

Universidade de Lisboa
Faculdade de Farmácia



Exploring the Intersection of Artificial Intelligence in Understanding Alzheimer's Disease

The Role of the Circadian Clock and Orexin Receptors

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Monografia orientada pela Professora Doutora Rita Alexandra Guedes,
Professora Associada.

Mestrado Integrado em Ciências Farmacêuticas

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**Trabalho Final de Mestrado Integrado em Ciências Farmacêuticas
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Declaro ter desenvolvido e elaborado o presente trabalho em consonância com o Código de Conduta e de Boas Práticas da Universidade de Lisboa. Mais concretamente, afirmo não ter incorrido em qualquer das variedades de fraude académica, que aqui declaro conhecer, e que atendi à exigida referenciação de frases, extratos, imagens e outras formas de trabalho intelectual, assumindo na íntegra as responsabilidades da autoria.

Resumo

A doença de Alzheimer é uma condição neurodegenerativa altamente complexa, responsável pela maioria dos casos de demência. Dado o envelhecimento progressivo da população e a diversidade dos fatores de risco, prevê-se que a sua prevalência aumente nas próximas décadas, o que irá sobrecarregar os sistemas de saúde em todo o mundo. Apesar das causas das alterações patológicas permanecerem em grande parte desconhecidas, as placas senis, os emaranhados neurofibrilares, o stress oxidativo, a disfunção mitocondrial, a sobre-estimulação glial, a neuroinflamação, a desregulação da homeostase do cálcio neuronal e a perturbação da neurotransmissão colinérgica e glutamatérgica são alguns dos fatores que contribuem para os danos neuronais e subsequente declínio cognitivo observado. É importante referir também que os distúrbios do sono são cada vez mais reconhecidos, não só como manifestações, mas também como fatores fisiopatológicos da doença de Alzheimer. O ciclo sono-vigília está intimamente relacionado com o relógio circadiano, que é um sistema autónomo de temporização das células, responsável por sincronizar inúmeros processos fisiológicos. As orexinas são um par de neuropéptidos amplamente distribuídos pelos sistemas nervosos central e periférico, que estão igualmente envolvidas na regulação deste ciclo, principalmente na promoção e consolidação da vigília. Curiosamente, estes dois sistemas exibem uma relação de auto-reforço, onde o desalinhar circadiano e a desregulação dos níveis de orexina exacerbam a progressão da doença de Alzheimer e vice-versa. Portanto, os antagonistas dos receptores de orexina têm sido investigados no tratamento das perturbações do sono para abrandar a progressão da doença de Alzheimer. A inteligência artificial, em particular a aprendizagem automática, a aprendizagem profunda e o processamento da linguagem natural, surge como uma ferramenta que permite analisar grandes bases de dados, para não só desvendar os processos biológicos subjacentes às relações entre estes sistemas e a doença de Alzheimer, bem como para acelerar a descoberta de biomarcadores, melhorar a precisão do diagnóstico e otimizar o desenvolvimento de fármacos. Apesar do seu potencial, a inteligência artificial enfrenta desafios significativos que limitam a sua aplicação na investigação e na prática clínica da doença de Alzheimer, os quais deverão ser abordados no futuro.

Palavras-chave: Inteligência Artificial; Doença de Alzheimer; Ciclo Circadiano; Recetores da Orexina

Abstract

Alzheimer's disease is a highly complex neurodegenerative disorder that accounts for most cases of dementia. Given the diversity of risk factors and the fact that the global population is aging, the prevalence of this disease is predicted to rise in the next decades, which increases the burden on healthcare systems worldwide. Despite the fact that the underlying triggers for the pathological changes remain unknown, senile plaques, neurofibrillary tangles, oxidative stress, mitochondrial dysfunction, glial overstimulation, neuroinflammation, dysregulation of neuronal calcium homeostasis and disruption of cholinergic and glutamatergic neurotransmission are some of the main pathways that are interrelated with the progressive neuronal damage and subsequent cognitive decline observed in Alzheimer's disease. Importantly, sleep disturbances are increasingly recognized not only as manifestations, but also as contributing factors to Alzheimer's disease pathophysiology. The sleep-wake cycle is closely related to the circadian clock, which is an evolutionarily conserved cell-autonomous timing system that synchronizes various physiological processes. The orexins are a pair of neuropeptides that are widely distributed throughout the central and peripheral nervous systems, and are also implicated in the regulation of the sleep-wake cycle, primarily by promoting long consolidated wakefulness. Interestingly, the circadian and orexin systems seem to be part of a self-reinforcing feedback loop, where circadian misalignment and dysregulation of the orexin levels exacerbate Alzheimer's disease progression, and vice versa. Thus, recent studies highlight the potential of targeting the orexin receptors to mitigate sleep impairments and slow Alzheimer's disease progression. Artificial intelligence, particularly through machine learning, deep learning and natural language processing algorithms, has emerged as a transformative tool for unraveling the intricate biological pathways that underlie the relationships between these systems and Alzheimer's disease, and for accelerating biomarker discovery, enhancing diagnostic accuracy and optimizing drug development by leveraging large and complex datasets. Despite its potential, artificial intelligence faces significant challenges that limit its widespread use in Alzheimer's disease research and clinical practice and these should be addressed in the future.

Keywords: Artificial Intelligence; Alzheimer's Disease; Circadian Clock; Orexin Receptors

List of Abbreviations

1-SORA	Selective Orexin 1 Receptor Antagonist
2-SORA	Selective Orexin 2 Receptor Antagonist
AD	Alzheimer's Disease
ADMET	Absorption, Distribution, Metabolism, Excretion and Toxicity
ADRD	Alzheimer's Disease Related Dementia
AI	Artificial Intelligence
APP	Amyloid Precursor Protein
A β	Amyloid- β
BACE1	Beta-site Amyloid Precursor Protein Cleaving Enzyme 1
BACE2	Beta-site Amyloid Precursor Protein Cleaving Enzyme 2
BBB	Blood-Brain Barrier
BMAL1	Brain and Muscle ARNT-Like Protein 1
CLOCK	Circadian Locomotor Output Cycles Kaput
CRY	Cryptochrome Circadian Regulator
CSF	Cerebrospinal Fluid
DL	Deep Learning
DORA	Dual Orexin Receptor Antagonist
DOTA	Drug Repositioning Approach using Optimal Transport for Alzheimer's Disease
EMA	European Medicines Agency
FDA	U.S. Food and Drug Administration
GPCR	G Protein-Coupled Receptor
INDRC	International Neurodegenerative Disorders Research Center
ipRGC	Intrinsically Photosensitive Retinal Ganglion Cell
ISF	Interstitial Fluid
KDE	Kernel Density Estimation
MAD	Multimodal Arousal Detector
MAPK	Mitogen-Activated Protein Kinase
MCI	Mild Cognitive Impairment
ML	Machine Learning
MRI	Magnetic Resonance Imaging
NFT	Neurofibrillary Tangle

NLP	Natural Language Processing
NMDA	N-Methyl-D-Aspartate
NN	Neural Network
NREM	Non-Rapid Eye Movement
OX-A	Orexin A
OX-B	Orexin B
OX ₁ R	Orexin 1 Receptor
OX ₂ R	Orexin 2 Receptor
OXR	Orexin Receptor
p-Tau	Phosphorylated Tau
PER	Period Circadian Regulator
PET	Positron Emission Tomography
PI3K	Phosphoinositide-3-Kinase
PKA	Protein Kinase A
PKC	Protein Kinase C
PLC	Phospholipase C
QSAR	Quantitative Structure-Activity Relationship
REM	Rapid Eye Movement
RF	Random Forest
SCN	Suprachiasmatic Nucleus
SHAP	Shapley Additive Explanations
TL	Time-Lag
TST	Total Sleep Time
XAI	Explainable Artificial Intelligence

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1 Introduction

Alzheimer's disease (AD) was named after the German psychiatrist Alois Alzheimer and it's a highly complex, heterogeneous and slowly progressive neurodegenerative disorder that accounts for 60-70% of all dementia cases [1,2]. It is characterized by the development of neuritic plaques and neurofibrillary tangles (NFTs), as a consequence of the build-up of amyloid-beta ($A\beta$) peptides and tau protein, as well as neuron loss, inflammation and brain atrophy, which can ultimately lead to a loss of cognitive abilities and independence in daily personal activities [3]. However, the underlying triggers for the pathological changes in AD remain unknown.

At present, there are multiple risk factors that have been associated with the development of AD, including increasing age, genetic factors, head trauma, vascular and metabolic diseases, infections and environmental factors [4]. So as the population ages, the prevalence of this disease is only expected to increase, placing an additional burden on national health systems [5]. Indeed, it is predicted that by the year 2050, the number of cases in Portugal will have reached around 347,000 [6].

In spite of all the efforts made by the scientific community, there are currently no curative therapies for AD and the two main classes of drugs available, the cholinesterase inhibitors [7] and N-methyl-D-aspartate (NMDA) antagonists [8], provide only modest clinical benefit as they alleviate the symptoms but do not prevent nor delay the progression of the disease. Over the last years, there were some breakthroughs on the development of disease-modifying treatments, such as monoclonal antibodies targeting $A\beta$, although questions remain regarding their clinical safety and effectiveness [9-12].

Moreover, due to a growing body of clinical evidence suggesting that sleep disturbances play a crucial part in the pathophysiology of AD [13], and that the orexinergic system, which is implicated in circadian rhythm homeostasis, becomes dysregulated in these patients [14,15], the orexin receptors (OXRs) emerged as promising targets for research and therapeutic interventions. This led to the recent approval of Suvorexant (Belsomra[®]/MK-4305) for the treatment of insomnia in mild to moderate AD by the US Food and Drug Administration (FDA) [16,17].

