzhu on April 17, 2021. The focus was upon interdisciplinary research and aligned with China's national strategy with the express aims of leading technological advances in four main areas i) General neuroscience; ii) Clinical diagnosis and treatment of brain-related disorders; iii) Brain-computer interfaces, and iv) Brain-inspired intelligence. A total of 28 principal investigators with representatives from 10 schools and departments gave reports. More than 400 teachers and students attended the events. From the Chinese Academy of Sciences, Professor Poo Muming, the Director of the Institute of Neuroscience (ION) and Senior Investigator for the Center for Excellence in Brain Science and Intelligence Technology (CEBSIT), was invited to comment on the academic reports and deliver the keynote speech. The meeting was considered to have been a wonderful success. From the enthusiastic reports, presentations, and discussions, many novel ideas have been forthcoming, and many new friendships and communication platforms have been established. These will form the basis for new interdisciplinary collaborations between colleagues and departments that are expected lead to imminent and exciting breakthroughs in research and development for this field.

The prospects for the co-development of brain science and brain-inspired research

In 2021, "the New Generation Artificial Intelligence Development Plan of China 2030" was officially launched with the focus of the development of "a new generation of artificial intelligence (AI)". This major project provided a substantial boost towards the further development of key aspects of brain science and the related endeavors to develop AI and artificial brain-inspired systems. In his keynote lecture, **Prof. Poo** introduced the basic structure of the nervous system and its complex functions. He then explained, not just how brain science can inspire artificial brain and AI research, but also how machine learning brain models can feedback to provide new theories for brain science and enabling us to understand our own brains more deeply and thoroughly.

Mu-ming Poo
Academician

The brain program of China, which has been in development for many years, is based on the basic principle of "one body with two wings", where the main body presents the neural basis of brain cognitive function that can promote the development of different levels of cognitive research, the wings are the powerful applications that stem from the body: one wing is the diagnosis and treatment of brain diseases, and the second is represented by the development of brain intelligence technologies. These two wings operate together and reinforce each other, with this co-development of the treatment of brain diseases and the development of machine learning, thus the whole research field may be lifted to a higher and higher potential. Breakthroughs in brain health care and brain-inspired intelligence industries are both confidently predicted as outcomes.

For such a huge system to operate autonomously, the wings would have to be a perfect fit. There are many types of neurons in the brain, and neural networks have complex connection patterns with high temporal and spatial specificity. Even so, the field of machine learning and artificial intelligence could be facilitated to introduce even more varieties and numbers of both neurons and connections to build even more detailed intelligent networks that are not restricted by biological parameters. However, in the brain, synapses and neural networks are structurally and functionally malleable in a way that is hard to emulate in artificial systems. The brain receives different sensory and cognitive electrical activities and realizes different functional malleability through learning and memory. This, in turn, causes further changes in cognitive behavior. Therefore, the key to brain-inspired intelligence is the ability to change the architecture and function of artificial networks through effective learning.

In newborns, for structural plasticity, synapses can be continuously repeated for self-improvement and pruned for efficiency and to avoid congestion. This enlightens that machine learning should also have the inbuilt capacity to change their own network structure, just like a "child-like machine". For example, the brain can transfer from short-term memory to long-term memory through memory storage, consolidation, and erasure. AI has yet to achieve this. At present, the Hebb neuron cluster concept is the most critical and widespread application for modelling the multiple mechanisms of plasticity in the brain. The brain stores sensory memories in strengthened synaptic connections between clusters, turning on entire clusters of cells when memory is retrieved. If we could learn the memory storage and retrieval patterns of the brain, carry out integrated perception of multi-modal information, and bind the neural network clusters of different regions through synchronous activities or different coupling activities, brain-inspired artificial intelligence could be vastly improved.

Recent studies have focused upon long-term enhancement and inhibition of ordered propagation in cultured hippocampal neurons. In vitro and in vivo neural networks have been deduced by combining the reverse learning algorithms of machine learning. Moreover, the natural back propagation of synaptic modification has been introduced into the pulse neural network. This has enhanced the efficiency of the data network and streamlined the calculation process, cutting down necessary computations considerably. This provides a fantastic case study of brain-inspired intelligence using brain science as a reference to realize "the fusion of two brains."

