

The exquisite details of dementia



Professors Zoya Leonenko, Michael Beazely and Melanie Campbell from the University of Waterloo discuss their research which aims to image the formation of amyloid fibrils in model systems and in eye retina and to discover the mechanisms by which melatonin protects against amyloid toxicity

Could you begin by outlining the background to your project?

Alzheimer's disease (AD) is one of more than 20 devastating neurodegenerative diseases for which there is currently no cure. They all have one common feature – the formation of amyloid β ($A\beta$) fibrils and plaques which impair cell function. Melatonin is a neuro-hormone produced in the pineal gland in the brain during sleep. The level of melatonin production significantly decreases with age, and also in AD patients. Interestingly, melatonin plays a protective role in age-related diseases such as Parkinson's and AD. Our hypothesis is that the protective mechanism of melatonin originates by enhancing the resistance of the cell membrane to amyloid attack.

How does the study of retinal ganglion cells help to understand the role of melatonin in resisting neurodegeneration?

Ganglion cells and visual function have been shown to be adversely affected in AD. Studying ganglion cell cultures can give us insight into neurodegeneration, $A\beta$ and the possible positive effects of melatonin.

What can measurements of circadian rhythm in the eye tell us about AD?

Melatonin sets the sleep-wake cycle and the circadian rhythm. In AD, sleep-wake cycles are often severely disrupted. Taking frequent measurements which show their variation within a circadian period shows the rhythms to be affected by light and by both melatonin and dopamine. Studies of these rhythms in the eyes of those with and without AD might reveal whether melatonin could help restore more normal rhythms.

Could you outline the imaging techniques you use?

Our research involves physics, optometry, biochemistry and pharmacology. We use atomic force microscopy (AFM), a powerful three-dimensional imaging technique, for imaging $A\beta$ in model membranes at nanometre-scale resolution. We have also developed a novel AFM approach to image $A\beta$ in flat mounted *ex vivo* retinas.

For diagnostic imaging of the retina, we use optical imaging methods which allow single cell resolution within the living eye. These techniques acquire their exquisite resolution through the use of adaptive optics correction of the imperfect optics of the eye. Adaptive optics is a technique originally developed in astronomy.

Your AFM studies show that melatonin changes the physical properties of model phospholipid membranes. What is the significance of this discovery?

It is important in understanding the roles of lipid membrane structure and physical properties and how these may control amyloid toxicity. It will help to shift the focus of new strategies for cure and prevention towards helping to modify the properties of the cell membrane.

You are the first group to demonstrate the appearance and morphology of amyloid- β in the retina in conjunction with AD using fluorescence and AFM nanoscale imaging. How does this contribute to current understanding of AD?

The retina is known to be a direct extension of the brain and there is increasing evidence that it may faithfully reflect the pathology of brain diseases including AD. We are currently

applying AFM in combination with fluorescence microscopy to visualise $A\beta$ deposits in postmortem retinas and plan to combine this with Raman spectroscopy and polarisation techniques in the future. This will help to move from the laboratory microscopy to real detection of amyloid in the living eye non-invasively. Considering the easier accessibility of retinas compared with the brain, and that there is no conclusive premortem diagnosis for AD, monitoring the retina for the pathophysiological signs of AD – mainly $A\beta$ deposits – may be effective for early diagnoses.

How would you assess your progress so far?

We were successful with AFM imaging and force measurements on model lipid membrane. We developed protocols for successful AFM imaging of the retinas in direct overlay with fluorescence.

We have established models for both $A\beta$ oligomer and $A\beta$ fibril toxicity in human neuronal cells lines and in rodent primary neurons. There is a great opportunity here to transfer previous work from our lab and from others to retinal cell models and for the first time to systematically determine the cellular mechanism(s) of melatonin neuroprotection.

The discovery of $A\beta$ in the retinas of people with AD and the ability to characterise its morphology are great advances. This has in turn resulted in the design and patenting of methods to image $A\beta$ in the living eye as a diagnostic of AD.