Finally, the recent rapid growth in computing power and memory storage, the unprecedented wealth of big biomedical data and the development of advanced

analytical processing algorithms have prompted the application of Artificial Intelligence (AI) methods within the medical and pharmaceutical fields [18-21]. And given the clinical heterogeneity of AD, the limited understanding of the underlying disease mechanisms, the cost and duration of traditional drug discovery and development methods [22-24] and the high failure rate of drug candidates [25,26], the scientific community has recognized AI as a promising tool to help overcome these challenges.

2 Methodology

The main objective of this monograph is to understand the relationships between Alzheimer's disease, the circadian clock and orexins, as well as to evaluate how Artificial Intelligence can contribute in this area of research.

To achieve the intended objectives, reviews of the literature were conducted.

Databases were used to allow access to scientific and review articles, including PubMed, Web of Science and Google Scholar, and some documents from other sources that have provided valuable and reliable information, such as the World Health Organization, the Alzheimer's Association, the Alzheimer Europe, the ClinicalTrials.gov, the U.S. Food and Drug Administration, and the European Medicines Agency.

This research was conducted from January to August 2024.

3 Alzheimer's Disease

3.1 Clinical Manifestations

Alzheimer's disease is characterized by a range of cognitive and behavioral symptoms, the so-called neuropsychiatric or behavioral and psychological symptoms of dementia. Examples include mood disorders, aggression, psychotic symptoms and behavioral problems [27]. However, the brain changes that cause them are thought to begin 20 years or more before their manifestation [5].

The disease progression, also known as Alzheimer's disease continuum, can be categorized into different phases: preclinical AD, mild cognitive impairment (MCI) due to AD and AD related dementia (ADRD). The dementia phase can be further divided into mild, moderate and severe dementia [28-31].

The preclinical stage is characterized by the absence of clinical symptoms and the presence of measurable brain changes that indicate early signs of AD [32]. The MCI phase features mild memory loss and early structural changes in the brain, but no significant functional impairment or any evident clinical symptoms [33]. In the mild or initial dementia phase, a variety of symptoms appear, including difficulties at performing daily activities, loss of concentration and memory, disorientation, mood swings and/or depression [34]. As the disease advances to the moderate dementia stage, it extends to the cerebral cortex, resulting in further memory loss, trouble recognizing family and friends, loss of impulse control and challenges at the reading, writing and speaking levels [34]. The severe or late dementia stage implies widespread of the disease through the entire cortex, which results in a significant accumulation of senile plaques and NFTs. At this stage, patients may lose the ability to recognize their family, often become bedridden, and find it particularly complicated to swallow and urinate, which can eventually lead to the patient's death [34].

3.2 Diagnosis

Those suspected of suffering from AD must undergo a thorough evaluation, including neurological examination, a magnetic resonance imaging (MRI) scan to assess neuronal integrity and laboratory analyses, in addition to a background check of their family and medical history [3].

In 1984, the National Institute of Neurological and Communicative Disorders and Stroke alongside the Alzheimer's Disease and Related Disorders Association established the following clinical criteria for the diagnosis of Alzheimer's disease [35]: probable Alzheimer's disease, diagnosed when dementia is present with no other systemic or brain diseases and confirmed by neuropsychological tests, progressive memory impairment, compromised daily functioning and other symptoms such as aphasia, apraxia and agnosia, which can manifest between the ages of 40 and 90; possible Alzheimer's disease, applied when neurological or psychiatric disorders are present, but are not the primary cause of dementia; definite Alzheimer's disease, determined by histopathological examination of a biopsy or autopsy.

More recently, the National Institute on Aging-Alzheimer's Association developed a new research framework designed to update the previous 1984 diagnostic criteria [36]. It adopts a biological instead of a syndromal perspective on AD, by using an A/T/N classification system that acknowledges relevant biomarkers. With "A" referring to the concentration of amyloid- β , "T" representing tau levels and "N" reflecting markers of neurodegeneration.

The updated criteria features the designations of probable and possible AD dementia for clinical use, and probable or possible AD dementia with supporting pathophysiological evidence to be used for research purposes. Additionally, AD biomarkers are classified under two groups: brain amyloid markers, such as positron emission tomography (PET) and cerebrospinal fluid (CSF); and neuronal injury markers, which include tau levels in CSF, fluorodeoxyglucose PET to assess metabolic activity, and MRI to detect atrophy.

Even so, there is an urgent demand for innovative diagnostic tools and biomarkers capable of detecting AD at an early stage, with high specificity and for a relatively low cost [37].

3.3 Pathology and Progression

The exact mechanisms of Alzheimer's disease are complex and remain under investigation, however two types of neuropathological changes provide insight into the course of the disease and its symptoms: the so-called positive lesions, which arise from the accumulation of deposits in the patient's brain; and the negative lesions,

characterized by major atrophy owing to neural, neuropil and synaptic loss [38]. As for positive lesions, the senile plaques formed by extracellular A β and the NFTs caused by hyperphosphorylated tau protein are considered the two main pathological hallmarks [39,40].

There are different types of A β peptides, which are synthesized through the proteolytic cleavage of the transmembrane amyloid precursor protein (APP) via β -secretase (BACE1 and BACE2) and γ -secretase, and these include both large, insoluble amyloid fibrils, as well as soluble oligomers [41]. And it has been found that mutations in genes such as APP, presenilin-1 and presenilin-2 disrupt the catabolism and anabolism of A β , resulting in a diminished degradation of A β , a higher A β_{42} /A β_{40} ratio - which promotes A β fibril formation, and an increase in soluble oligomers that can spread to the brain [41,42].

Although microglial cells normally play a protective role in eliminating A β and hyperphosphorylated tau, once amyloid fibrils have accumulated to form senile plaques there is a persistent microglial activation, thereby increasing the levels of inflammatory molecules, causing neurotoxicity and damaging axons and dendrites, which results in the loss of synapses and therefore cognitive impairment [43, 44]. Indeed, it has been reported that astrocyte dysfunction triggers the onset and progression of AD, given that A β and neuronal damage stimulate the production of astrocytic inflammatory cytokines, that in turn induce greater production of A β [45,46]. Moreover, A β is capable of binding to the calcium-sensitive receptor in astrocytes, initiating intracellular signaling and boosting the production of phosphorylated tau (p-tau) [46].

Furthermore, compromised astrocytes have trouble eliminating excess glutamate from the synapses, which means that this neurotransmitter, crucial in learning and memory processes, accumulates [47,48]. As a result, the glutamate surplus causes overstimulation of NMDA receptors, prompting a massive influx of calcium ions into the neuron, and contributing to mitochondrial dysfunction, reactive oxygen species production and cell death pathways activation, all of which culminate in neuronal death [49-52].

In addition to disrupting glutamatergic synaptic transmission, inducing mitochondrial malfunction and endosomal-lysosomal alterations, A β oligomers have

also been found to cause synaptic damage by constricting cerebral capillaries [53], thus reducing cerebral blood flow, and by binding to synaptic proteins [54].

On the other hand, it is well established that the ratio between phosphorylated and non-phosphorylated tau influences the stability of microtubules in the cytoskeleton, which maintain axoplasmic transport by acting as an intracellular transport system [55]. However, in AD the imbalance between protein kinases and phosphatases, such as protein kinase A, Ca^{2+} /Calmodulin-dependent protein kinase, protein kinase C (PKC) and mitogen-activated protein kinases (MAPK), leads to the hyperphosphorylation of tau and thereby reduces the binding force between the tau protein and tubulin, which consequently promotes the formation of double helix fibers [56]. The twisting and coiling of the double helix filaments leads to the formation of NFTs, that are not only associated with oxidative stress and synaptic loss, but also cause microtubule instability along with a decreased axonal transport, which disrupts the distribution of mitochondria, vital to meet the demand for high energy and regulate calcium homeostasis in neuronal cells [57,58].

Another major pathophysiological process is the impairment of cholinergic networks. In AD there's a substantial loss of cholinergic neurons, mainly in the basal forebrain which has projections to the hippocampus and cortex, as well as evidence of abnormalities affecting both nicotinic and muscarinic receptors [59]. Mounting evidence indicates that amyloid- β contributes to the lesion and malfunction of cholinergic neurons, causing a decline in both the production and release of acetylcholine, thus disrupting normal signaling pathways in brain regions that are critical for memory, attention, learning and other cognitive functions [60].

So in brief, senile plaques, NFTs, oxidative stress, mitochondrial dysfunction, glial overstimulation, neuroinflammation, dysregulation of neuronal calcium homeostasis and disruption of cholinergic and glutamatergic neurotransmission are some of the main pathways that are interrelated with the progressive neuronal damage and subsequent cognitive decline observed in Alzheimer's disease. All these pathological processes are believed to be fueled by complex genetic and environmental factors, of which apolipoprotein E (ApoE) represents a well-established genetic risk factor for the onset of Alzheimer's disease [61]. Furthermore, the ATP binding cassette transporter A1 [62], the clusterin gene [63], the bridging integrator 1 [64], the

evolutionarily conserved signaling intermediate in toll pathway [65], the estrogen receptor gene [66], and a variety of other genes have also been implicated into the pathological mechanisms of AD [67]. There is also increasing evidence associating AD with a prior history of infection [68,69], cardiovascular disease [70,71], obesity [72,73] and/or diabetes [74], providing additional support that inflammation is a possible initiating factor.