In 1958, the perceptron, the ancestor of deep learning, was born. With this, the prelude of artificial brain-related machine learning had arrived. In 2014, the chat program "Eugene Gustman" passed the Turing Test for the first time, offering a glimpse towards the infinite horizon of machine learning. Now, when we return to the Turing Test, we hope that the next generation of AIs will be able to stack the cups of language and perception and work in teams. If the definition of brain-inspired AI is defined as being able to do the work of ordinary professionals, we predict that perhaps half of this task will be completed by around 2040. This is when the brain program of China is striving to develop. We firmly believe that with the increasing development of frontal brain science research, more advanced brain-inspired intelligence tools will also spring up everywhere within the fertile soil of China's innovative academic environment. In the "one body with the two wings" approach, as each wing is developed and strengthened in a co-dependent manner, the heights to which brain science may fly are likely to be stratospheric.

Brainsmatics

Brain research is not just related to brain networks, brain functions and brain disease mechanisms but has many crossovers into studies of brain-like (artificial) intelligence and the development of related AI technologies. Additionally, it is important to understand the structure and function of the brain from spatial information and global perspectives. As an Academician of the Chinese Academy of Sciences and President of Hainan University, **Professor Qingming Luo** was invited to give a keynote speech entitled "Brainsmatics" at the Bi-Brain Center Lecture of Zhejiang University.

Qingming Luo
Academician

Inspired by geospatial informatics and incorporating cutting-edge technologies that enable the tracing of the whole brain, Professor Luo proposed "brainsmatics" as a discipline involving the

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comprehensive integration of three-dimensional spatio-temporal data of the whole brain with clear spatial information. Similar to the global positioning system (GPS), he proposed the concept of the brain positioning system (BPS), to enhance the repeatability and reliability of functional brain research. This development holds the potential to facilitate considerable improvements in the measurement of positioning accuracy for neurons in the brain and in related aspects of analyzing, processing, mapping, and presenting data.

Neuron connections exist with a high degree of complexity. Despite the projection of a single neuron being able to reach the whole brain, neurons have numerous projections. To understand the physical basis of consciousness at the neural level, higher-resolution three-dimensional brain mapping is required. Whilst being highly meaningful, such work is time consuming and involves many difficulties. Although it is not difficult to slice the brain and then perform analysis on the slice, it is extremely challenging to accurately match different slices in three-dimensional space. Adjusting the speed of cutting slices, sample hardness, knife edge design, cutting angles, and other parameters require exquisite fine-tuning to improve the accuracy of such three-dimensional matching.

Existing techniques and technologies have major limitations. Electron microscopy is difficult to employ on such a large scale, and nuclear magnetic resolution is also insufficient for the task. Undeterred, Luo and his team were able to independently develop a new high-resolution 3D whole brain imaging technique (MOST) in 2010. They then used this to integrate and analyze information from the spatial integration of the whole-brain. The technique met with great success. By utilizing it, they were able to obtain the first ever completed micrometer-scale tomography of a centimeter-sized whole mouse brain. Based upon this, the team then further developed a technique for high-resolution fluorescent whole-brain three-dimensional imaging (fMOST) in 2013, obtaining the first continuous whole-brain tracking and projection map of single neuronal axons of the mouse brain. In 2016, neural loop tracking and spatial localization technology based on whole-brain 3D imaging was further developed. This not only identified high-precision morphological imaging and tracking of neurons, but also synchronously recorded neurons and their precise projected localization information. In 2020, high definition and high throughput fluorescence imaging technology (HD-FMOST) emerged, which greatly improved the imaging dynamic range and other aspects. Following these years of continuous improvement and scientific breakthroughs, high-resolution whole brain connection atlas acquisition and analysis technologies, as developed by Luo and his team, have become irreplaceable and are considered as leading the field globally.

The MOST technology has a wide range of applications. In his talk at the BBMI Center, Luo demonstrated sagittal plane images of transgenic mice obtained with the fMOST technology and showed how these could then be presented to show reconstructed tracking of single neuron. Six steps were employed in this technology namely i) sample labeling; ii) sample imaging; iii) data processing and registration; iv) anatomical structure identification and labeling; v) anatomical structure boundary division marking; and vi) atlas publishing and database construction. In its development and application, MOST has resulted in a rapid and high-precision imaging technology enabling the field to leap from the tissue level to single-cell resolution, from two-dimensional to three-dimensional, and from local analysis to complete organ level analysis. Luo also hopes to integrate transcriptome and proteomics information into the atlas, making it visible (accurate and stable), clear (high resolution), complete (wide range), and comprehensible (knowledge formed by cross-level information integration). In the future, Luo and his team plan to not only to continue to develop and improve high-resolution 3D mouse brain maps, but also work with other neuroscientists to build high-resolution human brain maps and thus provide a significant contribution to the advancement of brain science research.