DR SIMON ATWOOD, PHD STUDENT YOUNGJIK CHOI, AND L EMTAGE WORKING WITH AFM

Images of Alzheimer's

Research at the **University of Waterloo** into the role of melatonin in neuroprotection has elucidated the molecular mechanisms of Alzheimer's, helping to improve brain disease diagnosis, treatment and monitoring

THE HORMONE MELATONIN, which is secreted at night by the pituitary gland, helps to regulate sleeping and waking patterns. As a person ages, the amount of melatonin produced by the pituitary gland and within the eye is reduced and their sleeping habits change. Ageing also increases the likelihood of vision problems as well as eye diseases.

Alzheimer's disease (AD) is an irreversible brain disease that is responsible for most cases of dementia. It is estimated that about 35 million people worldwide have the disease and the number is on the rise as longevity increases. It is most commonly, but not exclusively, found in the elderly; up to one half of all people over the age of 85 have Alzheimer's. Its onset is gradual and it affects memory, cognition and judgement, instigating confusion and changing the personality. It can also give rise to a specific form of cataracts. At present, there is no technique for unequivocally confirming a diagnosis of Alzheimer's in living people.

A project led by Professors Zoya Leonenko, Melanie Campbell and Michael Beazely at the University of Waterloo, in Ontario, has been studying the molecular mechanisms of melatonin neuroprotection, which is thought to help prevent AD, to establish just how the protection it affords is conferred. The early correlation of decreased melatonin and AD came from sleep studies where elderly AD patients displayed disrupted sleeping and waking cycles and showed impaired melatonin secretion; melatonin has been shown to protect

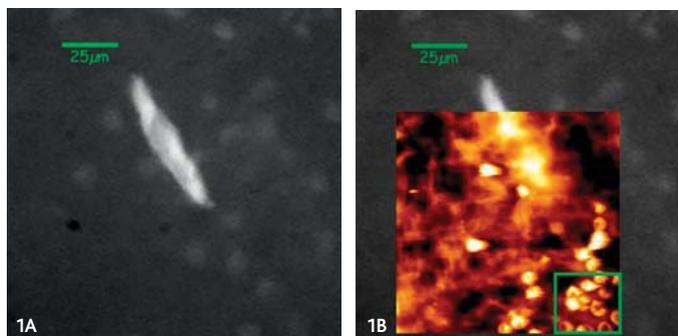


FIGURE 1. 1A – RETINA OF AN EYE POSITIVE FOR AD AND PARKINSON'S, FLUORESCENCE IMAGE, A β WAS STAINED WITH THIOFLAVIN S, WHICH LABELS A β ; **1B** – FLUORESCENCE IMAGE WITH AFM IMAGE OVERLAY ZOOMED FROM **1A**

against the disease, as well as glaucoma, but how it does this at a molecular level is unclear. In order to develop melatonin or a synthetic version for treating AD and other neurological diseases, a detailed understanding of how melatonin instigates protective action against them is first required.

COMBINING EXPERTISE

Leonenko is a Professor in the Department of Physics and Astronomy and Department of Biology at Waterloo; Campbell is a Professor in Physics and Astronomy and the School of Optometry, and Beazely is a Professor at the School of Pharmacy. Each of the Professors supports students participating in the research: "The project originated from a discussion that we and our students had at an annual conference where our graduate students present their work," reflects Leonenko.

The focus of the study is to explore the interactions of a fibril-forming protein, amyloid- β (A β), which has been found to be implicated in the onset of both AD and glaucoma, with cell and model membranes: "A β originates from the cell membrane," elucidates Leonenko. "A β forms fibrils and plaques which damage the cell membrane. Understanding the effects of A β peptides, and especially the most toxic, 1-42 form, on the lipid membrane, is crucial for understanding the overall mechanism and pathophysiology of AD." She goes on to emphasise the crucial role of the hormone melatonin within neuroprotection: "Melatonin slows down the amyloid fibril formation (figure 4); moreover, we hypothesised that melatonin may change the physical and chemical properties of cell membranes and thus may change the membrane resilience to amyloid toxicity".

STATE-OF-THE-ART RESOURCES

The combined resources of the laboratories of Beazely, Campbell and Leonenko comprise an array of high resolution scanning probe microscopy, pharmacological assays and advanced optical imaging techniques of the eye.

