**Abstract**

Alzheimer's disease (AD) is a debilitating neurodegenerative disease that is affecting an increasing number of people. It is characterized by the accumulation of amyloid-β and tau hyperphosphorylation as well as neuroinflammation and oxidative stress. The neurological damage of AD is thought to be irreversible upon onset of dementia-like symptoms, as it takes years until symptoms of cognitive decline manifest. Current AD treatments do not stop or reverse disease progression, highlighting the need for new, more effective therapeutics. Dysregulation of microRNAs (miRNAs) expression in AD is increasingly recognized and they have been suggested as potential biomarkers and therapeutic agents.

**Aims**: the overarching aim is to identify non-invasive and readily available biomarkers for early diagnosis of AD as well as finding drug treatments that are disease-modifying, and not only attenuating symptoms; we aim to examine in male and females rats, AD-induced alterations in miRNAs associated with AD and related regulatory mechanisms in the hippocampal-prefrontal (HPC-PFC) pathway and to detect abnormal expression of miRNAs in the brain and in peripheral circulation. We examine novel therapies against AD that include (i) the phytocannabinoid cannabidiol (CBD), and whether it could slow neurodegenerative processes through a bi-directional dialogue with miRNAs and the Wnt/β-catenin signaling pathway, and (ii) targeting specific miRNAs by their silencing or activation in the HPC-PFC.

In the **first experiment**, CBD is administered in a streptozotocin (**STZ**)-induced rat model of sporadic AD, after which cognitive and emotional function are correlated with alterations in the expression of miRNAs in the HPC-PFC pathway, as well as targets related to inflammation, CBD signaling, AD pathology and β-catenin. We also investigate peripheral miRNAs and inflammatory cytokines as potential biomarkers of AD and treatment response. These findings will reveal significant AD-related genetic changes that can be blocked by CBD treatment and highlight the potential value of peripheral microRNAs as biomarkers of AD. In the **second experiment** we ask whether HPC input to PFC is central in controlling the behavioral phenotype in AD rats using chemogenetic tools. In the **third experiment** we will explore whether different miRNAs are critically involved in AD-related cognitive and emotional dysfunction and the therapeutic effects of CBD by using agomirs and antagomirs to activate or inhibit specific miRNAs in the HPC-PFC pathway; after which the association between changes in miRNA expression, cognitive/emotional pathology, inflammatory markers, CBD targets, AD pathology-related targets, and β-catenin will be assessed.

**Successful completion of these experiments** (i) could suggest that targeting specific miRNAs (activating or silencing) has a therapeutic potential to restore memory and emotional deficits, and (ii) may define miRNA-regulated therapeutic role of CBD in the treatment of AD in males and females and thus propose how CBD may slow neurodegenerative processes. Ultimately, the establishment of a validated noninvasive biomarker of AD or associated targets will guide the future development of early diagnostic tools, preventive strategies, and effective pharmacological treatments for dementia.

Gaining a clear understanding of how a drug works before it enters clinical trials could increase the likelihood for drug success. CBD may have a therapeutic effect via mediation of microRNAs, but probably also through other different pathways. Elucidating specific microRNAs that predict the disease before the appearance of plaques and tangles will allow the development of specific drugs that will block the progress of AD. Moreover, identifying specific markers in males and females can guide the development of personalized, sex-specific medicine.

**Narrative (one paragraph?)**

Alzheimer's disease (AD) is a progressive and devastating neurodegenerative disorder in the world's rapidly growing aging population, characterized by cognitive decline and neuropsychiatric symptoms. The neurological damage of AD is thought to be irreversible upon onset of dementia-like symptoms, as it takes years until symptoms manifest. It is thus necessary to identify individuals at risk for the development of the disease to provide early intervention. We aim to elucidate specific microRNAs that predict the disease and allow the development of specific drugs that will block the progress of AD. We will study in a rat model for AD, whether the impact of Cannabidiol (CBD) is mediated via silencing or activation of specific miRNAs in the hippocampal-prefrontal pathway in males and females. Cannabidiol as an intervention drug can be immediately transferred to humans to relief symptoms while our research can identify specific miRNAs to be activated or silenced to start treatment before the outbreak of the disease.

**Specific aims- One page**

**Objective** No effective cure for Alzheimer Disease (AD) has been established to date, underscoring the need to identify novel effective compounds that can counteract the AD course. The establishment of a validated noninvasive biomarker of AD or associated targets will guide the future development of early diagnostic tools, preventive strategies, and effective pharmacological treatments for dementia. Our main objectives are to identify microRNAs (miRNAs) dysfunction in the hippocampal-prefrontal (HPC-PFC) pathway in AD in male and females rats, to examine whether silencing or activation of specific miRNAs can prevent or attenuate neurodegenerative processes, and whether the phytocannabinoid cannabidiol (CBD) can attenuate cognitive and emotional symptoms via miRNAs silencing or activation.

