Application note

Image data courtesy of Dr. Benjamin Engel, Dept. of Molecular Structural Biology, Max Planck Institute for Biochemistry, Martinsried, Germany.

Investigating the role of MCI in Alzheimer's disease with Volumescope SEM and Amira Software

Developing new treatments for Alzheimer's disease (AD) is an urgent challenge in modern healthcare. Most approaches to Alzheimer's therapeutics focus on the reduction of plaque-forming proteins in the brain – but these have, so far, proven ineffective. This application note will highlight a recent study that examines the therapeutic potential of the small molecule CP2, an inhibitor of mitochondrial complex I (MCI), for treating symptomatic AD in translational mouse models.[1]

Introduction

A Thermo Scientific™ Volumescope™ Scanning Electron Microscope (SEM) was used in combination with Thermo Scientific™ Amira™ Software to gather and process 3D volume EM data, enabling clear visualization of the changes in neuron morphology that result from MCI inhibition. Amira Software was able to handle multiple steps from segmentation to complex morphological measurements, saving time and improving workflow efficiency. The results demonstrate the efficacy of CP2 in improving synaptic activity, energy homeostasis, long-standing potentiation, proteostasis, dendritic spine maturation, and even cognitive function, ultimately preventing the characteristic neurodegeneration of Alzheimer's disease.

Alzheimer's disease

AD is the most common neurological disease in humans and has no known cure. It is characterized by the aggregation of the normally soluble proteins amyloid-β (Aβ) and tau into amyloid fibrils, which eventually form macroscopic tangles and plaques in the brain.[2] These abnormal protein deposits are thought to form the neurological basis of AD, whose symptoms include memory loss, confusion, mood swings, and, in more advanced stages, problems with eating, speaking, and moving.

With the prevalence of AD predicted to increase dramatically around the world over the coming decades, effective treatments are a major unmet clinical need.[3] The development of therapies based on the reduction of Aβ protein and its aggregates is a major theme in modern Alzheimer's research, and translational models suggest that such anti-Aβ therapeutics may be highly effective in the prevention or delay of AD in asymptomatic patients (or patients with very early signs of AD).[4]

Unfortunately, these therapies are not always effective in patients with AD, no matter at what stage of the disease they are,[5] as is implied by the constant failure of clinical trials that have focused on the reduction of Aβ and Aβ aggregation. This highlights an urgent need to identify new targets and develop novel therapies for Alzheimer's disease.

Exploring the metabolic foundations of Alzheimer's disease

Recent studies have demonstrated that a broad range of metabolic processes may trigger neuronal dysfunction in AD – including altered energy homeostasis that is coupled with decreased uptake and utilization of cerebral glucose, microglia and astrocyte activation, as well as altered mitochondrial function.[6–8]

A growing body of research indicates that non-pharmacological methods like intermittent fasting and exercise may help combat Alzheimer's disease by inducing an adaptive stress response that improves proteostasis while reducing oxidative stress and inflammation.[9]

The body's stress response is notoriously complex and consists of numerous metabolism-associated processes, indicating a range of potential therapeutic targets, such as mitochondrial complex I (MCI). In a previous study, when treatment was initiated prior to the onset of symptoms, it was found that CP2 (a small-molecule tricyclic pyrone compound) moderately inhibited MCI and prevented cognitive decline in transgenic AD mouse models.[10,11] In addition, CP2 allowed for the restoration of cellular energetics in neurons as well as mitochondrial dynamics and function.

However, the potential therapeutic performance of MCI in symptomatic patients remained unclear. In this application note, the efficacy of MCI in the treatment of mouse-model AD after major Aβ accumulation and progressive neurodegeneration is investigated. Amira Software was used to process 3D EM data of mouse brain tissue, visualizing and analyzing neuron morphology in detail. Using this approach, the effects of MCI on synaptic strength and plasticity could be directly viewed, analyzed, and quantified.

Methods

The role of synaptic development in Alzheimer's disease

Alzheimer's disease is known to produce a broad spectrum of cognitive impairments, including a reduction in neural long-term potentiation (LTP) – the strengthening of synapses based on patterns of activity. Closely related to the maxim "neurons that fire together wire together," LTP is believed to play a fundamental role in the formation of memory.