Transformation of brain functional imaging from scientific research to clinical practice

Individual differences are a basic and natural characteristic of the human brain. However, other differences may not be 'natural' but represent a brain disorder. Characterizing such differences in the diagnosis and treatment of brain diseases is somewhat of a challenge. So far, imaging methods for the human brain include nuclear magnetic resonance imaging (MRI), X-ray tomography, and positron emission tomography. Among these, brain structural imaging based on MRI is able to characterize the formation of tumors, vascular ruptures, gray matter and white matter abnormalities, and other structural lesions. Such applications are among the most important methods used for the diagnosis of brain diseases. However, it is still difficult to characterize functional deficits caused by changes in large-scale brain networks. The use and application of functional MRI (fMRI) therefore still remains limited and many aspects require further effort to improve. Such technical developments are also held back by the lack of direct application in clinical research.

Hesheng Liu
Professor

Professor Hesheng Liu has reported that the main shortcomings of fMRI is the poor reliability and low reproducibility. To solve this problem, he highlighted the essential requirement of understanding how to comprehensively evaluate individual differences across different brain regions, especially at the level of brain functionality. Using resting state fMRI, Liu and his team observed significant individual differences in the associative cortex and other higher functional areas of the human brain, while differences in the visual cortex and motor sensory cortex were noted as considerably subtle. The individual differences were found in newborns and further increased over time during development. Studies on primates have found similar individual differences and confirmed that individual differences are not occurred overnight, but are the result of natural selection and long-term evolution.

To accurately depict the functional architecture of the human brain, Professor Liu and his team clustered voxels (tiny brain regions) with similar functional signals, thus delineating a functional map for each individual brain. The function maps obtained using this method were 90% repeatable. In 2015, Professor Liu's team further designed and developed a new method for functional image processing. This process entailed drawing a map of 'whole brain function' for individual subjects. This was then applied to craniotomy electrical stimulation in patients undergoing craniocerebral surgery for clinical verification. This opened up a new direction for individualized analysis and diagnosis for the

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clinical application of fMRI.

In another important study, which was also published in Nature Neuroscience by professor Liu's team, they developed a noise reduction method based on a sparse noise model. This method can effectively reduce the noise of fMRI data and greatly improve the reliability of brain activation detection and the resting-state functional connectivity calculation. The emerges of such new techniques mapping individual brain imaging feature may be an important turning point in neuroimaging research field. Professor Liu and his team plan to apply this technology to assist clinical individualized treatment, such as language rehabilitation after stroke, Parkinson's disease treatment. It can also be used to guide precise brain surgery to remove lesions, tumors and other damaged or abnormal tissues whilst avoiding to damage important functional areas. Professor Liu commented that such a brain functional imaging technology, which accounts for precise individual markers, may inspire new methods for the identification of novel biomarkers for various diseases.

Phase separation and the formation and function of synapses

▲ Mingjie Zhang Academician

Professor Mingjie Zhang, (Chinese Academy of Sciences, Founder of the Hong Kong Academy of Sciences and the Dean of College of Life Sciences, Southern University of Science and Technology), has his main focus as a structural neurobiologist upon the aggregation of proteins in cells, with particular emphasis upon synapses. Correspondingly, his invited lecture in the 6th conference of the the BBMI Center was entitled "Phase separation and the formation and function of synapses".

Phase separation has become a recent topic of interest in cell biology. Linked to this, a team of Chinese researchers conducted a series of breakthrough studies on pre-synaptic and post-synaptic "biological separation".

At the beginning of the lecture, Professor Zhang introduced the general principle of the phase separation phenomenon in a biological system. "Biological macromolecules such as proteins and nucleic acids", he explained "can spontaneously form a highly ordered states under certain conditions. This state is correlated to the relationship between condensed and diluted phases in liquid-liquid separation. Since the condensed phases formed by phase separation have a physiological function, they are referred to as "membraneless organelles." Moreover, such membraneless organelles can also interact with other membraneless organelles to perform crucial functions."