3.4 Current Treatment Approaches and Challenges

Initially, the development of pharmaceutical drugs to tackle the deteriorating effects of AD was guided by the cholinergic hypothesis, which is the earliest theory proposed to explain the etiology and the mechanisms behind the neuropsychiatric symptoms, and states that both the loss of acetylcholine neurons and enzymatic functions in the regions of the amygdala, basal forebrain, cortex and hippocampus [75], as well as the decrease in nicotinic and muscarinic receptors in the pre- and postsynaptic neurons are determining factors in the pathology of AD [76,77].

For this reason, the cholinesterase inhibitors, such as galantamine, rivastigmine and donepezil, are a major therapeutic class in AD treatment, as they inhibit the hydrolytic action of acetylcholinesterase, thus resulting in a higher acetylcholine concentration and, in turn, enhancing neuronal communication and temporarily improving or stabilizing the symptoms of dementia [7,78].

Memantine, on the other hand, was designed based on the glutaminergic hypothesis, which suggests that the overactivation of NMDA glutamate receptors is the underlying cause for the neuronal damage in AD, and that targeting these receptors to inhibit the influx of calcium and the subsequent excitotoxicity can delay neurodegeneration [8,49].

Unfortunately, the efficacy of the abovementioned drugs is limited to the symptomatic treatment of AD, as they improve memory and alertness, but are incapable of preventing progression [10].

Nowadays, research is focusing on the development of disease-modifying treatments that can significantly improve the prognosis of the disease, placing a great emphasis on the A β immunotherapy [10,11,79]. In 2021, the FDA approved Aducanumab, an amyloid-targeting monoclonal antibody. However, there were some

concerns about its clinical efficacy, potential side effects and long-term safety, which is why it failed to get approval from the European Medicines Agency (EMA) [12]. By 2023, a second amyloid targeting monoclonal antibody, known as Lecanemab, received the FDA's approval and is currently undergoing regulatory review by the EMA [80].

Several other strategies and targets are under investigation, including gene therapy and nanoparticles [81], yet the lack of knowledge regarding the molecular mechanisms of AD continues to represent a fundamental barrier to the successful development of new drugs.

Finally, recent studies suggest a connection between Alzheimer's disease and the orexin system, which participates in the circadian regulation of the sleep-wake cycle. This relationship will be explored in detail in the next chapter.

4 Orexin Receptors and the Circadian Clock

4.1 Circadian Clock, Sleep and Alzheimer's Disease

The circadian timing system is an evolutionarily conserved cell-autonomous process that establishes a daily rhythm over a period of roughly 24 hours [82]. In mammals, this clock timing process is found in all organ systems and in the majority of cell types, ensuring the synchronization of various physiological processes including the regulation of sleep-wake patterns [83], hormone secretion [84], metabolism and energy balance [85], cognitive function [86], as well as various cellular processes, notably DNA repair, cell division and gene expression [87].

Lying at the core of this system is the suprachiasmatic nucleus (SCN), a small region located within the hypothalamus, that acts as the master pacemaker [82]. The main regulator of this clock is light, which is detected by specialized photoreceptor cells in the retina, called intrinsically photosensitive retinal ganglion cells (ipRGCs) [88,89]. There are also other environmental cues, such as social interactions, physical activity and dietary patterns, that can influence the circadian rhythm [82].

The ipRGCs then communicate the light information to the SCN, which triggers a series of molecular events through a transcriptional and post-translational feedback mechanism centered on the daily oscillation of the expression of the period (PER) and cryptochrome (CRY) genes [90].

The rhythmic regulation of PER and CRY gene transcription is driven by a heterodimeric E-box binding transcription factor, formed by circadian locomotor output cycles kaput (CLOCK) and brain and muscle ARNT-like protein 1 (BMAL1) [90,91]. As their cytoplasmic concentrations increase, the PER and CRY proteins dimerize and translocate to the nucleus, where they function as potent negative regulators of CLOCK-BMAL1-mediated transcription, thus regulating their own transcription and forming the core negative element of the transcriptional-translational feedback loop [92-95].

However, in certain circumstances these mechanisms lose their optimal synchronization, resulting in changes in the rhythmic transcription and expression of these clock genes, often referred to as "circadian misalignment", which has been linked to a wide array of detrimental health outcomes [96], including sleep and metabolic

disturbances [97], cardiovascular diseases [98] and even certain types of cancer [99,100]. More importantly, in recent years, increasing clinical evidence has suggested that circadian and sleep disturbances can also contribute to the pathophysiology of Alzheimer's disease [101-105].

In fact, sleep disorders are a common symptom in AD patients, and are often recognized as one of the first noticeable manifestations that precedes cognitive decline, appearing early in the preclinical phase and becoming increasingly prevalent as the disease progresses, thereby establishing themselves as a well-characterized feature of the mid to late phase of ADRD [106-109]. These sleep disturbances appear to be caused by circadian misalignment, changes in neuroendocrine mediators and impairment of the interstitial fluid (ISF) clearance system in the brain [110]. Furthermore, AD is also associated with alterations in the clock-regulation of core body temperature rhythms, activity rhythms, and the phasing of the pineal melatonin rhythm [111,112].

Clinically, individuals suffering from AD often display frequent and prolonged episodes of daytime sleepiness, sleep-maintenance insomnia, increased total sleep time, fragmented sleep with frequent awakenings and a typical phase-advance of the nocturnal sleep period [113-116]. All of these circadian abnormalities are closely associated with an accelerated cognitive decline, an aggravation of depressive symptoms and an altered nutritional intake [117,118]. And in the later stages of AD, these sleep disturbances can result in a virtual reversal of the day-night sleep pattern, which is a strong predictor of mortality and institutionalization, especially in the presence of sundowning syndrome (i.e. the exacerbation of behavioral symptoms in the afternoon and evening) [119,120].

Importantly, the recently acquired memories stored in the hippocampal network undergo offline reprocessing during sleep, in order to transfer the noteworthy information to the neocortex - the long-term memory center, allowing its integration into a greater neuronal network. And both rapid eye movement (REM) and slow wave sleep have a fundamental role in this process [121-124]. Furthermore, several studies have revealed that the synaptic growth that occurs during wakefulness requires a counteraction, to prevent the oversaturation of synapses and the loss of neural signals and memories, which can be best achieved through sleep [125-127]. Therefore, given that the circadian rhythm is such a crucial element in the hippocampus-dependent

memory formation and consolidation processes, the memory decline observed in AD may well be a reflection of the sleep-dependent memory consolidation impairment, rather than solely the consequence of the disease's progression [128].

In conclusion, the exacerbation of the sleep-wake disturbances already observed with normal aging can be considered an indicator of early AD stages. Interestingly, several lines of evidence suggest that circadian rhythm disturbances and Alzheimer's disease do not only overlap from a pathophysiological perspective, but are also part of a complex, self-reinforcing feedback loop, where the disruption of the biological clock's rhythm increases the risk of developing Alzheimer's disease, which in turn exacerbates the circadian misalignment [129,130]. Nevertheless, the relationship between the changes that occur in sleep patterns from the preclinical to the clinical stage of AD and their causal relationship with this disease remains unclear.

4.2 Overview of the Orexin System

Humans have approximately 50,000 to 70,000 orexin-synthesizing neurons that are exclusively located in the lateral and peripheral nerve regions of the hypothalamus, however their axons and their receptors are extensively distributed to multiple brain regions [131-133]. Additionally, orexin neurons also innervate various peripheral tissues such as adrenal glands, kidneys, lungs, testes, ovaries, pituitary gland, jejunum and thyroid [134,135]. Though a very small system considering the location, owing to its neural circuits integrated with other essential centers in different areas of the brain, orexinergic neurons have a wide range of physiological roles, which still need to be explored more clearly [136].

The best understood role of orexins in mammals is the regulation of sleep and wakefulness states, especially the maintenance of long consolidated wakefulness. Through activation of the wake-active monoaminergic system and the deactivation of the REM-on cholinergic groups, orexins promote arousal [136-138]. Orexins have also been implicated in the regulation of food intake, emotion, the reward system, the autonomic nervous system and the neuroendocrine system [131,136]. Furthermore, orexins have shown to modulate long term synaptic plasticity, learning and memory through their effect on cholinergic, glutamatergic, gabaergic and noradrenergic transmission within the hippocampus [139,140].

On the other hand, the activity of orexin neurons is modulated by inputs from several brain regions [141,142]. There are various neurotransmitters and neuromodulators involved in the activation and inhibition of the orexin neuronal activity. Glutamate, cholecystokinin, neurotensin, oxytocin and vasopressin activate orexin neurons via modulation of adenosine and CO₂ concentrations [143,144].

Lastly, the inhibitory factors of orexin neurons include GABA and serotonin, which directly hyperpolarize orexin neurons through activation of α 2-adrenoceptors, while catecholamines, such as noradrenaline, adrenaline and dopamine, regulate orexin neurons in a more complex manner [143-145]. Electrophysiological experiments on orexin neurons have revealed that around 30% of them are excited by acetylcholine, while others are conversely inhibited [146]. It has also been suggested that neuropeptides such TRH and CRH regulate their excitability [147]. This regulation by CRH and vasopressin suggests a link to the stress response. Furthermore, leptin inhibits orexin neurons while ghrelin increases their firing frequency, and they are also inhibited when extracellular glucose concentrations are elevated [148].