LTP depends on the morphology of dendritic spines: small, membranous protrusions from the neuron's dendrite, which function as postsynaptic compartments for excitatory synapses.[12,13] High-resolution images of neurons which show the morphology of their dendritic spines can offer insights into the progression of Alzheimer's disease and the potential effects of CP2.

Double-transgenic APP/PS1 mice and their non-transgenic (NTG) littermates were used for this study. APP/PS1 mice have a genetic predisposition towards developing elevated A"β" levels at an early age, resulting in fibrillar A"β" deposits in the cerebral cortex and hippocampus.[14]

To determine the effectiveness of chronic administration of CP2, symptomatic APP/PS1 mice were treated with 25 mg/kg/day CP2 in drinking water ad lib, from 9 to 23 months of age.

Imaging mouse neurons using 3D electron microscopy

A Volumescope SEM was used to obtain high-resolution 3D images of neurons in slices of mural brain tissue. The Volumescope SEM features an integrated serial block-face (SBF) microtome that automates the process of layer-by-layer mechanical sectioning and scanning, enabling straightforward 3D volume data collection of resin embedded biological structures. Additional multi-energy deconvolution, available on the Volumescope SEM, provides high z-axis resolution for truly isotropic imaging of large volumes.

3D volume EM data was collected for pieces of the hippocampal CA1 region, taken from the brains of CP2-treated APP/PS1 and NTG mice. This provided detailed insight into the complex 3D structures of intact neurons in their natural context. 400 sections were obtained from every region of interest, each with a depth of 50 nm; these were then registered, filtered, and adjusted to 5,000x magnification.

After initial processing (Gaussian blur, unsharp masking, and non-local means filters), image data was imported into Amira Software for additional processing. Neuronal features, including dendritic spines, dendrites, and synapses (i.e., the PSD and opposed synaptic membranes), were identified and segmented manually by contour tracing in successive micrographs, allowing every segmented synaptic junction or dendritic spine to be independently identified.

The processing and visualization tools in Amira Software offered a clear view of neuron structure and allowed neuron morphology to be quantified. Dendritic spines were removed from the dendritic shaft and analyzed using label analysis in Amira Software, measuring their surface area, volume, and 3D length. The measurement tool in Amira Software was subsequently used to determine the length of the neck as well as the length and width of the head of every individual dendritic spine.

Type	Criteria
Mushroom	Head >0.6 µm; length 1 < x > 2 µm
Long thin	Head <0.6 µm; length 1 < x < 2 µm
Branched	Two or more heads
Filopodium	No bulbous head with a length of >2 µm
Thin	Head <0.6 µm; length < 1 µm
Stubby	Length:width ratio <1; length < 1 µm

Table 1. Criteria for classifying dendritic spines.

