Opinion Paper

Cerebral Imaging – The Neurodegeneration Aspect

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Foreword

One of the major difficulties in AD research is the diagnosis at the earliest stages. Many diagnostic novel ideas are being pursued; one of them is the imaging of the inner brain focusing on the hippocampus where the dysfunction of the secretase cleavage to give the amyloid fibrils starts. Nowadays, there are two reports on agents that could be transferred to the inner living brain by injection to the blood stream [a, b]. The Korean group introduced 11C as an option for PET imaging, were as the Israeli group introduced the Biman unit which could be transferred to this, Glutathione for example in the inner brain.

As recently as the late 1980s, Alzheimer’s could only be diagnosed post-mortem through autopsies of the brain (paywall). In recent years, scientists have figured out how to identify signs of Alzheimer’s using spinal taps and PET scans, but because these tests are both invasive and expensive, they’re usually only given to patients who are already showing clear signs of the disease. In other words, they’re only used on patients for whom treatment will be too little, too late. A simple blood test could be given to anyone, at any age, and catch Alzheimer’s when it starts [2].

Use of Syn Biman Moiety as Markers for Alzheimer’s Parkinson’s Research Drug Delivery into the Brain –Introduction and Background

Among the diseases featuring cerebral peptides and proteins [3] the Parkinson’s disease (PD) is occurring in humans in about 0.3% of the population second after the Alzheimer’s disease AD (occurring in 0.5% of humans). Many disorders are classified in this feature, but only few like AD and PD cause direct brain damage. The Parkinson’s disease in a progressive disease. The progress reflect the damage in the brain caused by the deterioration of the brain tissues [4]. In 1817 a detailed medical essay was published on the subject by London doctor James Parkinson [5].

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The Amyloid beta (Aβ) featuring in the AD does not cause PD. Instead, another polypeptide namely human alpha-synuclein [6]. Normally an unstructured soluble protein, alpha-synuclein can aggregate to form insoluble fibrils in pathological conditions characterized by Lewy bodies, such as Parkinson’s disease, dementia with Lewy bodies and multiple system atrophy. These disorders are known as synucleinopathies. Alpha-synuclein is the primary structural component of Lewy body fibrils. Alpha-synuclein pentamer (Argonne National Laboratory) [7] Alpha-synuclein (Asyn) pentamer (various colors for each participating Asyn molecule) on the cell membrane interacting with beta-amyloid 1-42 (Abeta) [orange]. This interaction can contribute to Neurodegeneration during the combination of Parkinson’s and Alzheimer’s diseases.

Protein Aggregation in the Brain: The Molecular Basis for Alzheimer’s and Parkinson’s Diseases [8]. Lewy bodies [9] are abnormal aggregates of protein that develop inside nerve cells in Parkinson’s disease (PD) and Alzheimer’s disease (i.e. Lewy Body Dementia [10] ) and some other disorders. They are identified under the microscope when histology is performed on the brain. The researchers hypothesize that the development and/or progression of Parkinson’s disease begins with destabilization of the alpha-synuclein tetramer. The monomeric proteins then misfold and aggregate into Lewy bodies which accumulate with advancement of the disease. Accordingly, this finding could provide avenues to new therapeutic approaches for the treatment or prevention of Parkinson’s [6, a].
Treatment of Parkinson’s disease is mainly by application of drugs that target the nerve system in the brain. There is no cure for Parkinson’s disease, but medications [11], surgery and multidisciplinary management [12] can provide relief from the symptoms. The main families of drugs useful for treating motor symptoms are levodopa [13], usually Combined with a dopa decarboxylase inhibitor or COMT inhibitor), dopamine agonists and MAO-B inhibitors. Levodopa (L-DOPA) is the precursor to dopamine, the neurotransmitters nor epinephrine (noradrenalin), and epinephrine (adrenaline) collectively known as catecholamines [14,15].