4.2.1 Orexin Peptides and Receptors

The orexins are a pair of neuropeptides that, as previously stated, are widely distributed throughout the central and peripheral nervous systems.

Orexin A (OX-A) is a 33 amino acid peptide weighing approximately 3.5 kDa and orexin B (OX-B) is a 28 amino acid peptide weighing around 2.9 kDa [149]. They are derived from a common precursor gene, *prepro-orexin*, via differential hydrolysis and share 46% sequence identity [149,150]. Both of them are highly conserved in mammalian species, suggesting a strong evolutionary pressure that maintains their functionality [151].

Both peptides present a C-terminal amidation, but OX-A is further post-translationally modified with an N-terminal pyroglutamyl cyclization and two intrachain disulfide bonds between Cys⁶-Cys¹² and Cys⁷-Cys¹⁴ [149,154]. Through nuclear magnetic resonance studies, researchers successfully characterized the three-dimensional structure of the orexins, and determined that in aqueous solution OX-A and OX-B comprise two α -helices connected by a short, flexible loop: Cys¹⁴-His²¹ (helix I) and Asn²⁵-Leu³¹ (helix II) [152-154]; and Leu⁷-Gly¹⁹ (helix I) and Ala²³-Met²⁸ (helix II) [154-156], respectively.

Notably, these peptides orchestrate their diverse physiological effects by binding and activating two subtypes of class A G protein-coupled receptors (GPCRs), the orexin 1 receptor (OX₁R) and the orexin 2 receptor (OX₂R) [149,157,158]. The human OX₁R has 425 amino acids, while the human OX₂R has 444 amino acids, showing a sequence identity of 63.5%, and their genes are located on chromosomes 1 and 6, respectively [149,151]. They each consist of seven transmembrane helices, with the C-terminal portion of helices 2-4 providing distinct selectivity between the two receptors. Additionally, they both contain an N-terminal amphipathic α -helix that facilitates binding of orexin neuropeptides [159-161]. These receptors are highly conserved among mammals; for instance, human OX₁R shares about 94% identity with rat OX₁R, whereas human OX₂R shares about 95% identity with rat OX₂R [149,151].

Furthermore, through radioligand binding and intracellular calcium transient assays, the OXRs and their affinities for the orexin peptides were characterized. It was revealed that OX₂R is a nonselective receptor for both OX-A (IC₅₀ = 38 nM; EC₅₀ = 34nM) and OX-B (IC₅₀ = 36 nM; EC₅₀ = 60 nM), while OX₁R is more selective for OX-A (IC₅₀ = 20 nM; EC₅₀ = 30 nM) than for OX-B (IC₅₀ = 420 nM; EC₅₀ = 2500 nM) [151].

4.2.2 Signaling Pathways

Orexin receptor signaling pathways are very complex and our understanding of these is currently still small. Additionally, the existence of two orexins and two receptor subtypes is bound to create diversity within cellular signaling pathways.

Since orexin receptors are GPCRs, upon binding to an endogenous or pharmacological agonist they undergo a series of conformational changes, which propagate from the binding site to the intracellular side of the receptor, where they can recruit multiple transducer proteins to trigger distinct biological responses [162]. Classically, active GPCRs recruit heterotrimeric G proteins, which are composed of an α , β , and γ subunit, and these can be classified based on the sequence and functional similarities of their α subunit into four families: G α_s , G α_i , G α_q and G α_{12} [163,164].

However, the activation of GPCRs can also trigger the recruitment of β -arrestins, a family of proteins that promote the internalization of active GPCRs from the plasma membrane into intracellular endosomal membranes [162].

The two most recent large scale GPCR-G protein selectivity studies support the idea that $G\alpha_q$ proteins are the primary signaling transducer for orexin receptors [165,166]. Nevertheless, the so-called biased agonism (i.e. the preferential activation of different signal pathways by chemically different ligands), has been proposed for a number of GPCRs, including OXRs, supporting the idea that several types of G-proteins and other signal transducers, like β -arrestins, may be able to interact with a single GPCR [167,168]. There's also evidence suggesting that OXR subtypes can form dimers or oligomers with themselves, each other and other GPCRs [169,170].

Therefore, OXRs are likely capable of recruiting different transducers that trigger various downstream pathways and responses [171]. These include the activation of phospholipase C (PLC), which generates inositol-1,4,5-trisphosphate and diacylglycerol, and it's likely the main pathway. These messengers then stimulate Ca^{2+} influx into the cytosol and the activation of PKC.

Another central orexin response is the stimulation of Ca^{2+} fluxes, and several mechanisms are likely involved: PLC-dependent Ca^{2+} release or influx mediated by non-selective cation channels, voltage-gated Ca^{2+} channels or possibly Na^+/Ca^{2+} -exchangers. The physiological actions mediated by these Ca^{2+} fluxes are also largely unknown, although they are likely involved in the ability of orexin to promote depolarization of many types of neurons, transmitter release and postsynaptic plasticity. Furthermore, OXRs stimulation seems to regulate phospholipase A₂ enzyme activity and the consequent release of arachidonic acid; the phospholipase D isoform 1 pathway, via novel PKC; the phosphoinositide-3-kinase (PI3K) pathway, which is central in regulating cell growth and survival; and adenylyl cyclase, which may relate to their ability to couple to $G\alpha_s$ proteins.

In addition, OXRs can in principle activate some classical plasticity-regulating cascades, such as the extracellular signal-regulated kinase (ERK) and the p38 MAPK, however the exact mechanisms are yet to be better understood. Notably, ERK and PKC have also been associated with synaptic plasticity.

Finally, persistent OXR stimulation appears to induce programmed cell death and two different pathways have been suggested: the SHP-2-dependent cascade and the p38 MAPK mediated cascade, however there are several open questions relating to these pathways.

4.2.3 Circadian Regulation of the Orexin System

The orexin neurons receive circadian influence through abundant innervations from the bed nucleus of the stria terminalis, supraventricular zone and dorsomedial hypothalamus, which in turn receive inputs from the SCN [172,173].

Furthermore, the expression and release of prepro-orexin, orexins, and orexin receptors exhibit a strong diurnal fluctuation and a reciprocal relationship with circadian rhythm [174], suggesting that clock gene expression may be linked to the rhythmic expression of the orexinergic system components. During the active period of the day, orexin levels increase gradually and reach the peak before the beginning of the rest period. When orexinergic excitatory tone lessens, due to a combination of homeostatic and circadian mechanisms, the inhibition of sleep-promoting circuitry decreases, inducing sleep [175].

In rodents, lesions in the SCN eliminate the daily rhythms of orexin release [176], and forced wakefulness and REM sleep deprivation are observed to augment orexin levels [177], indicating that circadian rhythm may play a pivotal role in orexin transmission.

On the other side, orexin fibers and their receptors have been detected in the SCN [132,133], and the activity of the SCN clock cells and neurons has been found suppressed by orexins, suggesting that these peptides reciprocally regulate the activity of SCN neurons in an inhibitory way [178,179].

Thus, maintaining the normal orexinergic signal is key to ensure the circadian rhythm of the whole sleep-wake cycle. High CSF orexin levels are associated with more sleep fragmentation and nocturnal sleep impairment, such as increased wakefulness during the night, prolonged sleep latency, decreased sleep efficiency and decreased REM sleep [14,180], whereas excessive daytime sleepiness occurs when orexin levels are low. This is highlighted by findings that orexin deficiency causes narcolepsy [181,182], an orphan chronic disease characterized by excessive daytime sleepiness and cataplexy, in several mammalian species including mice, rats, dogs and humans.

4.3 Orexin System and Circadian Clock Relationship with Alzheimer's Disease Pathology

The role of the orexin system in the regulation of the sleep-wake cycle has significant implications for the pathology of AD, since a large number of studies have found that the orexinergic system is dysregulated in these patients.

Post-mortem studies revealed that the orexin-synthesizing neurons and the concentration of orexin in the CSF were significantly reduced in AD patients [183,184], indicating that orexin neurons were damaged during the process. Despite the reduced orexinergic neurons in AD brain, several other studies reported that orexin is increased in the CSF of patients with MCI and AD [14,180,185-187].

A possible explanation for these findings is that in AD there's a significant loss of neurons that promote awakening, especially basal forebrain cholinergic neurons [3,7], that contributes to the derangement of the sleep homeostasis. This impairment of the cholinergic networks may lead to the upregulation of orexin neurons as a compensatory mechanism, because of the absence of the damaged cholinergic feedback [188,189]. Meaning that the early AD neurodegeneration could be responsible for an increase in the orexin levels, but this compensation may decline with the progression of the disease, as is shown in post-mortem studies in which the orexinergic system is downregulated. Furthermore, the impact of the overexpression of the orexin system and whether it maintains or impairs cognitive function in AD is unknown [188].

On the other hand, the discrepancies in studies may also result from the differences in methodology, population characteristics, sample sizes and the influence of uncontrolled factors among different studies.

4.3.1 Amyloid- β

One of the mechanisms by which sleep, orexins and AD are related is the formation and clearance of senile plaques [185,190,191]. Although the whole process is largely unclear, amyloid plaque formation is modulated by the amount of wakefulness, which is by itself regulated by the endogenous orexins. Thus, perturbations in both orexin signaling and the sleep-wake cycle have acute effects upon A β dynamics.

A β burden is increased by enhancing intrinsic neuronal excitability during wakefulness, and less A β is produced during sleep time. Indeed, A β levels in ISF increase during sleep deprivation or during orexin infusion, while enhancing sleep via orexin receptor blockade markedly inhibits A β plaque accumulation [190,191].