**Background** In the search for new, more reliable biomarkers and potential therapeutic options, microRNAs have emerged as important players in the pathogenesis of AD. Studies suggest that cannabidiol (CBD) can provide symptomatic relief or even arrest AD progression through anti-inflammatory, antioxidative, and neurogenic effects. Therefore, understanding the underlying mechanisms of CBD effects in AD is necessary for intervening in AD pathology and for the translation of preclinical studies into clinical settings. CBD treatment can be immediately transferred to humans to relief symptoms while research on the activation and inhibition of miRNAs progresses.

**Hypotheses** We hypothesize that (i) AD will be associated with dysfunctional cognitive and emotional behaviors and abnormal expression of miRNAs and AD-associated genes and proteins in the HPC-PFC, (ii) AD will be associated with abnormal miRNAs expression in the blood of AD males and females at the early stages of AD development, (iii) CBD can ameliorate AD-induced cognitive and emotional symptoms as well as alterations in AD-associated genes and proteins, (iv) activating or inhibiting specific miRNAs can prevent AD phenotype, and (v) the preventing effects of CBD on AD phenotype may be mediated by miRNAs.

**Our specific aims:** Aim 1: (i) Identify significant dysregulation of miRNAs as well as genes and proteins that are associated with AD in male and female rats that may be restored with CBD treatment. (ii) Investigate peripheral miRNAs and inflammatory cytokines as potential biomarkers of AD and treatment response; Aim 2: examine whether the HPC-PFC pathway, that is highly associated with executive functions and neuropsychiatry symptoms, plays a fundamental role in abnormal cognitive and emotional behaviors associated with AD; Aim 3: (i) examine selected candidate miRNAs and their potential for reversing cognitive and emotional dysfunction by specifically activating and inhibiting microRNAs in the HPC-FPC of males and females, (ii) to examine whether the preventing or attenuating effects of CBD on AD phenotype are mediated via miRNAs.

Together, these experiments may suggest that targeting specific miRNAs (activating or silencing) may have therapeutic potential to restore memory and emotional deficits, and may define miRNA-regulated therapeutic role of CBD in the treatment of AD in males and females and thus propose how CBD may slow neurodegenerative processes. This study can bring forward novel pathways that suggest new treatments for implementation today as well as future strategies for reversing and preventing cognitive decline.

**Future directions** Current therapies only provide limited symptomatic relief and are ineffective in preventing AD progression. The fact that miRNAs function as key regulatory hubs that can influence several genes and entire biological pathways has made them attractive candidates for drug development. There is enormous potential of miRNAs for use as biomarkers in early detection and assessment of disease severity in AD, and possibly as therapeutics. There are challenges in using miRNAs as treatment, including developing drug formulation with higher stability and cellular availability in vivo and monitoring the widespread influence of miRNAs in gene regulation. Elucidating which miRNAs exert strong influence in AD laboratory model may have a potential therapeutic use in this disorder. While the delivery procedure of silencing and activation miRNAs in specific brain regions is invasive and only applied in animal studies, our study can identify specific miRNAs for future studies to deliver in a safe and specific route.

**Other parts**

The combined budget for direct costs for the two-year project period may not exceed $275,000. No more than $200,000 may be requested in any single year.

**Earliest Start Date** April 2023, The project period may not exceed two years.

Start date: July 2023

End date: June 2025

**FACILITIES & RESOURCES**

**Contribution of the scientific environment to the probability of success**:

The University of Haifa (UoH), located on Mount Carmel, was founded in 1963 to operate under the academic auspices of The Hebrew University of Jerusalem. In 1972, the University of Haifa declared its independence and became the sixth academic institution in Israel and the fourth university. The University of Haifa is the largest comprehensive research university in Israel's northern region. Its mission is to cultivate academic excellence, create a shared Israeli experience, and promote democratic values in an environment of tolerance and multiculturalism. Such an environment contributes to outstanding research and a community of exceptional, creative, and productive alumni. Over 18,000 students study there for undergraduate, graduate, and doctoral degrees. The University of Haifa is fully committed to academic excellence, which is expressed in its many and diverse interdisciplinary and international programs and collaborations with academic institutions around the world.

The PI’s lab is part of the School of Psychological Sciences. Neuroscience research at the School of Psychological Sciences of the University of Haifa is a unique enterprise, focusing on the interface between behavior and its neural substrates in psychopathology, psychiatric and neurodegenerative disorders. Each of the research groups, ranging across diverse neuroscience disciplines and model systems, focuses on particular behaviors and seek to unveil their underlying mechanisms. These explorations are conducted on multiple levels, from the molecular and cellular mechanisms to the study of whole neuronal systems. The methods employed by the School’s faculty include molecular and cellular biology, genetic manipulations, microscopy, in vitro and in vivo electrophysiology and human functional imaging. Together, illuminating topics in learning and memory, and cognitive processes in health and disease from diverse angles, ultimately aiming at understanding the neuronal processes yielding complex behaviors. The experiments for the proposed research project will be carried out in Prof. Akirav’s Learning and Memory Lab.