Dopamine is synthesized from tyrosine, which is first catalysed to L-DOPA by tyrosine hyroxylase. L-DOPA is then decarboxylated to dopamine by dopa decarboxylase, and stored in the vesicles. When released into synaptic cleft, dopamine binds to receptors (D1-D5 in the figure), which activates different second messenger systems inside the cell causing changes in excitability, metabolism and gene expression. Reuptake of dopamine is by dopamine transporter. If unsorted in the cytosol, dopamine is oxidized by monoamine oxidase (MAO). Probably the most compelling (and therefore overused) example is L-Dopa. In Parkinson’s disease, dopamine-releasing neurons die off and cause progressively severe trouble with movement. Dopamine doesn’t cross the blood-brain barrier, so it wasn’t possible to just replace the dopamine that way. Someone much more clever came up with the idea of delivering dopamine’s precursor, L-Dopa, as a pro-drug. L-Dopa crosses the BBB just fine, and once inside the brain gets metabolized into dopamine. Problem solved! Pro-drugs are a useful workaround for the BBB permeability issue when the option is available. Unfortunately, there is not such an elegant workaround for all cases. This is yet another reason we need more minds, more ideas in neuropharmacology—another problem that remains to be solved. Although Levodopa is used to treat PD patients, only 5–10% of Levodopa (L-DOPA) crosses the blood-brain barrier. Because of side effects resulting from L-DOPA that remains behind, Carbidopa
Current Treatments for PD

- Levodopa drugs
- Dopamine agonists
- Catechol-O-methyl transferase (COMT) inhibitors
- Anticholinergics
- MAO-B inhibitors
- Amantidine

Side-effects of Levodopa Drugs

- Short-term
  - Nausea and vomiting
  - Fainting
- Long-term
  - Reduced duration of action (end-of-dose akinesia)
  - Drug-induced dyskinesia and dystonia
  - “On-off” episodes
  - Mental disturbances

New PD Treatments on the Horizon

- Symptomatic drugs
  - Adenosine A2A antagonists
  - Opoid antagonists
  - NMDA antagonists
- Neuroprotective agents
- Neural tissue transplants
- Cell implants e.g. genetically engineered dopamine-producing cells
is used in combination in order to inhibit peripheral metabolism of levodopa. Novel drugs are aiming in part for a similar mode of therapy and may suffer from the same disadvantage. The crossing-the-BBB-for-treating-Parkinson. It is still a major obstacle in the delivery of drugs to the brain where they should act. DRUG CLASS AND MECHANISM: Levodopa-carbidopa is a combination of two drugs, levodopa and carbidopa. Levodopa-carbidopa is used in the treatment of Parkinson’s disease. Parkinson’s disease is believed to be related to low levels of dopamine in certain parts of the brain. When levodopa is taken orally, it crosses through the “blood- brain barrier.” Once it crosses, it is converted to dopamine. The resulting increase in brain dopamine concentrations is believed to improve nerve conduction and assist the movement disorders in Parkinson disease. Carbidopa does not cross the blood-brain barrier. Carbidopa is added to the levodopa to prevent the breakdown of levodopa before it crosses into the brain. The addition of carbidopa allows lower doses of levodopa to be used. This reduces the risk of side effects from levodopa such as nausea and vomiting. This combination medicine was approved by the FDA in 1988.

In summary

We would like to emphasize that we identify in this project two main issues:

• Distraction of polypeptides that kill brain cells – the bodies based on human alpha-synuclein. A Lewy body is composed of the protein alpha-synuclein associated with other proteins such as ubiquitin, neurofilament protein, and alpha B crystalline. Tau proteins may also be present, and Lewy bodies may occasionally be surrounded by neurofibrillary tangles. In this proposal we would like to create new compounds that will prevent the dissociation of the tetramer alpha-synuclein and the stacking of the various poly-peptides to Lewy Bodies and thereby stop the progress of the PD, and keep the tetramer alpha-synuclein precursor aggregated and stable.

• Improving current treatment: To find a suitable “Trojan horse” strategy [16]. For the better penetration of drugs promoting better treatment of motor symptoms.