The clearance of A β is mediated by the glymphatic system, which is a recently discovered macroscopic system responsible for the clearance of toxic waste products from the central nervous system. And the glymphatic system is primarily active during sleep, allowing the removal of more brain toxins, including A β , during this period and causing a natural oscillations of its levels in the ISF over a 24-hour period [185,192]. Therefore, orexins likely facilitate A β production and decrease glymphatic clearance of A β from ISF by promoting wakefulness.

Moreover, orexins are also found to directly affect the clearance of A β through the suppression of phagocytosis and autophagic flux in microglia [193]. By inhibiting the PI3K, the protein kinase B and the p38 MAPK signaling pathways, and by reducing the formation of actin filaments around the A β fibrils, orexins suppress the phagocytosis of A β , thereby obstructing its clearance. Orexin also disrupts the autophagosome-lysosome fusion process, impairing the degradation of extracellular A β fibrils.

In turn, the quality or amount of sleep is inversely associated with the amount of A β measured in the CSF, and of fibrillar forms of A β quantified by amyloid PET scan [194,195]. In addition, A β_{25-35} administration significantly decreases non-REM (NREM) sleep and increases wakefulness in mice [196]. These findings suggest that high A β presence in the brain tissue causes further disruption of the sleep-wake cycle, and that a positive feedback loop may exist between A β accumulation, orexinergic dysfunction and sleep impairment [191].

4.3.2 Tau

Another hallmark of AD, tau aggregation, also shows a sleep-wake cycle dependence. In AD patients, orexin levels in CSF exhibit an evidently positive correlation with total tau protein and p-tau protein, indicating that orexin may also play a role in tau protein metabolism [196-199].

Importantly, the OX-A downregulation dramatically decreases the levels of tau and p-tau induced by A β_{25-35} *in vitro* [196]. And mouse ISF and human CSF tau

increases during sleep deprivation [200], indicating that orexinergic signaling dysregulation may worsen tauopathy by increasing tau release in ISF and altering the process of tau phosphorylation, as a consequence of decreased restorative sleep stages.

Interestingly, tau knockout mice show significant alterations in their sleep/wake profile, suggesting a role of normal tau function in sleep regulation [201]. Moreover, tau inhibition seems to reverse the A β -induced increase in tau, phosphorylated tau and OX-A expression [196]. Together, these data indicate that tau pathology plays a pivotal role in the disruption of the sleep/wake system observed in AD, and suggest the existence of a network of reciprocal molecular interactions that contribute to AD pathophysiology.

4.3.3 Alzheimer's Disease Related Genes

Finally, orexins regulate both the hippocampal clock and the circadian oscillation of multiple neuroprotective and AD-risk genes [202]. Orexin signaling modulates the period of the hippocampal oscillator, which is involved in controlling the circadian expression of AD-risk genes that play a vital role in A β production and transportation, like BACE1, BACE2, and ApoE. The rhythmicity of these AD-risk genes indicates that AD is associated with circadian oscillation, and orexin signaling is involved in the reciprocal control of the core clock feedback loop and AD.

On the other hand, there's some evidence that orexins reduce hypothalamic cell death by downregulating caspase-3/7 apoptosis, stabilizing *Bcl-2* gene expression and decreasing *Bax/Bcl-2* gene expression, suggesting that these peptides may also play a neuroprotective role in AD [203]. Furthermore, it seems that orexins can increase brain-derived neurotrophic factor expression [204] and prevent oxidative stress [205,206], neurovascular damage [207,208] and neuroinflammation [209].

As a number of studies have shown contradicting roles of orexins in AD, future studies should focus on describing the pros and cons of global modulation of orexins in AD.

4.4 Orexin Receptors Pharmacology

Given the wide expression and distribution of OXRs throughout the central nervous system and peripheral areas, puls the range of physiological processes in which the orexin system is implicated, OXRs agonists/antagonists have broad therapeutic

potential, namely in the treatment of panic and mood disorders [210,211], obesity [212], metabolic syndrome [213], ischemia [207,208,214], chronic inflammation [215] and some forms of cancer [216]. Nevertheless, the spotlight of this discussion is the significance of orexin receptors modulation in the treatment of sleep disorders and neurodegenerative diseases.

Additionally, there is still a lack of understanding of the unique physiological roles of OX₁R and OX₂R activation. And the pharmacological relevance of specific receptor signaling pathways associated with stimulation or blockade of differential G proteins, cation channels and second messengers in well-defined brain regions has not yet been fully clarified [171]. This prompted many academic and pharmaceutical laboratories to design small molecules that can selectively activate signaling pathways to elucidate the OXR-mediated activation processes.

For all these reasons, the orexin system has gained remarkable attention in the last few years.

4.4.1 Orexin Receptor Antagonists

There are two main classes of orexin receptor antagonists, the selective OXR antagonists, which can be either selective for receptor 1 (1-SORAs) or receptor 2 (2-SORAs), and those with binding affinity for both receptors, the dual orexin receptor antagonists (DORAs) [217].

Both DORAs and 2-SORAs have been explored primarily for their sleep-promoting properties in humans. These antagonists promote sleep through the inhibition of the waking drive mediated by the orexin system [218]. Consequently, these drugs are used for the treatment of insomnia and disorders of impaired nocturnal sleep, and the following reasons have been proposed for its rationale: high use of genetic conservation of orexin neurons among mammals, better extrapolation, novel mechanism of action, less serious side effects, no impact on gait/balance/motor coordination, and the role of OX₂R > OX₁R in sleep regulation [219].

Suvorexant (Belsomra[®]/MK-4305), was the first DORA approved by the FDA, in 2014, for treatment of primary insomnia in adults [220]. It differs from existing hypnotics by its novel mechanism of action, which consists in the reversible binding to both OX₁R and OX₂R (K_i = 1.4 nM for OX₁R and 2.2 nM for OX₂R; IC_{50} = 8.8 nM for

OX₁R and 12.0 nM for OX₂R) [221], and thus inhibits the activation of the arousal system, causing sleep induction and sleep maintenance. Despite its potential side effects, which include abnormal thinking, behavioral changes and sleep paralysis, long-term studies with Suvorexant (up to 14 months) in adults and in elderly people with insomnia do not demonstrate a loss of efficacy (tolerance), dependence or withdrawal symptoms upon treatment cessation, making it desirable over other drugs that show higher potential for abuse, such as benzodiazepine receptor agonists or melatonin agonists [220].

Moreover, another two DORAs have been approved by the FDA so far, Lemborexant (Dayvigo®/E2006) in 2020 [222] and Daridorexant (Quviviq®/ACT-541468) in 2022 [223]. Pharmacologically, in comparison to Suvorexant, Lemborexant is slightly more selective for OX₂R than for OX₁R, but not enough to be considered a 2-SORA (K_i = 4.8 nM for OX₁R and 0.61 nM for OX₂R; IC_{50} = 6.1 nM for OX₁R and 2.6 nM for OX₂R) [221], and Daridorexant features a shorter half life ($t_{1/2}$ = 8 hours [224] vs. the 12 hours of Suvorexant [220] and the 17-19 hours of Lemborexant [225]), potentially sparing next-day effects.

While it is well established that DORAs increase total sleep time (TST), several studies have found that this effect occurs primarily through an increase in REM sleep without a proportional increase in time spent in NREM sleep [226]. By contrast, most studies utilizing 2-SORAs report that these compounds restore physiological sleep; that is, they proportionally increase NREM and REM sleep. If these results are replicated in future studies and broader clinical trials, such compounds may prove to be very advantageous in the treatment of sleep disorders in which the sleep disruption is not specific to a particular sleep-stage.

Seltorexant (JNJ-42847922; MIN-202) is an orally-active 2-SORA under development for the treatment of patients with major depressive disorder and insomnia [227]. Several other 2-SORAs have also shown promising sleep-promoting effects in preclinical testing [217].

While blocking OX₂R reduces wakefulness, the expected advantage of selectively antagonizing OX₁R is the ability to achieve clinical efficacy without the promotion of sleep, thus giving 1-SORAs a distinct advantage as potential addiction and anxiety therapeutics [228]. Nivasorexant (ACT-539313) represents the first 1-

SORA to enter clinical development and completed a first proof of concept phase II clinical trial in binge eating disorder in 2022. Furthermore, JNJ-61393215 is another 1-SORA currently in clinical testing [229].

4.4.2 Orexin Receptor Agonists

Since the discovery that the dysregulation of orexin signaling results in narcolepsy in mice, canines and humans, OX₂R-selective agonists have received significant attention as promising therapeutic options for this sleep disorder. While the early development focused on the derivatization of orexin peptides, only a few non-peptidic small-molecule agonists with weak activity were reported until 2015 [147].

The discovery of the first potent OX₂R-selective agonist YNT-185 (EC_{50} = 2750 nM for OX₁R vs. 28 nM for OX₂R), with an aryl sulfonamide moiety as a key structure, was reported in 2015 [230]. In 2019 and 2022, the pharmacological characteristics and discovery of the brain-permeable OX₂R-selective agonist Danavorexton (TAK-925) (EC_{50} > 100,000 nM for OX₂R vs. 5.5 nM for OX₁R), with a 3-amino piperidine and sulfonamide moiety, were reported [231]. Danavorexton has been under human clinical investigation for the treatment of narcolepsy [232,233], idiopathic hypersomnia [234] and sleep apnea [235-237].