**Laboratory**

The Learning and Memory Lab, directed by Prof. Irit Akirav, is affiliated to the School of Psychological Sciences and to the Integrated Brain and Behavior Center (IBBRC), University of Haifa, Israel. The research aim of this lab is to understand the neural mechanisms underlying the involvement of the endocannabinoid system in psychiatric disorders. Since the research is inherently multi-disciplinary, research approaches are combined from the fields of biology, and psychology, integrating data ranging from the molecular to whole animal level. The lab is 80m² and includes a room dedicated to biochemistry and molecular biology, several rooms for behavioral tests, and an electrophysiology room. We also use shared space in the labs’ complex for equipment.

The lab includes:

Staff

The lab includes: Staff:

Prof. Irit Akirav (Head of lab),

Lab manager

A research associate

4 Ph.D. students

3 M.A. students

3 research assistants

The lab is fully equipped with: Workstations for students, behavioral settings, 3 in vivo electrophysiology systems, Two lab-owned fully motorized stereotaxic apparatuses, Western blot equipment, and access to the departmental RT-PCR, Western blot reader…..incubators….. SHIRA

Computer: All lab members have their own personal computer with Windows 10 OS.

Akirav lab: Computer for each student + computers for equipment (total ## computers). We have licenses for all standard Office software on all computers, and licenses for Adobe Illustrator, GraphPad Prism 6.01, InStat Software (GraphPad Software, CA, USA), MATLAB, and SPSS.

**FOREIGN JUSTIFICATION**

The proposed project will be conducted by Prof. Irit Akirav, located and operating in the School of Psychological Sciences at the University of Haifa. Prof. Akirav comes with unique proprietary data, expertise, and knowledge available to her lab, for the purposes of this project.

The strength of this project is its comprehensive investigation of the research questions, using a wide range of methods, from the classical like behavioral testing, immunochemistry and pharmacology, to those at the forefront of science, like viral mediated gene transfer and chemogenetics. Such an approach is bound to yield a comprehensive picture where, if the hypotheses are correct, the outcomes from the different experimental approaches support each other, allowing greater confidence in the conclusions.

Suitability of Investigator Prof. Akirav is highly suitable for that study. For many years her research if focused on the neuronal, hormonal and molecular basis of emotions and memory. She has numerous publications on the role of the cannabinoids in regulating behaviors in models of psychiatric disorders such as stress, anxiety and post-traumatic stress disorder. Her lab is very well equipped to perform the present study, and her preliminary data presented in the proposal attest to her perfect suitability to conduct this project.

*The PI is an established researcher of the effects of cannabinoids in animal models. This proposal is considerably strengthened by the consultants, whose labs have the expertise to assist with the viral vector and chemogenetic approaches included in this proposal*

**BUDGET JUSTIFICATION (TOTAL $ for 2 years)**

a) Irit Akirav, Ph.D.: Prof. Akirav will be the Principal Investigator in this research proposal. She will have overall responsibility for the project with a special emphasis on leading the scientific team. Prof. Akirav brings unique expertise to this study through her vase expertise in bridging between behavior and neural mechanisms underlying learning and memory and emotions. Prof. Akirav will dedicate 1.2 calendar months per year to working on this project.

**Other Personnel**

b) Post doc or advanced PhD student (12 calendar months per year): A PhD student (to be recruited) will be in charge of all experiments. The PhD student will be responsible for brain microinjections and performing the molecular analysis.

c) MA student (8 calendar months per year): An MA student (to be recruited) will be responsible for behavioral experiments and pharmacology.

d) Research assistant (8 calendar months per year): A research assistant will help with the behavioral tests, i.p. injections and punching brain tissues.

**Travel**

One trip for Prof. Akirav to travel to a conference in Europe to present our findings in year two of this project. Expenses will cover the anticipated airfare and per diem cover of accommodations, meals, ground transportation, and incidentals (Cost: $2,500)

**Other Direct Costs**:

• Materials & Supplies:

Rats: We request $6,500 in year one and $6,500 in year two of this project for rat purchase and maintenance (adult and middle aged males and females, long periods of maintenance).

We request $80,000 in year one and $65,000 in year two of this project for biochemical reagents including reagents, primers, antibodies, agomirs, antagomirs, viral vectors for DREADDS, ELISA kits, western blot reagents including gel preparation antibodies, membranes, chemiluminescence reagent and general lab supplies, including: gloves, tips, tin foil, saran wrap, kimwipes, and lab glassware.

We request $2,000 per year for using departmental equipment (e.g. RT-PCR unit).

**Publication Costs**

 In accordance with NIH data sharing policies, the project’s results, collected and edited, will be published annually. We plan for one or two peer-reviewed publications for this project: 2,500 $.

**Indirect cost**

Indirect costs have been calculated at 8% of the indirect cost base that includes all costs for each Budget Period, in accordance with NIH policy for foreign applicants.

1

100 rats- M, F

Pharmacological agents (CBD, STZ, aCSF)

miRNA, mRNA, proteins, ELISA kits

2

100 rats

DREADDS

IHC

3

200 rats

Agomirs antagomirs

Pharmacological agents (CBD, STZ, aCSF)

miRNA, mRNA, proteins, ELISA kits