Chemicals that at are Applied in the Various Analytical Methods

The ability to image structure and function in the brain using tools as diverse as multiphoton fluorescence imaging and magnetic resonance imaging (MRI) hold the promise of providing insight into physiology and pathophysiological conditions but are greatly limited by the ability to deliver contrast agents with molecular specificity across the blood-brain barrier (BBB). In vivo effect of magnetic targeting on the extent and selectivity of nanoparticle accumulation in tumors of rats harboring orthotypic 9L-glio-sarcomas was quantified with MRI [17].

Positron Emission Tomography Scan

The diagnosis is based on the radioactive glucose that has F-18 in its structure. Fluorine-18 (18F) or other positron emitting agents. 11C, 18F are radioisotope which is an important source of Positrons. It has a mass of 18.009380(6) u and its half-life is 109.771 minutes. It decays by positron emission 97% of the time and electron capture 3% of the time. Both modes of decay yield stable oxygen-18. That is short lives and should be prepared in an adjacent isotopes chemical laboratory [18a-d] for the brain PET scan.

GSH as a Biomarker for Ad

Various lines of evidence indicate that brain OS (Oxidative Stress) is a key underlying factor behind AD etiology. GSH levels have been consistently shown to reflect the OS status. Furthermore [19], the literature reviewed thus far reveals a strong correlation between AD pathology and reduced GSH levels. These findings have spurred the development of assays for GSH levels as a biomarker for AD. Several methodologies have been developed to assess GSH levels in peripheral biological samples, such as blood. Recent progress in technology has also enabled noninvasive in vivo measurement of GSH directly in different brain regions using MRS. We discuss the latest findings from studies utilizing these various GSH measurement methodologies and evaluate their relative potential in serving as a reliable measure of GSH levels. It was postulated that either β-amyloids or Glutathione might serve as biomarkers in the diagnosis of the neurodegenerative diseases with AD as a priority. Polyphenols and resveratrol and curcumin were known for years of being agents for the repairing of dementia. Gazit has identified curcumin-β-amyloid complex and published the crystal structure of a congo red-amyloid complex.

Chasing Peptides in the Living Brain

Oxidative stress early stages and the Amyloid hypothesis early stages two main hypotheses are today directing neurodegenerative diseases research. The Amyloid hypothesis and the oxidative stress mechanism. Although accumulation of data supports the Amyloid beta aggregation hypothesis, in conclusion, the oxidative stress. Hypothesis of ADs still very much alive and viable, but a great deal of work needs to be done to design future studies and appropriate clinical trials that will conclusive establish the role of oxidative stress in AD pathogenesis. One high hurdle is the lack of a quantitative instrumental method to diagnose and follow the disease from early stages. In particular the development of new drugs depends a lot of such an instrumental devise. Alzheimer’s
Cerebral Imaging – The Neurodegeneration Aspect