Finally, various genetic and pharmacological studies have highlighted the involvement of the OX₁R in the regulation of sleep-wakefulness, feeding behavior, reward seeking, analgesia, energy homeostasis, emotion and the autonomic nervous system. Despite its physiological importance, the contribution of OX₁R to disease pathogenesis is not yet fully understood and, until recently, no small-molecule OX₁R agonists had been reported. Recent YNT-185-based derivatization has led to the discovery of the first dual agonist RTOXA-43 [238] and the first OX₁R-selective agonist (*R*)-YNT-3708 [239].

In conclusion, substantial advances have been recently made to elucidate the physiology and overall neuroscience associated with orexinergic transmission and OXRs. New compounds have been introduced, discussed and are currently under development at all stages of the drug discovery pipeline. These compounds provide invaluable chemical and pharmacological information for advancing the treatment of very complex pathologies and shed light on the underlying cellular mechanisms.

4.4.3 Targeting the Orexin Receptors for the Treatment of Alzheimer's Disease

Given the growing body of literature demonstrating the efficacy of DORAs in the management of chronic insomnia disorder, and the previously mentioned association between orexin system dysregulation and AD pathology, recent research has focused on assessing whether DORAs can improve sleep in AD patients with insomnia and delay the progression of the disease.

Herring *et al.* found that Suvorexant significantly decreased polysomnography-measured wakefulness after sleep onset and increased TST in patients with probable ADRD and insomnia, in comparison with placebo after four weeks of treatment [240]. The team also observed that Suvorexant was mostly effective in mild ADRD patients, had its largest effect on sleep maintenance in the latter part of the night, and did not significantly alter the overall sleep architecture, with mild-to-moderate somnolence being the most common side effect. Based on the data collected in this trial, in 2020 the FDA approved Suvorexant for the treatment of insomnia in patients with Alzheimer's disease of mild to moderate severity [16,17].

Furthermore, Zhou *et al.* observed that Suvorexant treatment effectively ameliorated the cognitive impairments, alleviated *in vivo* hippocampal long-term potentiation suppression, restored the circadian phosphorylated CREB expression in the hippocampus, and reduced amyloid- β protein deposition in the hippocampus and cortex in APP/PS1 mice [241].

More recently, Lucey *et al.* investigated the impact of Suvorexant on tau phosphorylation and amyloid- β concentrations in a randomized controlled trial, comparing acute administration of two Suvorexant dosages against a placebo in 38 cognitively unimpaired volunteers with ages between 45-65 years and without diagnosed or self-reported sleep disorders [242]. Participants treated with Suvorexant 20 mg exhibited a 10-15% decrease in the ratio of phosphorylated to unphosphorylated tau-threonine-181 (a measure of phosphorylation) when compared to the placebo group, and both Suvorexant groups displayed a 10-20% reduction in A β levels in comparison to the placebo.

In line with Lucey *et al.*'s work, an ongoing clinical trial [243] is currently investigating whether long-term treatment with Suvorexant 20 mg could potentially decelerate A β accumulation in the brain of cognitively intact subjects with biomarkers

consistent with AD pathology, further investigating the potential of DORAs as a preventing treatment for AD.

In addition, Suvorexant has also shown effectiveness in reducing the incidence of delirium in elderly intensive care unit-hospitalized patients [244]. And preliminary evidence from a case-series study further supports its possible usefulness for the management of nocturnal delirium in AD patients [245].

Regarding Lemborexant, a phase 2 trial was conducted by Moline *et al.* to evaluate the effect of various dosages versus placebo on actigraphic parameters in patients with mild-to-moderate AD with irregular sleep-wake rhythm disorder [246]. In this study, Lemborexant 5 mg appeared to be the most effective dosage in improving circadian rhythm, and no serious adverse events or worsening of cognitive functions were observed.

Furthermore, a clinical trial was designed to evaluate the efficacy of Seltorexant in reducing agitation symptoms, aggressive behaviors and sleep disturbances in AD patients, however the results have not been disclosed yet [247].

Finally, orexin receptor antagonists are also effective regulators for obesity and hypertension [248], both of which are modifiable risk factors for AD [70-73].

Based on the overwhelming positive clinical data collected in elderly insomnia patients, and positive preclinical data collected with OXR antagonists in various animal models for AD and tauopathies, there is an increasing number of clinical trials with DORAs conducted in mild to moderate AD patients.

Collectively, these findings support the hypothesis that antagonizing the orexin receptors may be an effective way to delay and/or prevent sleep disturbances, circadian rhythm dysfunction, A β accumulation, and thus, potentially, cognitive symptoms in AD. Importantly, OXRs antagonists appear to show the most benefit in treating insomnia in the early stages of AD, due to the relationship between sleep deprivation, the levels of A β and tau in CSF, and neuroinflammation. Therefore, ideally the treatment would start prior to the onset of cognitive symptoms, at the first signs of sleep disturbances.

5 Artificial Intelligence in Alzheimer's Disease Research

5.1 Overview of Artificial Intelligence Techniques

Artificial Intelligence encompasses a broad range of computational techniques and methodologies designed to simulate and extend human intelligence. In the realm of medical research, AI has emerged as a valuable tool to improve diagnosis, monitoring and to accelerate drug discovery and development [249]. Among the most prominent techniques within AI are Machine Learning (ML) and Deep Learning (DL), both of which have revolutionized how machines learn from data.

Machine Learning is a subset of AI that involves training algorithms to recognize patterns, make decisions or predict outcomes based on data. It focuses on developing models that can learn from and make predictions on data without being explicitly programmed for specific tasks. Supervised, unsupervised, semi-supervised, and reinforcement learning are key paradigms within ML [250].

Supervised learning algorithms, such as random forest (RF), naïve Bayes, K-nearest neighbor, decision tree and support vector machine are trained on labeled datasets, meaning that each training example is paired with an output label [251]. Unlike supervised algorithms, unsupervised algorithms, such as kernel density estimation (KDE) and k-means clustering, search for previously unknown patterns within unlabeled data sets [252]. Semi-supervised learning uses both labeled and unlabeled data, and it is typically used in scenarios in which there is a large amount of data available, yet only a small proportion of samples have been labeled [253]. Reinforcement learning involves training an agent by rewarding desired behaviors and punishing undesired ones, which is particularly useful in dynamic environments where the agent must learn to navigate and optimize its actions [254].

Deep Learning is a specialized subset of ML that uses neural networks (NNs) with many layers to model complex patterns in data, and does not require any human-designed rules to operate [255]. DL has gained prominence due to its ability to automatically extract features from raw data, making it particularly effective for tasks involving large and unstructured datasets. Convolutional NNs, recurrent NNs,

autoencoders and generative adversarial networks are some of the most common DL techniques.

Furthermore, DL has driven significant advances in areas like computer vision and natural language processing (NLP) [256]. The last of which involves the interaction between computers and human language, enabling the extraction of meaningful information from unstructured text data, such as clinical notes, research articles, and patient records.

These AI techniques, ranging from traditional ML to advanced DL and NLP, emerged as innovative tools that can aid in unraveling the underlying complex pathologies and clinical heterogeneity at an unprecedented speed and depth, allowing to transform data into improved knowledge in the AD research field.

5.2 Artificial Intelligence for Early Diagnosis and Monitoring of Alzheimer’s Disease Patients

As previously discussed, early diagnosis and continuous monitoring of AD are crucial for effective intervention and management. Importantly, the pre-symptomatic stage of AD has emerged as a key window of opportunity for detection and treatment.

Developments in high-performance computing and ML offer significant potential in identifying patterns and relationships in data that may be overlooked by other traditional methods, and can generate predictive models that can assist in the early detection and management of AD. Furthermore, AI can also be used to develop personalized risk profiles for individuals, which can facilitate early interventions and improve outcomes [257].

5.2.1 Clinical Pattern Recognition

AI algorithms excel in recognizing clinical patterns from patient data.

In the field of AD prediction, there is a wealth of biomedical images available for analysis, including MRI scans, PET scans, and other imaging modalities. By applying DL to these large datasets, it is possible to identify key structural and functional changes in the brain that are predictive of AD onset [258]. Indeed, M.S.K. Inan *et al.* proposed an integrated DL pipeline for early and accurate diagnosis of AD using structural brain MRI scans, focusing on automated slice selection and

classification [259]. The integrated pipeline demonstrated high accuracy in classifying AD from cognitively normal subjects and MCI cases, showing potential in improving AD diagnosis accuracy and offering hope for early detection and intervention.

Furthermore, AI can analyze patient electronic health records to pinpoint risk factors, identify high-risk patients, and build models to predict disease onset by combining clinical, imaging and biomarker data [260,261]. Accordingly, J.L. Ávila-Jiménez *et al.* developed an artificial neural network tailored to analyze longitudinal clinical data with the goal of automating AD diagnosis [262]. By leveraging the wealth of valuable and detailed information in medical records, the model provided accurate predictions, thus demonstrating significant potential in improving early diagnosis and supporting healthcare professionals in their decision-making processes, ultimately contributing to better patient outcomes and resource management in dementia care.

5.2.2 Biomarker Identification

Diagnosis of AD can be supported by PET and CSF biomarker analysis. However, the use of these confirmatory tests is limited due to perceived invasiveness, high costs, and lack of specialized equipment and/or clinical expertise [263].

Improving the clinical management of AD requires cost- and time-effective, minimally invasive and easy-to-interpret biomarkers, that can be widely implemented to aid in identifying high-risk populations, early diagnosis, accurate subtyping, and predicting prognosis, drug response or adverse events [37].