After leading the audience into the topic of "phase separation", Zhang then shared his team's achievements in the field of phase transition relating to synapses. He firstly presented their detailed biochemical and structural biological studies of two important proteins in the postsynaptic density (PSD), PSD-95 and SynGAP. The PSD is a semi-closed cell partition with high protein abundance which exists on the synaptic membrane and which continuously exchanges material with the cytoplasmic water-soluble environment of the surrounding synaptic spine in the postsynaptic submembrane.

Although the PSD was discovered under electron microscopy 60 years ago, it remains unclear as to how such a high concentration of protein can remain stable without diffusion and how it adjusts itself according to cell activity. In a compositional study of key proteins, it was unexpectedly found that a purified SynGAP and PSD-95 mixture could induce solution phase separation in vitro, forming "droplet" bodies. Based on this phenomenon, Zhang et al. hypothesized that PSD-95 could interact with other functional proteins to induce liquid-liquid separation and spontaneously form stable biomolecular condensates. These may be closely related to synaptic formation and plasticity. Therefore, through in vitro recombinant experiments, the team constructed a molecular platform for synaptic research to gain insight into how neuronal synapses are formed and into the mechanisms underlying their dynamic regulation. They subsequently found that both excitatory and inhibitory synapses undergo phase transitions, but with completely differing patterns.

Additionally, Zhang and his team investigated the localization mechanism of the synaptic vesicle (SV) in the active region of the presynaptic membrane. They found that both the synthetic small unilamellar vesicles (SUVs), and the SVs purified directly from the rat brain were directly adsorbed to the protein aggregates formed by RIM and RIM-BP. For such studies the team had prepared a giant unilamellar vesicle (GUV) to simulate the presynaptic membrane. In this, Suv-RIM/RIM-BP aggregate-GUV remodeling was achieved in vitro, thus successfully highlighting the different interactions with the "synaptic vesicle-active region-presynaptic membrane" structure. This interaction between the phase separation mediated membraneless organelles and classical membranous organelles, provides strong evidence for the formation of synapses as mediated by phase separation.

Phase separation and phase transformation of biological macromolecules is a frontier field of rapid development. Many recent studies have shown that phase separation is a relatively common mechanism of cell structure formation and that it is widely exhibited in numerous key physiological processes of cells including gene expression regulation, RNA interference, and autophagy. Previously, the biological theories relating to such molecular interactions had been based primarily upon the principles of dilute solution systems. However, phase separation studies have now brought such biological studies into the field of soft matter physics. The biggest related challenge currently being tackled in this field is the race to find a credible theory to represent the phase separation phenomenon. We are looking forward to increased cross- and multi-disciplinary cooperation, especially in the field of condensed matter physics, to guide subsequent research on physical theory and to jointly promote further development of phase separation research and its applications.

Diagnostic progress and clinical requirements for cognitive impairment

Cognitive dysfunction is a general term linking to cognitive impairment that can occur due to a wide range of causes. Dementia is more specific, referring only to intellectual dysfunction syndromes that are both chronic and acquired. In either case, the main symptoms involve impairment of cognitive function. Patients with mild cognitive impairment (MCI) are a group of individuals that exhibit normal aging but also aspects of dementia. In MCI, the level of cognitive impairment does not usually reach the severity of some other dementia disorders and conditions. In clinical practice, the classification of cognitive impairment is therefore both complex and diverse. It does not only refer to Alzheimer's disease (AD), which is familiar to many, but can be also

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overlap into many disease categories including diseases of the primary nervous system, diseases occurring outside of the nervous system, and diseases that affect both the nervous system and other organs.

For any patient, the diagnosis of dementia involves four steps. Firstly comes detailed collection of medical records and physical examination. This is followed by neuropsychological assessment and auxiliary examinations. The process is then completed with a comprehensive judgment that refers to the clinical diagnostic criteria for the potential diseases that correspond to that patient's situation.

To highlight the most familiar example of dementia, Professor Wu goes on to explain the development of the diagnostic criteria and therapies for AD. "In 1984," she explains, "the initial diagnostic criteria for AD were established, mainly based on clinical and pathological diagnosis. With continued progress in research, beyond 2018 the function of biomarkers in the early diagnosis of AD began to be emphasized. Together with examination of cerebrospinal fluid and corresponding imaging, the diagnosis of AD is now based on the diagnostic basis of A (Aβ), T (tau), and N (neurodegeneration/nerve damage), which stems from that 2018 diagnostic framework." As for therapies, non-pharmacological treatments remain as the main therapies for MCI related conditions. These include physical exercise, cognitive training, and lifestyle intervention. However, commonly used medicinal therapies for AD and other MCI conditions are also available include cholinesterase inhibitors, such as donepezil, rivastigmine, galantamine, and excitatory amino acid receptor antagonists, primarily represented by memantine.