Amyloid Beta Hypothesis

Oxidative stress Hypothesis

Glutathione

Secretase Inhibitors

Aggregation inhibitors

Aβ Immunization

Degradation Excretion

Oxidative Stress

Inflammatory Change

Tau Hyperphosphorylation

Neuronal Cell Death

Synaptic Injury
Disease is one of the many neurodegenerative disorders that are tormenting many, elderly people, and is in the unflavored situation where many theories on what initiates the cascade of events and when exactly it started to affect the life of the ill people [20]. Today, only a postmortem analysis of the patient brain can diagnose 100% the Alzheimer’s disease Schematic illustration of the Ab amyloid cascade from APP cleavage by secretase to generate Ab monomers, to plaque formation, via oligomers, protofibrils, and fibrils Causative factors for neuronal injury are indicated in italic letters under the Ab pathway. Anti-amyloid agents are also shown in solid white letters above the therapeutic targets in the Ab pathway [9] Schematic illustration [21] of the Ab amyloid cascade from APP cleavage by secretase to generate Ab monomers, to plaque formation, via oligomers, protofibrils, and fibrils. Causative factors for neuronal injury are indicated in italic letters under the Ab pathway. Anti-amyloid agents are also shown in solid white letters above the therapeutic targets in the Ab pathway [22] Oxidative Damage Is the Earliest Event in Alzheimer Disease [23] our observations indicate that increase oxidative damage is a nearly event in AD that decreases with disease progression and lesion formation. These findings suggest that AD is associated with Compensatory changes that reduced amage from reactive oxygen. The activities and expression of several antioxidant enzymes such as Cu/Zn- and Mn-superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase have been studied in AD and could be in part responsible for the decrease in oxidative damage we observed.Texas red is a red-purple fluorescent dye with a relatively low (MW 615) molecular weight. It is to the staining of cells. The agent does not cross the BBB. **A New Study is Changing How Scientists Think about Alzheimer’s Disease** However, Holtzman and others now believe that β-amyloid plays an important role in triggering the onset of Alzheimer’s disease, with tau deposits creating later damage. “Gone are the days when we had these competing camps” of tau versus amyloid, says Dennis Selkoe, a neurologist at Harvard Medical School in Boston and an architect of the amyloid hypothesis. “It’s both a double whammy.” But, he adds, the study reveals an important new target for treatments: the destructive conspiracy of pathogenic tau and ApoE4 [24]. People with Alzheimer’s disease die with brains riddled by both amyloid plaques and intracellular tau “tangles.” Yet the evidence linking tau to ApoE4 has been indirect and circumstantial, says Holtzman, a neuroscientist at the Washington University School of Medicine in St. Louis in Missouri. In any case, scientists doubted that tau normally a stabilizing protein within cells could escape from neurons to interact with the cholesterol-ferrying protein made by ApoE, he says. In contrast, Use of Thioflavin derivative resolve individual Ab plaques and cerebrovascular amyloid “in living” microscopy. Future studies will include imaging amyloid load in transgenic mice using newly developed high-resolution micro-PET [25], a technology that will provide a direct transition to PET imaging studies in human subjects. Amyloid beta is an antimicrobial peptide that is formed in the AD patient's brain by digestion of another polypeptide, amyloids precursor
peptide (APP). Amyloid beta shares similar structural feature with a natural antimicrobial peptide Magainin isolated from skins of African Todd. This may hint to a antimicrobials function of Aβ needed to eradicate invading microbes into the living brain. People tend to consider leaky gut and damaged BBB as causes for the penetration of the human microbiome in the onset of neuronal diseases [26].

It is still a great challenge to use either biomarker, β-amyloids or Glutathione or others that are produced in the brain, probably in the hippocampus gland in the early events of the neurodegenerative disease.

Quantitative Imaging of Glutathione in Hippocampal Neurons and Glia in Culture Using Mono-Chlorobimane

Glutathione (GSH) is a major antioxidant system in the mammalian central nervous system (CNS). Abnormalities of GSH metabolism have been associated with many disorders of the CNS, including Parkinson’s, Alzheimer’s, and Huntington’s diseases and ischemic / reperfusion injury. Investigation of GSH levels in the CNS generally relies on biochemical assays from cultures enriched for different cell types. Because of Glia influence neuronal metabolism, we have studied cultures in which neurons and Glia are co cultured. This approach demands fluorescence imaging to differentiate between the different cell types in the culture, permitted using m cell types decreased with time in culture. Inhibition of GSH synthesis by buthionine sulfoximine (BSO) caused a greater proportional depletion of GSH in Glia than in neurons. The depletion of GSH induced by BSO was significantly greater in cells cultured for .10 days. Furthermore, the release of GSH from glia and its breakdown by the ectoenzyme β-glutamyl transpeptidase (βGT) maintains [GSH] in neurons [27].