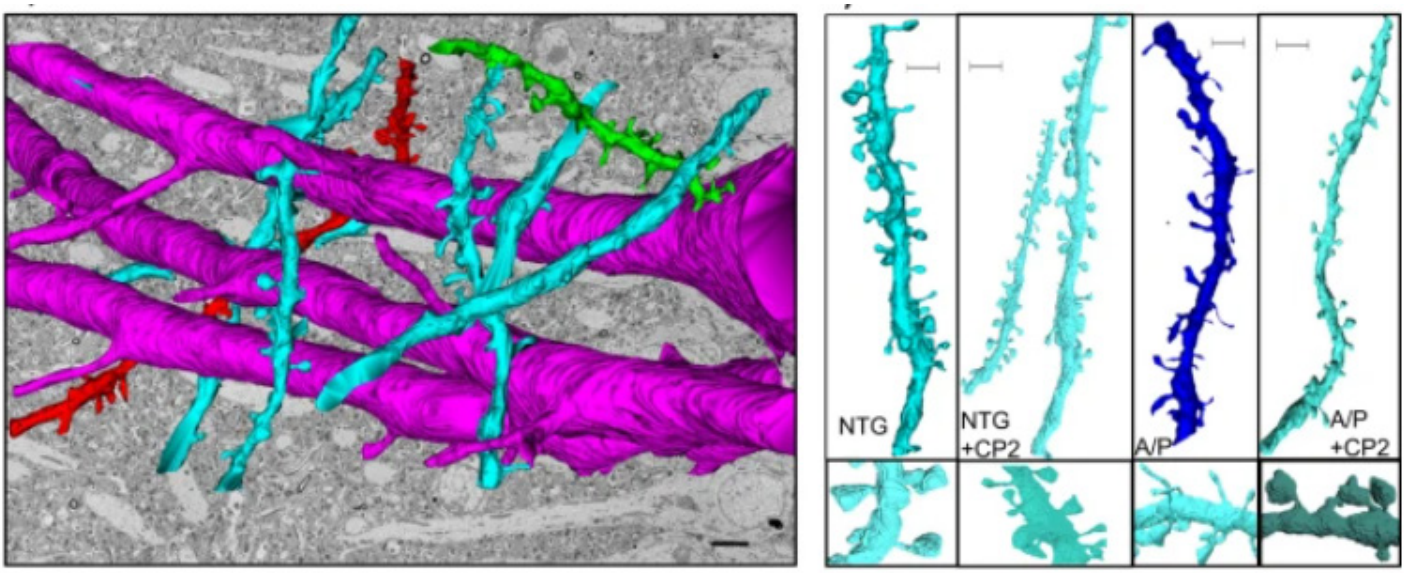

Figure 1. Left) 3D reconstruction of 3D EM data showing axons (red) and dendrites (green, blue) from the CA1 hippocampal region of a NTG mouse. The reconstruction is superimposed on a 2D EM image from the same brain. Scale bar = 5 µm. Right) Representative 3D EM reconstructions of dendrites from the CA1 region of NTG and APP/PS1 mice. Scale bar = 1 µm. *Image available through CC BY 4.0.*

Spine activity was also estimated based on the presence of synaptic vesicles. Finally, the "compartmentalization factor" was calculated for the neurons - a measure of the spine head depolarization during synaptic transmission based on simple geometric measurements. This can be used to estimate the impact of nanoscale changes in spine morphology as a result of the diffusional coupling between dendrites and spines that encourage LTP.

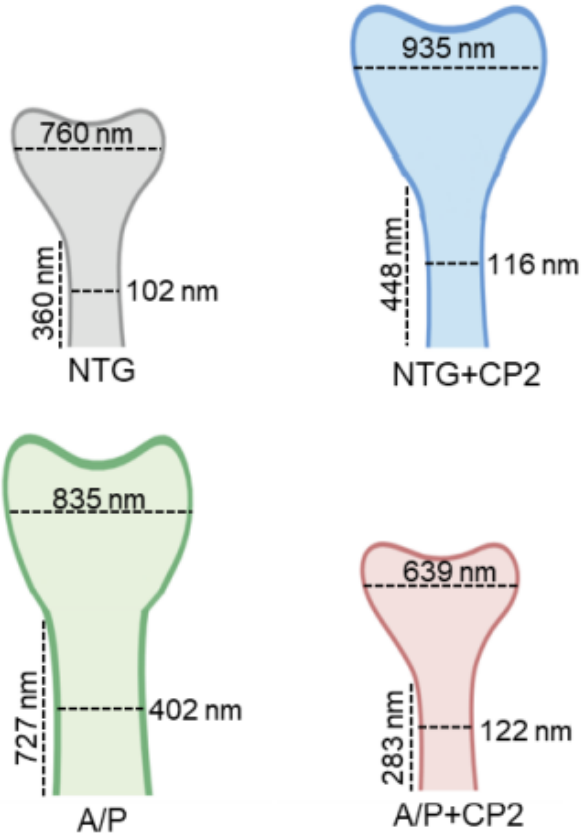

Figure 2. Schematic illustration of the morphological alterations in dendritic spines observed in CP2-treated NTG and APP/PS1 mice. Numbers indicate mean values.[1] *Image available through CC BY 4.0.*

Results and discussion

The 3D EM images showed a marked difference between NTG and APP/PS1 mice: while the majority of dendritic spines in NTG mice were mature (i.e., "thin," "stubby," "mushroom," and "branched"), immature "filopodia" and "long thin" spines were prevalent in APP/PS1 mice.

However, treatment with CP2 resulted in significant morphological changes to the dendritic spines, including increased spine maturation in APP/PS1 mice. Additionally, CP2 resulted in significant improvements in spine geometry (in terms of the length and width of spine necks and heads as well as the compartmentalization factor) in both APP/PS1 and NGT mice – indicating an increased ability to maintain LPT.