Blood-based data is gaining ground as promising AD biomarkers, since blood samplings are affordable and can be easily performed. Such data is typically generated with omics techniques and exhibits high complexity, large volumes and high dimensionality, making it challenging to extract meaningful information manually. Therefore, the use of DL and ML processes has become increasingly important for analyzing and interpreting blood-based omics data, allowing researchers to detect new disease relationships and identify early biomarkers of AD [264,265].

For example, it has been recently shown that AI in association with omics data can be used to detect new blood biomarkers, such as microRNAs, that predict early stages of dementia, namely MCI [266].

5.2.3 Wearable Technology and Remote Monitoring

Advancements in wearable technology have revolutionized the early prediction and monitoring of AD. These devices can collect data on various parameters, such as language and verbal fluency or executive functions, which can be analyzed by AI algorithms to detect changes indicative of disease progression [267-269]. Owing to their accessibility, safety and cost-effectiveness, digital data from wearables and biosensors is emerging as promising non-invasive tools for AD onset prediction and remote monitoring [257].

Importantly, these advancements have also enabled continuous monitoring of sleep patterns in individuals, allowing for AI models to identify patterns indicative of circadian disturbances associated with AD.

For instance, Brink-Kjaer *et al.* proposed a fully automatic method that concurrently detects arousals and wake using convolutional and long short-term memory NNs, named the Multimodal Arousal Detector (MAD) [270]. This study confirms the viability of using a fully automatic method for scoring arousals, which could lead to more consistent and efficient evaluations in sleep studies. The MAD's performance suggests that it can replace manual scoring, reducing the time and cost associated with sleep disorder diagnostics. The model predictions were correlated with the mean sleep latency, showing the potential in clinical applications as a fully automated scoring tool that predicts next day sleepiness, a characteristic manifestation of circadian misalignment in AD patients.

In addition, Khosroazad *et al.* proposed an innovative method for MCI and ADRD detection using an overnight recording of movements associated with sleep, combined with advanced signal processing and artificial intelligence [271]. Time-Lag (TL), is a parameter extracted from the correlation between high frequency sleep-related movements and respiratory changes during sleep with potential for early detection of MCI and ADRD. By implementing NNs and KDE algorithms, the TL parameter was found to be a significant discriminator in MCI detection, with NN achieving 86.75% sensitivity, 89.25% specificity and 88% accuracy. KDE achieved 65% sensitivity, 100% specificity and 82.5% accuracy. This study further supports the hypothesis that circadian disruption is an early driver of AD, and that sleep signal

analysis combined with AI provides a reliable non-invasive approach for early detection of AD.

All in all, AI is emerging as a potential resource for improving the accuracy and timeliness of AD diagnosis. By leveraging ML, DL and NLP, researchers have developed powerful tools to analyze complex datasets, recognize clinical patterns, identify early biomarkers, detect sleep patterns and continuously monitor the disease dynamics. Therefore, AI models can serve as support tools for healthcare professionals, as they provide additional information that complements their clinical expertise.

5.3 Artificial Intelligence for Drug Discovery and Development for Alzheimer's Disease

Alzheimer's disease is becoming increasingly common as the global population ages, which is bound to raise the financial burden of healthcare [5]. Thus, there's an urgent need for new and highly effective drugs at preventing, delaying the onset, slowing the progression and improving the symptoms. Unfortunately, drug development for AD has proven to be very difficult, partly due to the lack of understanding about the multifaceted pathological mechanisms and its therapeutic modulation, which results in a high rate of failure of drug candidates entering the AD drug-development pipeline [25,26]. Moreover, traditional drug discovery processes are time-consuming and costly, often taking over a decade and billions of dollars to bring a new drug to market [22-24].

Artificial Intelligence offers transformative potential in accelerating drug discovery and development for AD. By leveraging AI techniques, researchers can efficiently analyze vast datasets, identify novel drug targets, optimize drug design, and streamline clinical trials [272,273].

5.3.1 Target Identification and Validation

With recent advances in the data science and ML field, both academia and pharmaceutical companies have begun implementing these approaches within their early drug discovery process.

Indeed, feature extraction from published literature using NLP has started to be explored in target discovery. For example, IBM's Watson, a data analytics processor that uses NLP, was successful at identifying potential novel targets for amyotrophic

lateral sclerosis, highlighting the use of NLP to accelerate scientific discovery in neurodegenerative disorders [274].

Beyond human genetic databases, multi-omics resources offer biologically meaningful information to prioritize potential drug targets [275]. These resources have motivated the application of advanced AI to help scientists identify promising drug targets and accelerate progress towards effective treatments for AD and other challenging diseases. In fact, network analysis with peptides and/or mRNA transcripts have already been used to identify additional potential AD targets [276]. Moreover, a transformative network model was developed for identifying potential targets of late-onset AD by leveraging multi-omics data [277].

Importantly and as already mentioned, the challenges in developing novel therapeutics for AD result from the etiological heterogeneity, the multifaceted often polygenic nature of genetic risk and the complexity of the human brain. The International Neurodegenerative Disorders Research Center (INDRC) is dedicated to overcoming these challenges through an interdisciplinary collaboration, leveraging AI and ML to enhance drug discovery and develop new treatment pathways, since these techniques are suited for modeling complex multi-dimensional systems, like the brain architecture and disease pathogenesis [278]. The INDRC aims to develop a high-performance AI/ML platform to integrate dispersed knowledge and data, in order to generate comprehensive models that consider the diverse and non-linear nature of neurodegenerative diseases.

Hence, advances in artificial intelligence offer promising avenues to unravel the complex interactions between the circadian clock, orexin system and AD, enabling the identification of novel pathological pathways and therapeutic targets. For example, AI models can be employed to classify circadian genes that exhibit altered expression in AD patients compared to healthy controls, in order to gain more understanding about the circadian disruption mechanisms [279]. These algorithms can also be applied to integrate multi-omics data to construct comprehensive models of orexinergic signaling pathways, so that we can clarify its role in AD pathology [278,280].

Moreover, ML models have been developed to predict ligand binding affinity for OXRs. These predictions help to understand how orexinergic signaling may be altered in AD, and have the potential to identify therapeutic agents that can modulate

orexinergic activity and ameliorate sleep disturbances in AD patients. V.Y. Zhang *et al.* developed and validated a quantitative structure-activity relationship (QSAR) model using RF algorithms and molecular descriptors to predict the binding affinity of ligands for the OX₁R [281]. The predictive capacity of the QSAR model was assessed using an external test set and enrichment study, confirming its high predictivity, and the practical applicability of the final model was demonstrated through virtual screening of the DrugBank database. The developed model facilitates virtual screening and drug discovery, offering a valuable tool for identifying new therapeutic agents, and for repurposing existing drugs for disorders associated with the orexin system. In the future, the team intends to extend the QSAR models to differentiate between OX₁R and OX₂R binding affinities, explore additional ML techniques for enhanced predictivity, and address the challenge of predicting ligand functionality (agonist vs antagonist).

5.3.2 Drug Design and Optimization

Recently, there has been substantial progress in drug design and optimization through AI and DL techniques [282], which facilitate the strategic design of novel molecular structures both *de novo* and based on information gleaned from large databases.

AI-driven virtual screening methods can rapidly screen vast chemical libraries to identify compounds that are likely to interact with the target protein, significantly reducing the time and cost associated with experimental screening [283]. Furthermore, molecular docking simulations powered by AI further refine the selection of potential drug candidates, by predicting the binding modes and interactions between the target protein and the compounds [284]. For example, Moon *et al.* introduced a novel DL model named PIGNet, that creatively predicts the atom-atom pairwise interactions via physics-informed equations parameterized with NNs [285]. Comparative analysis demonstrated that PIGNet outperformed other models in both docking and screening tasks, highlighting its superior predictive capabilities.

In addition, once a desired molecular structure is identified, the next question is whether it can be readily synthesized from already-existing molecules. This is known as retrosynthesis prediction, and AI can accelerate this process by predicting synthesis pathways for new compounds [286]. For instance, Liu *et al.* formulated retrosynthesis prediction as a machine translation problem by representing molecules as simplified

molecular-input line-entry system strings [287]. And Shi *et al.* proposed a graph-to-graph framework for retrosynthesis prediction by treating both the product and reactant molecules as graphs [288].

Another fundamental challenge in drug discovery and lead optimization is evaluating the efficacy and safety of a drug candidate, especially its absorption, distribution, metabolism, excretion, toxicity (ADMET) and blood-brain barrier (BBB) penetration properties. AI has recently enabled major progress in this area [289]. For example, ImageMol, an unsupervised pre-training DL framework, was pretrained on 10 million molecular images of unlabeled drug-like bioactive molecules to predict molecular targets, pharmacokinetic and pharmacodynamic properties of candidate compounds [290]. ImageMol outperformed existing state-of-the-art approaches in various benchmark datasets. Furthermore, some existing ADMET models have incorporated BBB permeability prediction, such as admetSAR 2.0 [291].

5.3.3 Drug Repurposing

Drug repurposing involves finding new therapeutic uses for existing drugs, offering a faster and cost-effective alternative to traditional drug development. AI techniques have proven effective in identifying repurposing opportunities in several diseases [292].

Notably, a recent ML-based framework named DRIAD has been developed to quantify the potential associations between AD biological processes and linked genetic datasets, thereby prioritizing drug candidates for repurposing [293].

Furthermore, Chyr, J. *et al.* developed a novel and robust network-based DL method called DOTA to repurpose FDA-approved drugs for AD [294]. This framework considers drug targets, side effects and associations with other diseases in its predictions, increasing the discovery of mechanistically effective drugs for AD. DOTA successfully identified several FDA-approved drugs with potential efficacy in treating AD, including Suvorexant, emphasizing the importance of using advanced DL techniques and optimal transport theory in drug repositioning.