The Enzyme Glutathione Peroxides

As for glutathione, its production in the hippocampus is reduced when oxidative stress takes place at the initial stages of AD. It is reported that use of images can aid in the detection of glutathione that is excreted from the brain. In sections from rat brain GPx (GPx, glutathione peroxidases:) immunoreactivity has been found predominantly in neurons in the cortex, hippocampus and cerebellum. Glutathione in living organisms can be identified due to the transfer of Bimane with the catalysis of Glutathione S-Transferases [28].

The detection of the bimanyl-glutathione adduct is carried out as follows:

Confocal microscopy was carried out using an inverted confocal microscope (Zeiss LSM 510) with a water-immersion633 objective lens. The 364 nm line of the argon UV laser (Coherent, Inc.) was used to excite the MCB-GSH adduct, and fluorescence was collected between 440 and 490 nm to construct the MCB image. A 488 nm laser line was used to excite calcein, and the image was acquired between 505 and 550 nm. Again, data were digitized to 12 bits. The pinhole of the confocal was set to acquire optical slices of; 1 mm for all images.

Applying Glutathione S-Transferase as Biomarker

Glutathione S-transferase, commonly abbreviated GST, refers to a group of enzymes which employ glutathione in many reactions that contribute to the transformation of numerous compounds such as therapeutic drugs, carcinogens and products involved in oxidative stress. Glutathione is an essential metabolic molecule that is produced in the liver of humans and animals. GST (Glutathione S-transferase) family of detoxification enzymes contains many microsomal, cytosolic and mitochondrial proteins which form significant parts of the enzyme body. They are present in prokaryotes and eukaryotes where they play the role of catalyzing different reactions and at the same time accept xenobiotic and endogenous substrates. Every member of
the eukaryotic species has multiple GST isoenzymes that are bound by cytosolic and membranes. Each of them portrays unique catalytic and noncatalytic binding characteristics. The is decreased glutathione transferase activity in brain and ventricular fluid in Alzheimer's disease. Thiols in general, which are components of many proteins and simple molecules, such as glutathione (GSH) and cysteine (Cys), play an important role in the cellular antioxidant defense system. 1 GSH is the most abundant intracellular nonprotein thiol (1-10 mM). 2 It has a pivotal role in maintaining the reducing environment in cells and acts as the redox regulator because thiols exist in redox equilibrium between sulphydryl and disulfide forms. 3-5 Intracellular thiol levels change dramatically in the response to oxidative stress. 1 Thus, the quantitative detection of intracellular thiols is of great importance for investigating cell functions. [29]. Blood-Brain Barrier-Penetrating 6-Halogenopurines Suitable as Pro-Probes for Positron Emission Tomography are Substrates for Human Glutathione Transferases. Scientist developed a spectrophotometric assay for the glutathione conjugation and determined specific activities with a range of human GSTs as well as some rat GSTs for comparison. The ubiquitous GST P1-1 showed the highest activities with the 6-halogenopurines, which bodes well for the application of pro-probes for human investigations. Since bromo- (or chloro-) bimanes were shown to have a very useful and sensitive application in reacting with thiols to produce fluorescent labeling, attempts were made to stain brain tissues. Glands and galea with the direct use of the halo-bimanes. The enzymes of the Glutathione S-Transferases family may become instrumental in choosing glutathione Transferases level as a biomarker for Alzheimer's disease. Slicing brain in the laboratory serves research abundantly. However, Dealing with living brains is the way to go. In respect to the biogenesis of brain compounds, Nedergaard and coworkers found out in mice that while seeping, the waste is excreted from the brain via the spinal fluids and then transported in the blood system to the regular way the living organism gets rid of such wastes. Scientist observe the decrease of free SH groups in proteins extracted from the hippocampus of AD patients provides additional evidence for increased oxidative damage of proteins a vulnerable region of the AD brain [31].