CP2 treatment was also able to restore the spine head width, volume, and length of the "mushroom" in the neurons of APP/PS1 mice to the measurements expected of NTG mice. CP2 significantly enhanced the compartmentalization factor in NTG and APP/PS1 mice, which is controlled by spine neck length and signifies a greater level of synaptic plasticity. In CP2-treated APP/PS1 mice, a more active synapse was a result of increased spine maturation, which meant that synaptic activity was back up to the level expected in NTG mice.

An extensive battery of other tests was carried out in addition to 3D volume EM, including spectrophotometry of electron transport chain (ETC) activity, in vitro pharmacology and pharmacokinetic studies, as well as postsynaptic potential analysis (fEPSP).

Conclusions

The results show that inhibition of mitochondrial complex I (MCI) using CP2 improves synaptic function, long term potentiation (LTP), dendritic spine maturation, and mitochondrial dynamics – effectively halting the neurological degeneration associated with Alzheimer's disease.[1] These findings open up a new avenue of research on MCI as a new therapeutic target for Alzheimer's disease treatment in humans, even after the onset of symptoms.

Amira Software proved to be a powerful and versatile toolbox for visualization, processing, and analysis of 3D volume EM data obtained with the Volumescope SEM. Easily adapted into any workflow, Amira Software enables researchers to save time and improve efficiency by handling multiple steps from visualization, to segmentation, to complex morphological measurements.

References and further reading

1. Stojakovic, A et al. *Partial inhibition of mitochondrial complex I ameliorates Alzheimer's disease pathology and cognition in APP/PS1 female mice* **Commun Biol** 4, 61 (2021)
2. Goedert, M and Spillantini, MG *A Century of Alzheimer's Disease.* **Science** 314, 777–81 (2006)
3. *Alzheimer's Facts and Figures Report.* **Alzheimer's Association** https://www.alz.org/alzheimers-dementia/facts-figures
4. Golde, TE, Schneider, LS, and Koo, EH. *Anti-Aβ therapeutics in Alzheimer's disease: The Need for a Paradigm Shift.* **Neuron** 69, 203–213 (2011)
5. Mullard, A. *Alzheimer prevention hopes continue to dim.* **Nature** Reviews Drug Discovery 19, 226–226 (2020)
6. Trushina, E et al. *Identification of altered metabolic pathways in plasma and CSF in mild cognitive impairment and Alzheimer's disease using metabolomics.* **PLoS One** 8, e63644 (2013)
7. Arnold, M et al. *Sex and APOE ε4 genotype modify the Alzheimer's disease serum metabolome.* **Nat Commun** 11, 1148 (2020)
8. Johnson, ECB et al. *Large-scale proteomic analysis of Alzheimer's disease brain and cerebrospinal fluid reveals early changes in energy metabolism associated with microglia and astrocyte activation.* **Nat Med** 26, 769–780 (2020)
9. de Cabo, R and Mattson, MP. *Effects of Intermittent Fasting on Health, Aging, and Disease.* **New England Journal of Medicine** 381, 2541–2551 (2019)
10. Zhang, L et al. *Modulation of mitochondrial complex I activity averts cognitive decline in multiple animal models of familial Alzheimer's Disease.* **EBioMedicine** 2, 294–305 (2015)
11. Zhang, L et al. *Corrigendum to "Modulation of mitochondrial complex I activity averts cognitive decline in multiple animal models of familial Alzheimer's disease"* [**EBioMedicine** 2 (2015) 294-305]. **EBioMedicine** 42, 532 (2019)
12. Nimchinsky, EA, et al. *Structure and Function of Dendritic Spines.* **Annu Rev Physiol** 64, 313–353 (2002)
13. Nakahata, Y and Yasuda, R. *Plasticity of Spine Structure: Local Signaling, Translation and Cytoskeletal Reorganization.* Frontiers in Synaptic **Neuroscience** 10, (2018)
14. Holcomb, L et al. *Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes.* **Nat Med** 4, 97–100 (1998)
15. Palop, JJ and Mucke, L. *Amyloid-beta-induced neuronal dysfunction in Alzheimer's disease: from synapses toward neural networks.* **Nat Neurosci** 13, 812–818 (2010)

Learn more at **thermofisher.com/amira** or at **thermofisher.com/volumescope**