Scanning probe microscopy methods used in the project include atomic force microscopy (AFM) combined with fluorescence microscopy and AFM-based force measurements. The principle of AFM is sensing atomic forces of interaction of a sharp probe (needle) with a sample's surface while the probe scans the surface (figure 2). "Nanoscale imaging is a new approach for studying the effects of melatonin, which previously were largely unknown at the molecular level and at the nanoscale," asserts Leonenko. "We believed that direct nanoscale visualisation, in combination with more traditional biochemical assays, would help to reveal its protective mechanisms."

The imaging techniques are combined with pharmacological assays and calcium channels expertise from Beazely's lab. Cell viability studies from the latter have been optimised for testing the protective mechanism of melatonin against A β toxicity in retinal cells.

Novel AFM and fluorescence imaging studies of A β in human retina have allowed the deposits to be characterised as to their morphology and location. This in turn has motivated Campbell to patent new, high-resolution *in vivo* methods for imaging A β in the retina within the living eye. Campbell and her collaborators have developed technology for high resolution optical imaging of the eye, including polarisation and adaptive optics techniques. She will work with a company to combine these techniques with spectroscopy. The aim is to perfect an early diagnostic of Alzheimer's

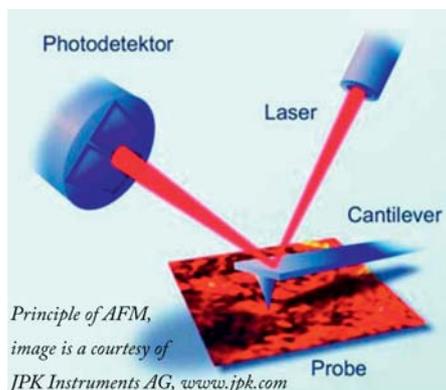


FIGURE 2. PRINCIPLE OF AFM (IMAGE COURTESY OF JPK INSTRUMENTS AG WWW.JPK.COM)

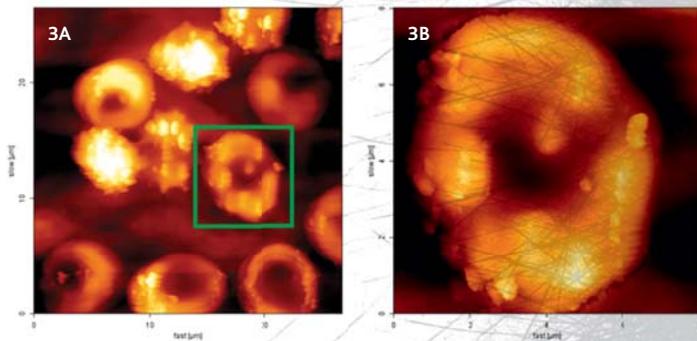


FIGURE 3.
3A AND 3B ARE AFM IMAGES SHOWING THE NANOSCALE DETAILS OF A β DEPOSITS IN RETINA OF THE EYE ZOOMED FROM IMAGE 1B

which will also allow the tracking of disease therapies, including the effects of melatonin.

FINDINGS TO DATE

The group has made good progress, obtaining AFM and fluorescence images of *ex vivo* retinas (figure 1, 3). They have confirmed the presence of A β deposits in the retinas of people who were diagnosed as having AD; moreover, using micrometre scale fluorescence imaging in combination with nanoscale AFM imaging, they have demonstrated for the first time both the appearance and the morphology of A β in the retinas of people with AD. Significantly, they have also characterised the A β deposits and are currently developing and patenting techniques for imaging these deposits in living AD patients. It was a challenge to conduct AFM imaging in the retina, Leonenko and Campbell recall, due to the thickness of the tissue: "We overcame this by using flat mounts," Campbell notes.

The analysis of AFM force measurements has shown that melatonin changes the physical properties of model phospholipid membrane. The success of the AFM imaging and force measurements within the project has led to much clearer understanding of the effect of melatonin on the physical properties of cell membranes: "We found that at saturation, melatonin decreases membrane thickness and increases the forces required to break it. Interestingly, it seems that this effect is opposite to the effect of cholesterol, which has been shown to

have multiple implications for lipid membranes," affirms Leonenko. Based on these results, she is confident that future AD therapies should include melatonin: "The fact that melatonin is not toxic to the brain makes it a perfect candidate for designing nontoxic preventative approaches".