**Optimal parameters for near infrared fluorescence imaging of amyloid plaques in Alzheimer’s disease mouse models**

Amyloid-β plaques are an Alzheimer’s disease biomarker which present unique challenges for near-infrared fluorescence tomography because of size (<50 µm diameter) and distribution. We used high-resolution simulations of fluorescence in a digital Alzheimer’s disease mouse model to investigate the optimal fluorophore and imaging parameters for near-infrared fluorescence tomography of amyloid plaques. Fluorescence was simulated for amyloid-targeted probes with emission at 630 and 800nm, plaque-to-background ratios from 1–1000, amyloid burden from 0–10%, and for transmission and reflection measurement geometries. Fluorophores with high plaque-to-background contrast ratios and 800nm emissions performed significantly better than current amyloid imaging probes. We tested idealized fluorophores in transmission and full-angle tomographic measurement schemes (900 source-detector pairs), with and without anatomical priors [32].

**Officials with the US Food and Drug Administration (FDA) have recently approved the use of a new fluorescent dye in Alzheimer’s detection. The substance binds to amyloid plaques in the brain, structures that are hallmarks of this neurodegenerative form of dementia**

The plaques are formed by accumulations of the beta-amyloid protein in the human brain, and they interfere with the normal functioning of neurons. Detecting elevated concentrations of this molecule before it starts forming plaques has been a focus of Alzheimer’s research for many years. Now, investigators at the University of California in Los Angeles (UCLA) are able to use the new dye to reveal the plaques wherever they form. This means that doctors will soon have access to a test that they can apply to patients suspected of developing dementia. After the weakly radioactive dye is injected into the brain, a simple positron emission tomography (PET) scan can reveal their number and size, as well as their exact locations. When it hits the market, the dye will most likely be first used on people exhibiting cognitive decline or impairment [33].
Alzheimer’s disease ‘could be detected by eye test

A simple eye test might be able to detect Alzheimer’s and other diseases before symptoms develop, according to UK scientists.

These findings have the potential to transform the way we diagnose Alzheimer’s.

Rebecca Wood, Alzheimer’s Research Trust This new technique enables scientists to track the progress of brain disease by looking at dying cells in the retina. The cells show up as green dots because they absorb the fluorescent dye [34].

Throwing Light on Parkinson’s and Alzheimer’s disease. Nau, Bremen

Fluorescent dyes (or labels) are fluorescent molecules used in medical samples to detect and visualize various biological structures. Most fluorescent labels are by nature hydrophobic (i.e. they do not mix with water) and they get attracted by hydrophobic surfaces. As a result, they accumulate in the hydrophobic area of interest allowing its visualization. The research group led by Prof. Werner Nau at Jacobs University Bremen has successfully used the fluorescent labels Thioflavin T and congo red to visualize insoluble (hydrophobic) plaques of amyloid proteins found in the brains of Parkinson’s and Alzheimer’s disease patients. (Watch Roy D’Souza talking about amyloid plagues) The researcher’s team currently works on improving the properties of fluorescent dyes by placing them in special molecular carriers - also called biomimetic macro cyclic containers. (Watch Prof. Nau talking about the research project) [35]

Thioflavin T in Alzheimer’s Early Detection

When it binds to beta sheet-rich structures, such as those in amyloid aggregates, the dye displays enhanced fluorescence and a characteristic red shift of its emission spectrum. [1] This change in fluorescent behavior can be caused by many factors that affect the excited state charge distribution of Thioflavin T, including binding to a rigid, highly-ordered amyloid structure, or to specific chemical interactions with a protein.
CLARITY

Scientists at Stanford University published in 2013 on the development of the imaging system “Clarity”. The method is best represented in the article’s abstract in “Nature”.