Nevertheless, the clinical diagnosis of cognitive impairment still faces great challenges. The application of traditional diagnostic biomarkers can only target the onset period and depends on the particular pathological index of the patient. Similarly, implementing a cerebrospinal fluid biomarker detection is invasive and not always painless or without risk, which often results in reluctance to undergo the test for the patients and their family. The clinical universality of PET-CT is also not high because of the high expense and that it contains risks associated with radioactivity. The lack of blood biomarkers with diagnostic values and warning values; the lack of prognostic biomarkers; the lack of an internationally standardized cohorts and biological samples; and other key issues; all add to the challenge. Moreover, many treatment gaps exist and the requirement still exists for the development of newer more efficient medicines.

Zhiying Wu
Professor

In order to address these clinical problems, the team of **Professor Zhiying Wu** has established a full set of clinical cohorts of cognitive impairment from cases related to common neurodegenerative diseases as contrasted with their normal elderly aging controls. These cohorts include those of AD, Frontotemporal dementia (FTD), dementia with Lewy bodies (DLB), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS). The team has also carried out a series of studies related to the inheritance and fluid biomarkers for the above diseases. These may contribute to clinical precision, diagnoses and treatment relating to the many differing forms of cognitive impairment.

Diagnostic challenges and opportunities in neurodegenerative diseases

Jing Zhang
Professor

Professor Jing Zhang of the First Affiliated Hospital of Zhejiang University School of Medicine gave a detailed presentation in the online forum of the Bi-Brain Center on the challenges and opportunities associated with the diagnosis of neurodegenerative diseases.

He outlined that the diagnosis of neurogenerative disorders is often problematic. Taking Alzheimer's disease (AD) and Parkinson's disease (PD) as examples, he explained how these diseases often display remarkable individual differences in their pathology. Such inconsistencies of pathological change naturally result in low clinical diagnostic accuracy. The lack of accurate diagnosis, in turn leads to the almost impossibility of accurately determining precise and optimal treatments.

He then turned to similar issues and problems of diagnosis and treatment that had historically been overcome in other diseases. He took his main example from the research processes that had successfully resulted in the molecular targeted therapy for non-small cell lung cancer. His premise was how such a productive research process, that was able to overcome such difficulties in this cancer example, could be then applied back into the development of more precise diagnosis and improved treatment for neurodegenerative diseases.

At present, specific gene mutations and molecular markers are used for the accurate classification and treatment of lung cancer. By contrast, the common diagnostic method used for AD or PD is to detect the associated biomarkers in samples of cerebrospinal fluid and then to use imaging, aimed at the detection of amyloid beta (Aβ) or Tau as targets/markers for AD, or of α-synuclein for PD. However, these tests are invasive, expensive, radioactive, and have poor clinical popularity. Therefore, there is an increasing number of studies on the diagnosis of AD and PD that use blood samples instead. A major barrier to this (literally!) is the blood-brain barrier, where proteins in the blood cannot always reflect changes in proteins in the cerebrospinal fluid of the central nervous system (CNS). This therefore limits such studies that attempt to investigate aspects of the CNS using only blood serum or plasma.

The discovery of extracellular microvesicles (EVs) solves this problem to some degree. The screening of neurogenic EVs allows for not just for the detection of proteins in the plasma, but also for these to be matched with changes in differential expression of protein in the cerebrospinal fluid. This technique may be able to uncover biomarkers for the precise diagnosis of these diseases which can then be derived from blood alone. Such a breakthrough, it is hoped, would promote increasing accuracy and earlier diagnosis and corresponding treatment of such neurodegenerative diseases. The process has already begun. Plasma EVs from neurons, astrocytes, oligodendrocytes, and microglia have begun to be identified using specific markers.

Such research may also help for an additional number of related challenges, such as for the various comorbidities associated with different types of neurodegenerative diseases and when early stages of clinical symptoms often correspond with the middle and late stages of pathology. In learning from previous experience, in which earlier diagnosis and more precise treatment of lung cancer was made possible, it is hoped that a similar path and approach may enable more accurate and earlier AD and PD diagnoses and to facilitate more precise treatment of such neurodegenerative conditions at an earlier stage.