NEXT CHALLENGES

The Waterloo collaboration is set to continue to develop new methodologies to support investigations into the biological mechanisms of ageing and of cognitive impairment due to Alzheimer's disease. The group hopes that, in the long term, the techniques and protocols developed in the project will provide a foundation for the development of new approaches that build upon the principle of non-invasive AD detection and diagnosis. They are confident, too, that the knowledge gained in their work will advance understanding of melatonin's neural protective mechanisms both in ageing and during the progression of AD and other neurological diseases.

Leonenko, Campbell and Beazely are aiming to secure funding to support preclinical imaging of A β in the retinas of living AD patients. Meanwhile, they have a grant to explore at what stage A β is deposited in the retinas of AD patients in comparison with brain deposits imaged with positron emission tomography (PET) imaging methods. This may lead to new techniques for early diagnosis and monitoring of AD and effects of therapies.

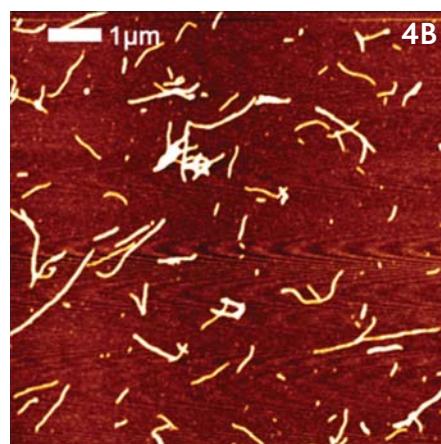
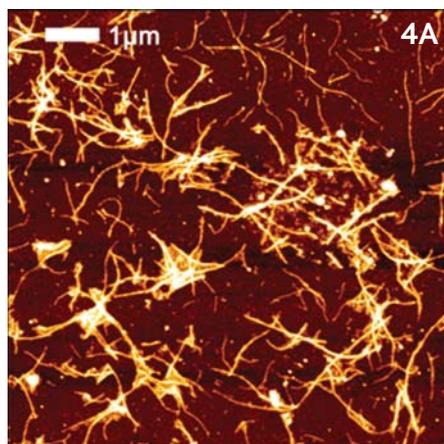


FIGURE 4. 4A and 4B are AFM images showing the effect of melatonin on amyloid fibril formation. 4A – 1 hr incubation of A β in solution without melatonin resulted in large fibrils and fibril clusters; 4B – A β incubated for 31 hrs with melatonin shows significant slow-down of fibril formation and accumulation.

INTELLIGENCE

THE ROLE OF MELATONIN IN NEUROPROTECTION DURING AGEING AND IN ALZHEIMER'S DISEASE

OBJECTIVES

To establish a cross-disciplinary collaboration at the University of Waterloo to conduct a multilevel investigation into nanoscale mechanism(s) of melatonin neuroprotective effects in AD and in other age-related neurodegenerative diseases involving A β .

THE TEAM

Professor Zoya Leonenko, Physics and Astronomy Department; Biology Department, **Youngjik Choi**, Biology PhD student, and **Simon Attwood**, PhD, Physics, working with her.

Professor Melanie Campbell, Physics and Astronomy Department; School of Optometry, and **Laura Emptage**, Biotechnology undergraduate student working with her.

Professor Michael Beazely and **Dr Maryam Vasefi**, working in the Pharmacy School.

FUNDING

Canadian Institutes of Health Research
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ZOYA LEONENKO has a PhD in Chemical Physics (Russian Academy of Sciences, Novosibirsk, Russia). Her research includes scanning probe microscopy and biophysics of lipid membrane and lipid-protein interactions.

MICHAEL BEAZELY has a PhD from Purdue University, 2004. His research focuses on understanding cross-talk pathways between G protein-coupled receptors (GPCRs) and neurotrophic factor receptors in the CNS.

MELANIE CAMPBELL has a PhD in Applied Mathematics and Physiology from the Australian National University. She is well-known for her work in scanning laser and polarisation methods for imaging the eye and biological tissues.