5.3.4 Preclinical Development and Clinical Trials

AI techniques are also transforming preclinical and clinical development by optimizing experimental design, patient selection and trial monitoring.

Despite the widespread adoption and necessity of experimental models in preclinical research, they present significant limitations both in terms of reproducibility of results within models, and in translation from models to human patients. ML can be used to aid in the design, reproduction, interpretation and translation of studies in experimental models, even prior to investment of extra time and resources in new experiments or clinical trials [295].

On the other hand, disentangling the heterogeneity of AD is critical to get the right drug to the right patient at the right time, including during clinical trials. Clustering tools, as unsupervised learning applications, can allow for the identification of biologically meaningful subphenotypes that exhibit more homogeneous characteristics and disease progression profile, allowing not only for better patient stratification and selection, but also more personalized and successful therapies [296-299].

Additionally, ML algorithms can analyze large amounts of medical data to identify optimal participants with specific requirements needed for recruitment in clinical trials, and refine inclusion eligibility criteria for more efficient trial design [300]. This use of AI can help reduce recruitment time and screening procedures.

Furthermore, AI can be used to design clinical trials. Using simulation models, AI can help researchers identify key variables to be monitored during the trial, maximizing trial efficacy and reducing the risk of errors [261].

Finally, AI can be used for pharmacovigilance during the trial [301]. Through mobile phone applications, AI can monitor adherence to medications and symptoms in real time and report any changes to doctors and researchers [302]. This can help to improve adherence, minimize patient attrition, identify side effects, and monitor drug performance and responses to treatments.

In conclusion, AI holds significant promise in transforming AD drug discovery and development by integrating complex datasets, accelerating target identification, optimizing drug design and improving clinical trials. By leveraging ML, DL and NLP techniques, researchers can save time and costs in the discovery of effective treatments for AD, offering new hope for patients and their families.

5.4 Limitations and Future Perspectives

Artificial Intelligence has emerged as a transformative tool in Alzheimer's Disease research, offering novel approaches for early diagnosis, targets and biomarkers identification, drug discovery and development, and patient monitoring.

Despite the remarkable progress already made, the application of AI in AD research is still fraught with challenges. Addressing these limitations is crucial to fully harness the potential of AI and to pave the way for future advancements.

5.4.1 Data Quality and Availability

One of the most significant challenges in AI applications is the quality and availability of data, since models, especially DL ones, require large high-quality datasets to train effectively.

However, AD data is often fragmented, inconsistent and plagued with missing values. The heterogeneity of data sources, including clinical records, neuroimaging, multi omics and wearable device outputs, further complicates the integration and standardization processes [303]. Thus, expert curation, data wrangling and domain expertise are needed to integrate data from multiple sources in an optimal way. Furthermore, adopting common standards for data collection and reporting can certainly enhance the quality and consistency of the datasets [304,305].

Moreover, access to comprehensive datasets is frequently restricted due to privacy concerns and proprietary limitations by pharmaceutical companies and publishers. Inaccessible datasets means that AI cannot contribute value by applying data mining and predictability, hindering the development of robust models [306]. Therefore, in order to maximize the impact that big data and AI can bring to drug discovery and eventually the patient, there is a need for sharing data in an open non-competitive manner with multiple parties in the healthcare ecosystem. In other words, collaboration between academic institutions, industry and government agencies can facilitate the sharing of resources, expertise and data, which in turn accelerates the development and implementation of AI technologies in AD research.

Importantly, federated learning [307,308] and swarm learning [309] can be used for data collection and sharing between entities in a decentralized way, ensuring patient privacy and data security.

5.4.2 Generalization and Bias

As we develop novel computational models to understand AD, issues of reproducibility (i.e. obtaining the same results from the same model and data) and replicability (i.e. applying the same model to independent datasets and observing generalizability of the findings) become increasingly important.

AI models are susceptible to overfitting, where they perform well on training data but fail to generalize to new unseen data, meaning that models often require retraining and tuning for a new problem/dataset [310]. This not only affects the time- and cost-effectiveness of the proposed solution, but also may lead to changes in the model performance, often caused by discrepancies in data distribution or feature space. Thus, the code and all other relevant parts of the prediction modeling pipeline should be made available to others in order to facilitate replicability [311]. Furthermore, this problem is exacerbated by the inherent variability in AD progression and presentation across different populations, which contributes to dataset shift and impacts model generalizability [312]. One way to overcome these challenges is by applying transfer learning. This technique allows the re-use of pre-trained models for domain-specific tasks, offering a time-saving alternative to improve accuracy, replicability and generalizability of results, as well as to prevent overfitting when dealing with relatively small datasets [313,314].

Additionally, biases present in the training data can lead to biased AI models, which may perpetuate health disparities [315]. There have been significant efforts on methods to assess and mitigate bias in existing data. For example, the Global Biobank Meta-analysis Initiative will power genetic and target discovery, while reducing ethnic disparities [316]. Furthermore, existing automated AI methods that quantify and correct for known sources of bias should be routinely implemented [315].

5.4.3 Ethical and Legal Concerns

The widespread use of AI in clinical settings necessitates compliance with regulatory standards and guidelines, which are still evolving in response to the rapid technological advancements. Current regulatory papers lag the wider usage of ML techniques, leading to regulatory uncertainty with risks for stakeholders and companies looking to implement these methodologies [317-319].

Developing comprehensive ethical frameworks and regulatory guidelines for AI in medical research is imperative, as this will help identify the circumstances where each model can be appropriately applied [320,321]. Engaging patients, caregivers, healthcare providers and policymakers in the development and implementation of these frameworks can ensure that AI technologies are used responsibly and ethically.

The FDA has begun to encourage the harmonization of Good ML Practice Guiding Principles to describe a set of AI/ML best practices [322-325]. Similarly, the EMA's 2025 strategic document outlines their plans to establish similar guidelines, as well as acceptability metrics and success factors for the approval of these paradigms [325].

5.4.4 Interpretability and Transparency

The complexity of AI models, particularly DL algorithms, poses significant challenges regarding interpretability and transparency. These models often operate as “black boxes”, making it difficult to understand the decision-making processes underlying their predictions, as the mathematical relationships between variables are learned from the data and are inaccessible to the user [249].

In a clinical context, the lack of interpretability impedes the adoption of AI tools, because healthcare professionals require clear and understandable rationale for AI-driven recommendations to trust and act upon them [327-328]. Efforts to develop more interpretable AI models, such as explainable AI (XAI), can provide insights into the decision-making processes of these models [329].

Techniques like attention mechanisms, feature importance scores and visualization tools can help elucidate how models arrive at specific predictions, thereby increasing trust and transparency [330]. Also, methods such as local interpretable model-agnostic explanations [331] and Shapley additive explanations (SHAP) [332], allow clinicians to interrogate the decision-making process of the models, meaning that they can be used to explain the decisional processes of black-box models.

Indeed, El-Sappagh *et al.* used SHAP to enhance the interpretation and increase the impact in clinical practice of a classification model for both early diagnosis of AD and MCI-to-AD prediction [333]. The model outputs a sentence in natural language, explaining the involvement of the attributes in the model's classification output, and

exhibits a good performance, while balancing the accuracy-interpretability trade-off in both AD classification and MCI-to-AD prediction tasks, allowing for actionable decisions that can enhance physician confidence, contributing to the realization of XAI in healthcare.

Overall, AI offers multiple innovative solutions to improve the diagnosis and treatment of Alzheimer's disease, as well as to accelerate the discovery of new therapeutic targets. However, many challenges remain for its implementation, including the lack of harmonized high quality data, issues in model's reproducibility, replicability, interpretability and transparency, ethical concerns and the infancy of standardized guidance from regulatory authorities.

6 Conclusion

Alzheimer's disease is a growing global concern for which there is an urgent need for early diagnostic tools and disease-modifying treatments.

Mounting evidence supports that disruptions in the orexin and circadian systems contribute to AD pathology by forming a feedback loop, where sleep impairments - one of the first symptoms - exacerbate AD progression and vice versa. However the underlying molecular mechanisms responsible for these changes and relationships remain elusive. Nevertheless, novel treatments that modulate the orexin receptors, namely DORAs, have shown potential in improving sleep, cognition and slowing AD progression. Future studies should be designed to confirm these promising findings, and the recent advancements in artificial intelligence offer powerful tools to do so.

Machine learning, deep learning and natural language processing can be employed in AD research, diagnosis and drug discovery. These techniques provide innovative solutions to address the complexity of AD; elucidate the underlying pathophysiological triggers, including the orexin system's role and the specific clock-controlled transcriptional pathways involved; support healthcare professionals in the decision-making process; accelerate the identification of therapeutic targets and biomarkers; monitor the AD continuum; optimize drug development and improve clinical trials design, all in a more time- and cost-effective way.

However, challenges remain in harnessing AI's full potential, including the lack of harmonized high quality data, issues in model's reproducibility, replicability, interpretability and transparency, ethical concerns, and the fact that guidance from regulatory authorities has not yet been standardized. A clear opportunity exists for researchers, clinicians and other stakeholders to work together to overcome these hurdles, and develop trustworthy models that can be successfully implemented in clinical practice. By addressing these current limitations, the field can move towards more effective and personalized approaches to AD management, ultimately improving patient outcomes and quality of life, and alleviating the healthcare systems' burden.

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