As a researcher with an engineering background, Dr. Li hopes that he can make use of this advantage to serve the center.

Honghao Li

Dr. Haohong Li received his Doctor of Philosophy Degree from Fudan University and performed his postdoctoral research at the Cold Spring Harbor Laboratory. In 2014, he joined the Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology as a team leader. He had the opportunity to work with many renowned engineers from the optical and electronic engineering fields. This experience largely changed his view on how to conduct biological research. To be closer to the clinic, he joined the BBMI center in 2020. His research team mainly uses in vitro and in vivo electrophysiology and live animal brain imaging technology, combined with animal genetic manipulation, to study the regulation mechanism of wakefulness and sleep and the neural circuits underlying brain oscillations.

Dr. Lin Yao's research expertise is in the brain-computer interface (BCI) field, which fits well with the newly developed BBMI. He wants to have a deeply collaborative relationship with talented researchers at the BBMI center, to investigate and develop new technologies for brain-machine intelligence, and to make more discoveries in the neuroscientific field using BCI technologies. The BBMI is a new home after several years of research in numerous foreign countries. He thinks he will make many friends in the BBMI center and build his career as a competitive researcher in this new platform.

"Hopes that the BBMI center will grow to be an outstanding research platform both nationally and internationally and will be a shining star in the neuroscientific and brain–machine integration field."

Lin Yao

"The BBMI center provides a good working environment and academic atmosphere for cutting–edge research in brain science and brain–machine intelligence. It will place China at the forefront in terms of BMI technology."

Jian Xu

Dr. Jian Xu decided to join the BBMI center because that it has formed a multidisciplinary research team in the fields of medicine, engineering, information, and science. In addition, it has built the most complete rat-primate-clinical patient brain-machine interface (BMI) research platform in China. This would provide a good working environment and academic atmosphere for cutting-edge research in brain science and brain-machine intelligence. After joining the center, he intends to independently develop high-performance bidirectional closed-loop BMIs for drug-resistant epilepsy, treatment-refractory depression, and motor dysfunction; and explore high-efficiency, safe, and reliable personalized closed-loop neuromodulation treatment methods to improve the treatment and diagnosis of refractory neurological diseases in China. In the near future, he hopes that many top talents will gather in the center, which will cultivate a number of top-notch innovative young researchers who will help in overcoming the cutthroat rivalry facing the current development of BMI in China. The ultimate goal is to place China at the forefront in terms of BMI technology.

Dr. Yu Qi received her BS degree from the Mixed Class, Chu Kochen Honors College, Zhejiang University. She received her PhD degree at Qiushi Academy for Advanced Studies (QAAS) and the College of Computer Science, Zhejiang University where she witnessed and participated in the development of brain-computer interfaces at Zhejiang University. Thanks to training in the College of Computer Science, she received a "digital brain "; thanks to the research experience at the QAAS, she received a "neuroscience brain." She has now joined the BBMI center with her "double brains," to investigate how to integrate the two through brain-computer interfaces and brain-inspired computing, both of which are very exciting and frontier areas.

She believes that the BBMI center is in an ideal situation to foster break–through innovations in brain–machine integration. She is excited to join the team of talented researchers here and looks forward to adding her unique contributions.

Yu Qi

"The BBMI center is a young and exceptional center. With the endeav–ors of its talented members, it should become an icon for brain sci–ence/engineering in China and globally within the next few years."

Zhiguo Ma

Dr. Zhiguo Ma was a former postdoctoral fellow in Dr. Marc Freeman's lab at the Oregon Health & Science University, United States. His groundbreaking work on dissecting the role of astrocytes in neural circuits revealed important implications of glial cells in neuromodulation and neurodegenerative diseases. Zhiguo is excited to join the faculty of the BBMI center. "It's always thrilling for me to think that one day we will be able to decode our brains. We could then wire some electronic chips together to improve our physical and mental health and even better understand ourselves. Scientists have made great progress towards this; however, more challenges remain ahead of us. The BBMI center has pioneered the collaboration of neuroscientists, engineers, computer scientists, psychologists, and physicians to explore how our brains work, why they fail in disease, and to find cures for patients."


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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.

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