Obtaining high-resolution information from a complex system, while maintaining the global perspective needed to understand system function, represents a key challenge in biology. Here we address this challenge with a method (termed CLARITY) for the transformation of intact tissue into a nano porous hydrogel-hybridized form (cross linked to a three-dimensional network of hydrophilic polymers) that is fully assembled but optically transparent and macromolecule-permeable. Using mouse brains, we show intact-tissue imaging of long-range projections, local circuit wiring, cellular relationships, sub cellular structures, protein complexes, nucleic acids and neurotransmitters. CLARITY also enables intact-tissue in situ hybridization, immune histochemistry with multiple rounds of staining and de-staining in non-sectioned tissue, and antibody labeling throughout the intact adult mouse brain. Finally, we show that CLARITY enables fine structural analysis of clinical samples, including non-sectioned human tissue from a neuropsychiatric-disease setting, establishing a path for the transmutation of human tissue into a stable, intact and accessible form suitable for probing structural and molecular underpinnings of physiological function and disease [36].
Imaging Brain Amyloid in Alzheimer’s Disease with Pittsburgh Compound-B

“...We and others have worked to develop in vivo amyloid-imaging agents for use with a variety of brain imaging techniques. Modification of the amyloid binding histological dye, thioflavin-T, led to the finding that neutral benz well. The basic properties of the prototypical benzothiazole amyloid binding agent 2-(4-methyaminophenyl) benzothiazole (termed BTA-1) and related derivatives have been described in detail. These studies showed that these compounds could bind to amyloid with low nanomolar affinity, enter brain in amounts sufficient for imaging with positron emission tomography (PET), and clear rapidly from normal brain tissue. At the low nanomolar concentrations typically used in PET studies, the binding of BTA-1 to postmortem human brain was shown to be a good indication of A⁺ amyloid deposition but did not appear to detect the presence of neurofibrillary tangles."

Two-Photon Laser Scanning Fluorescence Microscopy

Molecular excitation by the simultaneous absorption of two photons provides intrinsic three-dimensional resolution in laser scanning fluorescence microscopy. The excitation of fluorophores having single-photon absorption in the ultraviolet with a stream of strongly focused subpicosecond pulses of red laser light has made possible fluorescence images of living cells and other microscopic objects. The fluorescence emission increased quadratic ally with the excitation intensity so that fluorescence and photo-bleaching were confined to the vicinity of the focal plane as expected for cooperative two-photon excitation. This technique also provides unprecedented capabilities for three-dimensional, spatially resolved photochemistry, particularly photolytic release of caged effect or molecules.

New Imaging Technologies Namely Two-Photon Microscopy

The brain's process of clearing waste had long eluded scientists for the simple fact that it could only be observed in the living brain, something that was not possible before the advent of new imaging technologies, namely two-photon microscopy. Using these techniques, researchers were able to observe in mice – whose brains are remarkably similar to
The study, which was published today in the journal Science, reveals that the brain's unique method of waste removal – dubbed the glymphatic system – is highly active during sleep, clearing away toxins responsible for Alzheimer's disease and other neurological disorders. Furthermore, the researchers found that during sleep the brain's cells reduce in size, allowing waste to be removed more effectively [39].

**In Vivo Fluorescence Imaging with High-Resolution Microlenses**

Micro-optics are increasingly used for minimally invasive in vivo imaging, in miniaturized microscopes and in lab-on-a-chip devices. Owing to optical aberrations and lower numerical apertures, a main class of micro lens, gradient refractive index lenses, has not achieved resolution comparable to conventional microscopy. Here we describe high-resolution micro lenses, and illustrate two-photon imaging of dendritic spines on hippocampal neurons and dual-color nonlinear optical imaging of neuromuscular junctions in live mice[18b].
Concluding Remarks

The imaging of the living inner brain is currently practiced in many clinics based on positron tomography [40]. This procedure uses an in most cases an isotope laboratory to prepare on site 18F glucose to administer via the bloodstream and BBB penetration the 18F glucose which is the energy fuel for the brain [41].

There are only few fluorescent agents with low molecular weight that can be employed. Recently, such small molecules that could be supplied to the brain via the bloodstream have been reported by an Israeli and a Korean group. Japanese and American scientists have shown that brains could be made transparent and substructures of the inner brain could be identified in detail. “Clarity” has been developed. Nedergaard investigated the bidirectional fluxes of agents and their metabolites from the brain while asleep. These extrusions are accumulated in the bloodstream and further elaborated to extrusions in the normal metabolic manner.
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